LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

Eglė Balčiūnienė

THE EFFECT OF GENES RELATED TO TUMOR PROGRESSION AND METASTASIS ON CERVICAL CANCER PROGNOSIS

Doctoral Dissertation
Medical and Health Sciences,
Medicine (M 001)

Kaunas, 2024

Dissertation has been prepared at the Institute of Oncology of Medical Academy of Lithuanian University of Health Sciences during the period of 2018–2024 year.

Scientific Supervisor

Prof. Dr. Arturas Inčiūra (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

Consultant

Prof. Dr. Rasa Ugenskienė (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

Dissertation is defended at the Medical Research Council of the Lithuanian University of Health Sciences:

Chairperson

Prof. Habil. Dr. Virgilijus Ulozas (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

Members:

Prof. Dr. Sandra Lejniece (Riga Stradins University, Medical and Health Sciences, Medicine – M 001);
Assoc. Prof. Dr. Audrius Ivanauskas (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001);
Assoc. Prof. Dr. Vilius Rudaitis (Vilnius University, Medical and Health Sciences, Medicine – M 001);
Prof. Dr. Rasa Jančiaukienė (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

The dissertation will be defended in an open session of the Medical Research Council of the Lithuanian University of Health Sciences on December 17, 2024, at 1 p.m., in the Didžioji Auditorium at the central building of the Hospital of Lithuanian University of Health Sciences Kauno klinikos.
Address: Eivenių 2, LT-50161 Kaunas, Lithuania.

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS

Eglė Balčiūnienė

GENŲ, SUSIJUSIŲ SU NAVIKŲ PROGRESAVIMU IR METASTAZAVIMU, REIKŠMĖ GIMDOS KAKLELIO VĖŽIO PROGNOZEI

Daktaro disertacija
Medicinos ir sveikatos mokslai,
medicina (M 001)

Kaunas, 2024

Disertacija rengta 2018–2024 metais Lietuvos sveikatos mokslų universiteto Medicinos akademijos Onkologijos institute.

Mokslinis vadovas

prof. dr. Arturas Inčiūra (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Konsultantė

prof. dr. Rasa Ugenskienė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija ginama Lietuvos sveikatos mokslų universiteto Medicinos mokslo krypties taryboje:

Pirmininkas

prof. habil. dr. Virgilijus Ulozas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Nariai:

prof. dr. Sandra Lejniece (Rygos Stradinio universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
doc. dr. Audrius Ivanauskas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
doc. dr. Vilius Rudaitis (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
prof. dr. Rasa Jančiaukienė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija bus ginama viešame Lietuvos sveikatos mokslų universiteto Medicinos mokslo tarybos posėdyje 2024 m. gruodžio 17 d. 13 val. Lietuvos sveikatos mokslų universiteto ligoninės Kauno klinikų Didžiojoje auditorijoje.
Adresas: Eivenių g. 2, LT-50161 Kaunas, Lietuva.

CONTENTS

ABBREVIATIONS

ACC1	–	acetyl-CoA carboxylase 1
ACLY	–	ATP citrate lyase
AID	–	activation-induced cytidine deaminase
AF6	–	afadin
Akt	–	protein kinase B (PKB)
ALL	–	acute lymphoblastic leukemia
ALY	–	Aly/REF export factor
AMPK	–	AMP-activated protein kinase
ANRIL	–	antisense non-coding RNA in the INK4 locus
AP1	–	activator protein 1.
APOLO	–	apolipoprotein L protein
AQP2	–	aquaporin-2
ARF	–	alternative reading frame gene
ART	–	artesunate
AS-Uchl1	–	antisense UCH-L1 lncRNA
ATP	–	adenosine triphosphate
BACE1	–	beta-secretase 1 antisense RNA
Bax	–	Bcl-2-associated X protein
Bcl-2	–	B-cell lymphoma 2 protein
Bcl-xL	–	B-cell lymphoma-extra large protein
Bcr	–	breakpoint cluster region
Bcr-Abl	–	breakpoint cluster region – Abelson murine leukemia viral oncogene homolog
β-TrCP	–	beta-transducin repeat containing protein
BIN1	–	bridging Integrator 1 protein
BM	–	bone marrow
BRD4	–	bromodomain containing 4 protein
BRMS1	–	breast cancer metastasis suppressor 1
C9-I6	–	isoform or variant of the C9 protein
CA-125	–	cancer antigen 125 protein
CaMKK	–	calmodulin-dependent protein kinase
Cas-II-gly	–	caspase-2 (CARD domain)
CC	–	cervical cancer
CD44	–	cluster of differentiation 44
c/EBP	–	CCAAT/enhancer binding protein
cfDNA	–	cell-free DNA
CFTR	–	cystic fibrosis transmembrane conductance regulator
CHASERR	–	cancer-associated SEMA3A-enhancing lncRNA
CHD2	–	chromodomain helicase DNA-binding protein 2
CIN3	–	cervical intraepithelial neoplasia grade 3
circSMARCA5	–	circular SMARCA5 gene
CK7	–	cytokeratin 7
CK17	–	cytokeratin 17
CK19	–	cytokeratin 19
Clk/Sty	–	clock/shaggy proteins
COOLAIR	–	COOLAIR lncRNA

CONCR	–	conserved RNA
CRC	–	colorectal cancer
CREB	–	cAMP response element-binding protein
CRISPR	–	clustered regularly interspaced short palindromic repeats
CTCs	–	circulating tumor cells
CTL	–	cytotoxic T lymphocytes
Cxcl1	–	C-X-C motif chemokine ligand 1
Cxcl2	–	C-X-C motif chemokine ligand 2
Cxcl9	–	C-X-C motif chemokine ligand 9
DAMPs	–	damage-associated molecular patterns
DBF4B-FL	–	DBF4B full-length protein
DCUN1D5	–	DCUN1D5 E3 ubiquitin ligase
DDX11	–	DEAD-box helicase 11 protein
DFS	–	disease-free survival
DGCR5	–	lncRNA (stands for DiGeorge critical region 5)
DHT	–	dihydrotestosterone
dilncRNAs	–	damage-induced long non-coding RNAs
DNR	–	deoxyribonucleic acid
DOCK1	–	dedicator of cytokinesis 1
E2F1	–	E2F transcription factor 1
EBRT	–	external beam radiation therapy
ECDC	–	the European Centre for Disease Prevention and Control
ECM	–	extracellular matrix genes
ELEANORs	–	extensive lncRNA associated networks of RNA sequences
elncRNAs	–	enhancer-associated long non-coding RNAs
EOC	–	epithelial ovarian cancer
EPAS1	–	endothelial PAS domain protein 1
ER	–	estrogen receptor
ERBB2	–	receptor tyrosine-protein kinase erbB-2 (human epidermal growth factor receptor 2)
ERK	–	extracellular signal-regulated kinase
eRNA	–	enhancer RNA
ESCC	–	esophageal squamous cell carcinoma
EZH2	–	enhancer of zeste homolog 2
FABP4	–	fatty acid-binding protein 4
FAK	–	focal adhesion kinase
FAST	–	focal adhesion assembly and stabilization transcriptional regulation protein
FGF2	–	fibroblast growth factor 2
FIGO	–	International Federation of Gynecology and Obstetrics
FZD2	–	frizzled class receptor 2
G	–	degree of tumor differentiation
GADD45A	–	growth arrest and DNA damage-inducible protein alpha
GAP	–	GTPase activating protein
GBM	–	glioblastoma multiforme
GRB2	–	growth factor receptor-bound protein 2
GRD domain	–	GTPase-regulating domain
GTPase	–	guanosine triphosphatase
H3K27me3	–	trimethylation of histone H3 at lysine 27 protein

hESC	–	human embryonic stem cells
HDR	–	homology-directed repair
HGF	–	hepatocyte growth factor
hnRNPA1	–	heterogeneous nuclear ribonucleoprotein A1
HICs	–	high-income countries
HIF-1α	–	hypoxia-inducible factor 1-alpha, transcription factor
HOTAIR	–	HOX transcript antisense RNA
HOXA	–	homeobox A (HOXA) cluster
HOXD4	–	a member of the homeobox D (HOXD) gene cluster
HP1α	–	heterochromatin protein 1-α
HPV	–	human papillomavirus
HSD17B2	–	hydroxysteroid dehydrogenase 17-beta type 2
ICC	–	invasive cervical cancer
IGF1-R	–	insulin-like growth factor 1 receptor
Igf2r	–	insulin-like growth factor 2 receptor
IL-2	–	interleukin-2
INK4a/INK4b	–	member of the INK4 family of cyclin-dependent kinase
IRAK	–	interleukin-1 receptor-associated kinase
IRES	–	internal ribosome entry site
IRF	–	interferon regulatory factor
ISE	–	insertion sequence element
ITGB1	–	integrin beta 1
JARID2	–	Jumonji and AT-rich interaction domain 2 protein
JNK	–	c-Jun N-terminal kinase
KEAP1	–	Kelch-like ECH-associated protein 1
KHPS1	–	KH domain containing, putative spliceosome associated protein 1
Ki67	–	antigen identified by monoclonal antibody Ki-67
LD	–	linkage disequilibrium
LIG1	–	DNA ligase 1
LMICs	–	low- and middle-income countries
lncRNAs	–	long non-coding RNAs
LPL	–	lipoprotein lipase
LSCC	–	laryngeal squamous cell carcinoma
LSD1	–	lysine-specific demethylase 1
LVSI	–	lymphovascular space invasion
LZ	–	leucine zipper
M	–	metastasis spread
MAF	–	minor allele frequency
MALAT1	–	metastasis-associated lung adenocarcinoma transcript 1
MAPK	–	mitogen-activated protein kinase
MAT2A	–	methionine adenosyltransferase 2A
MDM2	–	mouse double minute 2 homolog
MDS	–	myelodysplastic Syndromes
MEG3	–	maternally expressed gene 3
MET	–	mesenchymal epithelial transition factor
METTL3	–	methyltransferase-like 3
MFS	–	metastasis-free survival
MICB	–	MHC class I chain-related protein B
miR-10b	–	microRNA-10b

MIR155HG	–	MIR155 host gene
miR-320a	–	microRNA-320a
miRNA	–	microRNA
MMPs	–	matrix metalloproteinases
MMTV	–	mouse mammary tumor virus
Mnk2	–	MAPK-interacting serine/threonine-protein kinase 2
MPN	–	myeloproliferative neoplasms
mTOR	–	mammalian target of rapamycin
MVD	–	microvascular density
Myc	–	myelocytomatosis virus proto-oncogenes
MYH9	–	myosin heavy chain 9
MyD88	–	myeloid differentiation primary response 88
N	–	lymph node involvement
NEAT2	–	nuclear-enriched transcript 2 lncRNA
NF-kB	–	nuclear factor kappa-light-chain-enhancer of activated B cells
NHEJ	–	non-homologous end joining
NK	–	natural killer cells
NKG2D	–	natural killer group 2 member D
NKILA	–	nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) interacting lncRNA
NMD	–	nonsense-mediated mRNA decay
NORAD	–	non-coding RNA activated by DNA damage
NPM1	–	nucleophosmin 1
NRF	–	nuclear respiratory factor
NSCLC	–	non-small cell lung cancer
NXF1	–	nuclear export factor 1
OPN	–	osteopontin
OS	–	overall survival
OSCC	–	oral squamous cell carcinoma
p16INK4a	–	inhibitor of cyclin-dependent kinase 4a
p53	–	tumor protein 53
p65	–	a subunit of the NF-κB transcription factor complex
p70S6K	–	p70 S6 kinase
PABPN1	–	poly(A)-binding protein nuclear 1
PAMPs	–	pathogen-associated molecular patterns
PBL	–	peripheral blood lymphocytes
PCa	–	prostate cancer
PCDH10	–	protocadherin 10
PCK2	–	phosphoenolpyruvate carboxykinase 2
PD-1	–	programmed death-1 protein
PD-L1	–	programmed death-ligand 1 protein
PDGFRβ	–	platelet-derived growth factor receptor beta
PDZ domain	–	postsynaptic density-95/disc-large/zonula occludens-1 domain
PI3K	–	phosphoinositide 3-kinase
PIK3CA	–	phosphatidylinositol 3-kinase catalytic subunit alpha
PLK1	–	polo-like kinase 1
PNUTS	–	PP1 nuclear targeting subunit
Pol II	–	RNA Polymerase II
PP1	–	protein phosphatase 1

PP2A	–	protein phosphatase 2A
PR	–	progesterone receptor
pRb	–	retinoblastoma protein
PRC2	–	polycomb repressive complex 2 protein complex
PRRs	–	pattern recognition receptors
PSMA	–	prostate-specific membrane antigen
PTBP1	–	polypyrimidine tract binding protein 1
PTC	–	premature termination codon
PTEN	–	phosphatase and tensin homolog
PTPMT1	–	posttranslational modification type 1
PWS	–	Prader-Willi syndrome (PWS) region
QTL	–	quantitative trait locus
RAF	–	rapidly accelerated fibrosarcoma kinases
Ran	–	Ras-related nuclear protein
RAS	–	rat sarcoma proteins
Rasa3	–	Ras GTPase-activating protein 3
RBFOX2	–	RNA-binding protein Fox-2
RBP	–	RNA binding protein
RNA	–	ribonucleic acid
RNF43	–	RING finger protein 43
RPL	–	ribosomal protein large subunit
RRMs	–	RNA recognition motifs
RRP	–	ribosomal RNA Processing Protein
RRP1B	–	ribosomal RNA processing 1B protein
RS	–	arginine-serine
RSK	–	ribosomal S6 kinase
SAA3	–	serum amyloid A3 protein
SAMMSON	–	survival associated mitochondrial magnesium-dependent protein
SCD1	–	stearoyl-CoA desaturase 1
SCC-Ag	–	squamous cell carcinoma antigen
Ser	–	serine
SETDB1	–	SET domain bifurcated 1 protein
SHM	–	somatic hypermutation
SIPA1	–	signal-induced proliferation-associated 1 protein
sno-lncRNAs	–	small nucleolar long non-coding RNAs
SNP	–	single nucleotide polymorphism
SOX17	–	SRY-related HMG-box gene 17
SPHK1	–	sphingosine kinase 1
SREBP-1c	–	sterol regulatory element-binding protein 1c
SRPK	–	serine/arginine-rich protein kinase
SRSF1	–	serine/arginine-rich splicing factor 1 protein
SRY	–	sex-determining region Y
STAT3	–	signal transducer and activator of transcription 3
STAU1	–	Staufen double-stranded RNA binding protein 1
STIs	–	sexually transmitted infections
SUZ12	–	suppressor of zeste 12 protein
SWINGN	–	single-cell weighted interactions of non-coding genes
SYK	–	spleen tyrosine kinase
T	–	tumor size

TAP	–	transporter associated with antigen processing
TARID	–	T-cell acute lymphoblastic leukemia-associated RNA induced by DNA damage
TCF21	–	transcription factor 21
TDP43	–	TAR DNA-binding protein 43 protein
TEAD1	–	TEA domain family member 1, a transcription factor
TET1	–	Tet methylcytosine dioxygenase 1
TIR	–	Toll/interleukin-1 receptor
TIRAP	–	Toll/interleukin-1 receptor (TIR) domain-containing adaptor protein
TJ	–	tight junction
TLR4	–	Toll-like receptor 4 protein
TLRs	–	Toll-like receptors
TMA	–	tissue microarrays
TNBC	–	triple-negative breast cancer
TNM	–	tumor, node, metastasis (staging system)
TNF	–	tumor necrosis factor
TPA	–	tissue plasminogen activator
TRIF	–	TIR-domain-containing adapter-inducing interferon-β
TRIM28	–	tripartite motif-containing protein 28
UMLILO	–	upregulated in mitochondria lncRNA
UNICEF	–	the United Nations International Children's Emergency Fund
UPP1	–	uridine phosphorylase 1
VEGF	–	vascular endothelial growth factor
WHO	–	the World Health Organization
Wnt	–	wingless/integrated
XIST	–	X-inactive specific transcript (lncRNA)
YBX1	–	Y-box binding protein 1
ZEB	–	zinc finger E-box binding proteins
ZO-1	–	zonula occludens-1

INTRODUCTION

Cervical cancer is a widely spread health problem and one of the most common oncological diseases. According to the World Health Organization, it is women's fourth most commonly diagnosed cancer. It also ranks fourth in the world for cancer-related deaths among women [1]. Despite advancements in prevention and treatment, it continues to be a leading cause of cancer-related morbidity and mortality among women, particularly in low- and middle-income countries [2, 3].

The pathogenesis of cervical cancer is complex and multifactorial, involving both environmental and genetic factors. While persistent infection with high-risk human papillomavirus (HPV) is the primary etiological factor, genetic variations also play a crucial role in disease susceptibility, progression, and treatment response [4].

Among the myriad of genetic factors implicated in cervical cancer, Toll-like receptor 4 (TLR4) has emerged as a key player in innate immunity and inflammation. TLR4 activation has been shown to modulate the host immune response to HPV infection and influence the development and progression of cervical cancer [5, 6].

Similarly, the *RRP1B* gene, encoding the ribosomal RNA processing 1B protein, has been implicated in various cellular processes, including cell proliferation, migration, and invasion. Genetic alterations in *RRP1B* have been associated with tumor aggressiveness and metastasis in cancer [7, 8].

SIPA1, a gene involved in signal transduction pathways, has also been implicated in cervical cancer progression. Studies have demonstrated a role for *SIPA1* in promoting tumor cell proliferation, migration, and invasion, highlighting its potential as a therapeutic target in cervical cancer [9].

Furthermore, splicing factor SRSF1 has garnered attention for its role in mRNA processing and alternative splicing in cancer. Dysregulation of SRSF1 expression and its genetic variants have been linked to aberrant splicing patterns and tumor progression [10].

Long non-coding RNAs (lncRNAs) HOTAIR and MALAT1 have also been implicated in cervical cancer pathogenesis. These lncRNAs play critical roles in epigenetic regulation, metastasis, and treatment resistance, making them attractive targets for further investigation [11, 12].

Despite advancements in our understanding of the genetic basis of cervical cancer, significant gaps remain in our knowledge. Further research is warranted to elucidate the precise roles of genes such as *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR*, and *MALAT1* in disease pathogenesis and clinical outcomes. While challenges in cervical cancer prevention and treatment

persist, recent advancements in molecular biology and genetics offer promising opportunities for improvement. Gaining a deeper understanding of the genetic determinants influencing cervical cancer susceptibility, progression, and response to treatment is crucial for developing targeted interventions and personalized treatment strategies.

Emerging research is increasingly focused on unraveling the molecular mechanisms that drive cervical cancer development and progression. As our knowledge of these genetic and molecular factors expands, the potential for more precise and individualized approaches to cervical cancer care becomes more attainable, offering hope for better prevention, early detection, and treatment in the future.

In this context, our study aims to investigate the influence of germline polymorphisms in key genes associated with cervical cancer progression. By elucidating the role of genetic variants in tumor development and response to therapy, our research seeks to identify novel biomarkers with prognostic value and inform precision medicine approaches for cervical cancer management. The primary objective of this dissertation is to explore the associations between genetic variants in these six genes and cervical cancer susceptibility, progression, and treatment response. By unraveling the genetic determinants of cervical cancer, this research aims to identify novel biomarkers for risk stratification and personalized therapeutic approaches, ultimately contributing to improved patient outcomes.

The aim of the study

The aim of this study was to evaluate the significance of germline polymorphisms in the *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR*, and *MALAT1* genes on cervical cancer prognosis.

Objectives

1. To identify the polymorphisms of the immune response modulator *TLR4* gene and assess their correlations with the progression and prognosis of cervical cancer.
2. To assess the associations of the *RRP1B*, *SIPA1*, and *SRSF1* gene variants, involved in the metastasis process, with the course of cervical cancer.
3. To analyze the variants of the epigenetic modifiers *HOTAIR* and *MALAT1* genes and investigate their relationship with the progression of cervical cancer.

Novelty and relevance of the study

Our study focused on patients with cervical cancer, a disease with one of the highest incidence rates among cancers affecting women.

The investigation of 27 SNPs of *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR*, and *MALAT1* genes represents a novel and highly relevant approach in the study of cervical cancer. Each of these genes plays crucial roles in various aspects of cancer development, progression, and treatment response.

The inclusion of *TLR4* in this study is particularly noteworthy due to its involvement in the innate immune response and its potential impact on tumor microenvironment regulation. Understanding the role of *TLR4* polymorphisms in cervical cancer progression could provide valuable insights into the interplay between the immune system and tumor development.

Similarly, *RRP1B* and *SIPA1* are genes with emerging significance in cancer biology, implicated in cell proliferation, migration, and invasion. Investigating genetic variants within these genes in the context of cervical cancer could shed light on their specific contributions to tumor aggressiveness and metastasis.

SRSF1, a splicing factor, has been increasingly recognized for its role in alternative splicing patterns associated with cancer progression. Exploring the correlations between *SRSF1* variants and the course of cervical cancer could reveal novel mechanisms of tumor growth and therapeutic targets.

The study of long non-coding RNAs (lncRNAs) encoded by *HOTAIR* and *MALAT1* represents a cutting-edge area of research in cancer biology. These lncRNAs have been implicated in various aspects of tumorigenesis, including epigenetic regulation, metastasis, and treatment resistance. Analyzing genetic variations within *HOTAIR* and *MALAT1* genes may uncover novel biomarkers for cervical cancer prognosis and potential therapeutic targets.

Previous studies have indicated that inherited polymorphisms are associated with specific tumor characteristics and subsequent outcomes in human cancer. Recognizing the potential impact of germline polymorphisms on disease pathomorphological features and disease progression, we examined the associations between twenty-seven functional SNPs in the *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR*, and *MALAT1* genes and the clinicopathological profiles and survival rates in a cohort of women with cervical cancer. To our knowledge, our study is the first to analyze these gene SNPs to assess clinicopathological features and the progression of cervical cancer. It establishes a link between SNPs and CC, suggesting these genetic variants as predictive biomarkers for prognosticating the development of the disease in the future.

The study boasts several strengths, including a comprehensive dataset comprising genetic data, tumor phenotype information, and survival data.

Notably, the absence of comparable studies examining the associations between these polymorphisms and the clinicopathological characteristics of cervical cancer prevents a direct comparison of our results. Consequently, our investigation aimed to elucidate the effect of these polymorphisms on the clinical manifestations and outcomes of the disease.

Overall, by investigating the genetic variants of these six genes and their associations with cervical cancer progression, this study not only addresses gaps in current understanding but also holds promise for the identification of novel prognostic markers and therapeutic targets, ultimately contributing to improved patient outcomes in the management of cervical cancer.

Author's personal contribution

The author has been a part of the team collaborating on the "Oncologic Disease Tissue Biobanking" project (started in 2014) at the Oncology Institute of the Lithuanian University of Health Sciences.

The author's contributions to this project include:

1. Designing the study;
2. Conducting a literature review;
3. Selecting SNPs for analysis;
4. Enrolling 172 cervical cancer patients;
5. Collecting blood samples for genetic analysis;
6. Gathering patient clinical data;
7. Collecting patient survival follow-up data;
8. Creating a patient database;
9. Performing statistical analysis;
10. Preparing publications related to the study.

1. LITERATURE REVIEW

1.1. Prevalence of cervical cancer

Cervical cancer (CC) ranks among the most frequently diagnosed cancers and stands as a leading cause of cancer-related mortality in women worldwide. It presents a significant global health challenge, with marked disparities in incidence and mortality rates between regions, particularly affecting women in low- and middle-income countries (LMICs). According to the World Health Organization (WHO), cervical cancer is the fourth most common cancer among women worldwide, with an estimated 660,000 new cases and 350,000 deaths reported in 2022 alone [1]. These statistics underscore the urgent need for continued research and intervention efforts to mitigate the burden of this disease.

The distribution of cervical cancer incidence and mortality rates varies substantially across regions, with the highest burden observed in LMICs. In sub-Saharan Africa, for instance, cervical cancer ranks as the leading cause of cancer-related mortality among women, with incidence rates as high as 40 per 100,000 women [13]. Similarly, regions such as South Asia and Latin America experience significant morbidity and mortality attributable to cervical cancer [14].

These disparities in cervical cancer burden are largely attributed to disparities in access to preventive measures such as vaccination against human papillomavirus (HPV) and screening programs for early detection of precancerous lesions. In high-income countries (HICs), widespread implementation of HPV vaccination programs and organized screening initiatives has led to notable reductions in cervical cancer incidence and mortality rates [2]. However, such preventive measures remain largely inaccessible in many LMICs, contributing to the disproportionate burden of cervical cancer in these regions. In addition to disparities in preventive measures, challenges in accessing timely diagnosis and treatment further exacerbate the burden of cervical cancer in LMICs. Limited infrastructure, shortage of skilled healthcare providers, and socioeconomic barriers often impede women's access to essential cervical cancer screening, diagnosis, and treatment services [15–18].

In Europe, cervical cancer incidence and mortality rates vary widely, reflecting differences in healthcare infrastructure, screening programs, and access to preventive measures such as HPV vaccination. While many countries in Western Europe have implemented organized screening initiatives and achieved substantial reductions in cervical cancer burden, disparities persist in Eastern and Southern Europe, where access to screening services may be

limited and screening uptake rates remain suboptimal. While some countries are close to reaching the target for reducing cervical cancer incidence, significant disparities remain in incidence, mortality, and access to quality care across Europe. The annual world-age standardized incidence rates vary significantly, ranging from 6.8 per 100,000 women in Western Europe to 16 per 100,000 women in Central and Eastern Europe [19, 20].

Recent data from the European Centre for Disease Prevention and Control (ECDC) indicate that although cervical cancer incidence rates have been declining in most European countries, mortality rates remain a concern, particularly in regions with lower screening coverage and vaccination rates [21].

The success of HPV vaccination programs in Europe also varies across countries, with higher coverage rates reported in Northern and Western European nations compared to Central and Eastern European countries. As a result, disparities in HPV vaccine uptake contribute to disparities in cervical cancer burden across the continent [2].

In addition to geographical disparities, socioeconomic factors play a significant role in shaping cervical cancer outcomes in Europe. Women from disadvantaged socioeconomic backgrounds are less likely to participate in cervical cancer screening programs and may face barriers to accessing timely diagnosis and treatment services. Figure 1 displays the age-standardized incidence and mortality rates for cervical cancer across different regions worldwide. Figure 2 provides estimates of cervical cancer incidence and mortality for European countries.

According to data from the Lithuanian Institute of Hygiene, there were 154 recorded deaths due to cervical cancer in 2023, accounting for 0.42% of all causes of death. Based on data from the Cancer Institute, 354 new cases of cervical cancer were reported in 2017 (the most recent available data). Cervical cancer was the sixth most common type of cancer among women in Lithuania. In that year, there were 200 recorded deaths due to cervical cancer.

Despite these challenges, recent advancements in molecular biology and genetics offer promising avenues for improving cervical cancer prevention and treatment. Understanding the genetic determinants of cervical cancer susceptibility, progression, and treatment response is crucial for developing targeted interventions and personalized treatment strategies.

In this context, our study aims to investigate the influence of germline polymorphisms in key genes associated with cervical cancer, with a particular focus on European populations. Our research seeks to identify novel biomarkers with prognostic value and to inform precision medicine approaches for cervical cancer management in Europe.

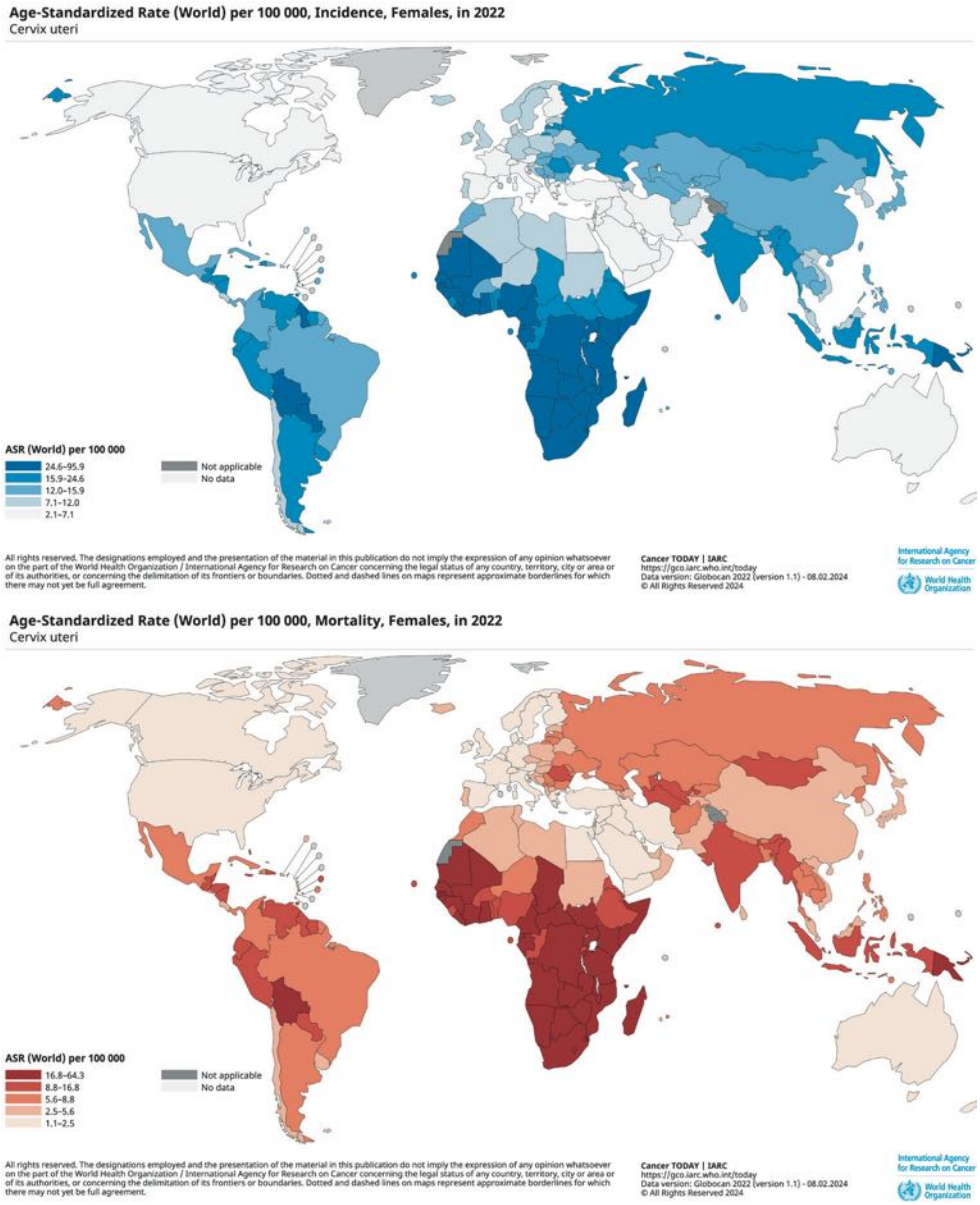

Figure 1. *Region-specific age-standardized incidence and mortality rates for cervical cancer in 2022.*

The rates are presented in descending order based on the world (W) age-standardized incidence rate, with the highest national age-standardized rates for both incidence and mortality highlighted. Source: GLOBOCAN 2022: https://gco.iarc.fr/today/en/dataviz/maps-heatmap?mode=population&sexes=2&cancers=23&types=0; https://gco.iarc.fr/today/en/dataviz/maps-heatmap?mode=population&sexes=2&cancers=23&types=1.

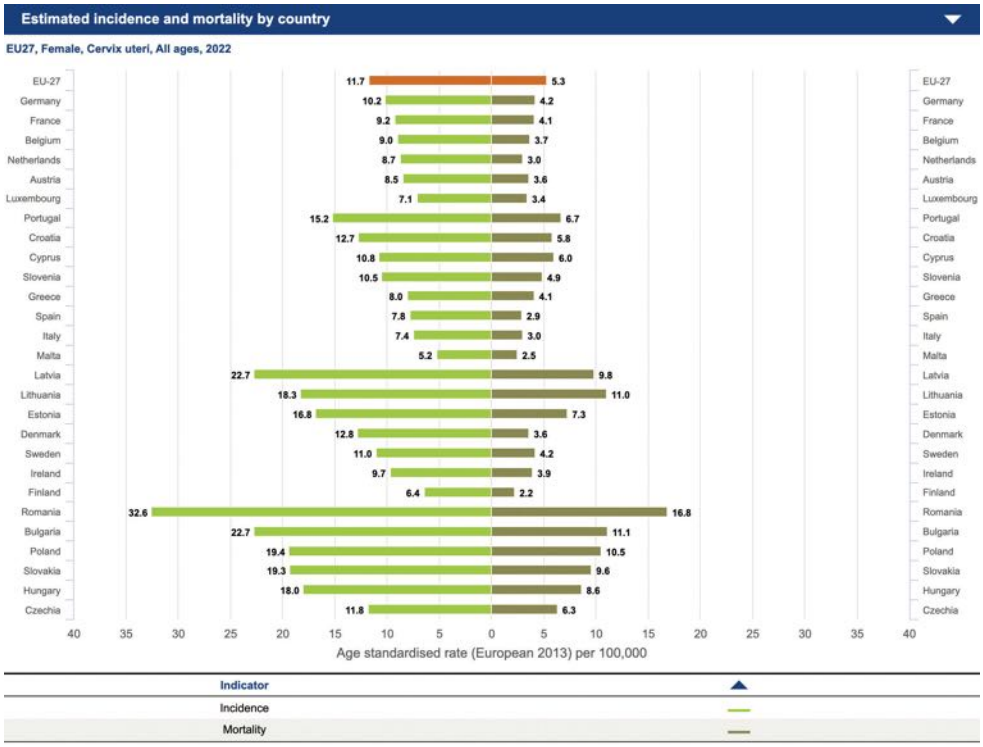

Figure 2. *Estimates of cervical cancer incidence and mortality in 2022, for all countries.*

Source: ECIS – European Cancer Information System: https://ecis.jrc.ec.europa.eu/explorer.php?$0-0$1-All$2-All$4-2$3-30$6-0,85$5-2022,2022$7-7,8$CEstByCountry$X0_8-3$X0_19-AE27$X0_20-No$CEstBySexByCountry$X1_8-3$X1_19-AE27$X1_-1-1$CEstByIndiByCountry$X2_8-3$X2_19-AE27$X2_20-No$CEstRelative$X3_8-3$X3_9-AE27$X3_19-AE27$CEstByCountryTable$X4_19-AE27.

1.2. Cervical cancer risk factors

The risk factors for cervical cancer yields valuable insights into the epidemiology and determinants of this prevalent disease. The complex factors interplay of socioeconomic, behavioral, and healthcare-related factors influences the development of cervical cancer.

Cervical cancer remains a significant global health challenge, particularly affecting women in developing countries. Across the world, certain risk factors have been consistently identified as contributing to the development of this cancer. The findings regarding the association between sexually transmitted infections (STIs) and cervical cancer highlight the need for comprehensive sexual health education and STI prevention programs.

Human papillomavirus (HPV) infection stands out as the primary risk factor. One of the most significant scientific discoveries of the past 30 years is the establishment of a causal link between human papillomavirus infection of the cervix and cervical cancer, spearheaded by Harald zur Hausen and his team, leading to the development of highly effective prophylactic vaccines preventing 70–80% of cervical cancer cases, for which zur Hausen was awarded the Nobel Prize in Physiology or Medicine in 2008 [22].

Human papillomavirus types are classified as either carcinogenic or probably carcinogenic, with type 16 being significantly more likely to persist and lead to CIN3 and cervical cancer [23, 24]. Roughly 70% of all cervical cancer cases are attributed to Human Papillomavirus types 16 and 18 [25, 26]. Specific HPV types such as 16, 18, 31, 33, and 45 being strongly associated with invasive cervical carcinoma [27].

Global randomized clinical trials have demonstrated that bivalent, quadrivalent, and nonavalent vaccines provide protection against precancerous lesions associated with human papillomavirus, with high efficacy observed in individuals not previously infected with HPV, although efficacy decreases in those with prior HPV exposure. Additionally, these vaccines offer cross-protection against non-vaccine HPV types [28–37].

Human papillomavirus (HPV) infection is the primary risk factor for cervical cancer, with certain high-risk HPV types, notably HPV 16 and 18, being responsible for the majority of cases [38, 39]. Advances in screening methods, such as HPV testing and Pap smears, have significantly improved early detection and prevention efforts, leading to a decrease in cervical cancer incidence and mortality rates in many countries [40]. Vaccination against HPV has emerged as a powerful strategy for cervical cancer prevention, with vaccines targeting the most oncogenic HPV types, demonstrating high efficacy in preventing HPV infection and subsequent cervical lesions [41]. While many countries in Western Europe have implemented organized screening initiatives and achieved substantial reductions in cervical cancer burden, disparities persist in Eastern and Southern Europe, where access to screening services may be limited and screening uptake rates remain suboptimal. Recent data from the European Centre for Disease Prevention and Control (ECDC) indicate that although cervical cancer incidence rates have been declining in most European countries, mortality rates remain a concern, particularly in regions with lower screening coverage and vaccination rates [42].

WHO/UNICEF data from 2010 to 2019 show progress toward the 90% HPV vaccination target by 2030, but significant challenges remain. By June 2020, 107 of 194 WHO Member States had introduced HPV vaccination, with the Americas and Europe leading. Despite a surge in introductions, especially

in low- and middle-income countries, global coverage for the final HPV dose in 2019 was only 15%. Achieving the 2030 target will require continued efforts, particularly in high-population countries, to improve and expand vaccination programs [43].

Other notable risk factors include low socioeconomic status, smoking, early age at first sexual intercourse, having multiple sexual partners, and giving birth to multiple children. Moreover, the impact of early marriage and limited access to menstrual hygiene products, such as sanitary pads, in exacerbating the risk of cervical cancer, particularly among women in rural areas [44, 45].

Researches indicates that these risk factors often intersect, amplifying the likelihood of cervical cancer development. One of the most striking observations is the disproportionate burden of cervical cancer in developing countries, particularly in regions with low socioeconomic status and limited access to healthcare services. For instance, women from disadvantaged socioeconomic backgrounds may have limited access to healthcare services, leading to lower rates of HPV vaccination and cervical cancer screening. Additionally, cultural and societal factors may influence behaviors such as early marriage and childbearing, which further compound the risk. Furthermore, poor knowledge and awareness about cervical cancer prevention and screening exacerbate the problem, particularly among marginalized communities. Women with limited education may be less likely to seek preventive measures or recognize early symptoms of cervical cancer, delaying diagnosis and treatment.

Countries with a high prevalence of cervical cancer highlight the urgent need for targeted interventions and awareness campaigns in such settings. The role of education emerges as a critical determinant of cervical cancer risk, with illiteracy identified as a significant risk factor. This underscores the importance of education in empowering women with knowledge about preventive measures and early detection strategies. By targeting high-risk populations and addressing the social determinants of health, it is possible to reduce the burden of cervical cancer globally and improve outcomes for women worldwide.

1.3. Cervical cancer treatment

Cervical cancer treatment has evolved significantly in recent years, incorporating advances in surgery, radiotherapy, chemotherapy, and targeted therapies to improve patient outcomes. The choice of treatment depends on the cancer's stage, histological type, and the patient’s overall health.

Surgery remains a cornerstone of cervical cancer treatment, particularly for early-stage disease. The primary surgical approaches include conization, trachelectomy, and hysterectomy. Conization is often used for very early-stage cancers and involves removing a cone-shaped piece of tissue from the cervix to both diagnose and treat the cancer. Radical trachelectomy is a surgical procedure that involves the removal of the cervix, the upper part of the vagina, and surrounding tissues, while preserving the uterus. Hysterectomy is total or radical hysterectomy involves the removal of the uterus, cervix, and sometimes surrounding tissues. Recent advancements include minimally invasive techniques like laparoscopic and robotic-assisted surgeries, which have been shown to reduce recovery times and surgical complications [46, 47].

Radiotherapy is a critical treatment modality for cervical cancer, especially in combination with chemotherapy for locally advanced stages. External beam radiation therapy (EBRT) and brachytherapy (internal radiation) are the primary forms used. Recent studies emphasize the importance of personalized radiotherapy planning to maximize efficacy while minimizing side effects [48, 49].

Chemotherapy is often used in conjunction with radiation therapy for advanced cervical cancer or in the adjuvant setting. Platinum-based regimens, such as cisplatin, remain the standard. Recent developments include the exploration of new drug combinations and agents, such as the addition of targeted therapies to enhance treatment efficacy [50].

Targeted therapies and immunotherapies are an expanding area of research and clinical application, designed to specifically target cancer cells or the mechanisms driving tumor growth. Recent advancements include checkpoint inhibitors, which target PD-1 and have shown promise in treating recurrent or metastatic cervical cancer, particularly in patients with high PD-L1 expression. Additionally, anti-VEGF therapies, which inhibit tumor blood vessel growth, are increasingly used in combination with chemotherapy for advanced stages of the disease. These innovative approaches are enhancing treatment options and improving outcomes for cervical cancer patients [51, 52].

The field of cervical cancer treatment is increasingly moving towards personalized medicine, where treatment is tailored based on genetic, molecular, and clinical characteristics of the tumor. Advances in genomic profiling and biomarker identification are leading to more precise and effective treatment strategies [53, 54].

1.4. Cervical cancer biomarkers

Tumor development is linked to the expression of tumor suppressor genes and oncogenes. Identifying biomarkers for early diagnosis and prognosis, along with developing effective therapies, is crucial to improving patient survival.

Liquid biopsy biomarkers are emerging as promising tools for the non-invasive detection and monitoring of cervical cancer. These biomarkers, which include circulating tumor cells (CTCs), cell-free DNA (cfDNA), microRNAs (miRNAs), proteins, and exosomes, can be found in blood or other body fluids. CTCs, for example, provide insights into tumor behavior and metastasis, while cfDNA analysis reveals tumor-specific genetic alterations. MiRNAs, which regulate gene expression, and protein biomarkers like p16INK4a and SCC-Ag, offer diagnostic and prognostic information. Exosomes, containing various molecular signals from cancer cells, also contribute to understanding tumor dynamics [55–60].

Protein biomarkers for cervical cancer are specific proteins with altered levels or expression patterns in cancer tissues or fluids like blood or cervical fluid. These biomarkers can indicate disease presence, progression, or response to treatment. Key examples include p16INK4a [61], which is elevated in cervical cancer due to HPV infection and used to identify high-grade lesions, and HPV E6/E7 oncoproteins [62], which drive cancer development and serve as indicators of HPV-associated cancer. Cytokeratins like CK7, CK17, and CK19 [63, 64] help distinguish cancer subtypes, while VEGF [65, 66], SCC-Ag [67], and CA-125 [68] levels are associated with tumor growth, progression, and metastasis. NF-kB is a key transcription factor in immune response and cervical cancer progression, reactivated by HPV to promote tumor growth and metastasis [69]. Additional, Ki67 [70] and MMPs [71] are protein biomarkers under investigation for cervical cancer detection, as their dysregulation is linked to key processes like apoptosis, cell proliferation, invasion, and metastasis.

Epigenetic biomarkers, including DNA methylation patterns [72], histone modifications [73], and chromatin remodeling [74] have all been linked to cervical cancer progression.

Cervical cancer development and progression are driven by complex genetic and molecular alterations. Persistent HPV infection, particularly with high-risk types like HPV16 and HPV18, initiates the process by disrupting cell cycle control through the E6 and E7 oncoproteins, which inactivate tumor suppressors p53 and pRb [75, 76]. Several key signaling pathways are also involved. The PI3K/Akt/mTOR pathway, often activated by mutations such as PIK3CA or PTEN loss, promotes cell survival, proliferation, and meta-

stasis [77]. The RAS/RAF/MAPK and Wnt/β-catenin pathways further contribute to tumor growth and spread by promoting cellular proliferation and invasion [78, 79]. Additionally, dysregulation of the Hedgehog [80, 81] and Notch [82] pathways supports cancer cell survival and stem cell maintenance. Understanding these pathways not only sheds light on the disease's biology but also offers potential targets for new therapies and biomarkers for diagnosis and treatment response.

Genetic alterations in cervical cancer, including mutations, amplifications, deletions, and rearrangements, serve as key biomarkers for detection, diagnosis, prognosis, and treatment response. Common mutations, like those in the TP53 tumor suppressor gene, are linked to advanced cervical cancer [83], while PIK3CA [84] and Kras mutations activate critical pathways like PI3K/Akt/mTOR [85] and RAS/RAF/MAPK [86–88], influencing tumor growth and treatment resistance. PTEN loss [89] and alterations in other genes like Myc and ERBB2 further drive tumorigenesis [90]. Identifying these genetic changes through various molecular techniques can help tailor treatment and improve patient outcomes, though further validation is needed for routine clinical use.

Advances in genomic [91], transcriptomic [92], and proteomic [93] technologies have significantly deepened our understanding of cervical cancer and enabled the discovery of new biomarkers for diagnosis, prognosis, and treatment assessment. High-throughput genomic methods like whole-genome and whole-exome sequencing reveal critical mutations and genetic alterations, while transcriptomic technologies such as RNA sequencing uncover gene expression patterns and non-coding RNAs linked to tumor progression. Proteomic techniques, including mass spectrometry and antibody-based assays, identify key proteins and signaling networks involved in cervical cancer [93–95]. These integrated multi-omics approaches offer a comprehensive view of cervical tumor biology, enhancing early detection, patient stratification, and personalized treatment strategies.

1.5. Inflammation, immune response, and cervical cancer

In today's oncology, the genetic features of the host that determine the pathophysiology of cancer and the course of the disease are intensively studied. The genetic influence on cancer is multifaceted; some factors are known for their roles in cell cycle function, apoptosis, and cell differentiation. The risk of cancer is increased by additional factors that activate the immune system and cause inflammation. Although the role of inflammation is to resolve infection and injury, when inflammation becomes chronic, it can

contribute to the development and progression of cancer. Inflammatory mediators can promote neoplasia by inducing mutations, adaptive responses, resistance to apoptosis, and environmental changes such as stimulation of angiogenesis [96–98].

1.5.1. The multifaceted functions of Toll-like receptor signaling pathways in immunology and oncology

Scientific studies suggest that membrane-associated innate Toll-like receptors (TLRs), as pattern recognition receptors (PRRs), play a major role in activating the immune response associated with autoimmune diseases, inflammation, and tumor-associated diseases. The human TLR family consists of 10 members (TLR1–TLR10). They are expressed in human immune cells and many tumors. Each of their expressions elicits a different response. These transmembrane proteins can recognize pathogen-associated molecular patterns (PAMPs) or host damage-associated molecular patterns (DAMPs) to activate innate and adaptive immune responses by triggering the activation of NF-κB, AP1, CREB, c/EBP, and IRF transcription factors. TLRs mediate changes in the expression of chemokines and pro-inflammatory cytokines and activate the response of cytotoxic lymphocytes, thereby eliminating pathogens and host debris [99–102].

The signaling pathway of TLRs begins in the cytoplasmic TIR domain, which contains adaptors such as MyD88, TIRAP, and TRIF. These adaptors modulate TLR signaling pathways, helping to recognize antigenic molecules (lipopolysaccharides, nucleic acids). This activates protein complexes, such as NF-κB, IRFs, and MAP kinases, via the MyD88-dependent pathway through the recruitment of members of the IRAK family, the TRIF-dependent pathway, or the MyD88-independent pathway. This regulation of the production of cytokines, chemokines, and type I interferon aids in eliminating antigens. Negative regulation of the signaling pathway helps protect the host from inflammatory damage [103–106]. Figure 3 illustrates the Toll-like receptor pathways involved in the progression of cervical cancer [5].

Studies have shown that TLRs can produce the desired antitumor effects by inducing apoptosis, autophagy, and necrosis in tumor cells [107–109]. TLR expression correlates with cancer prognosis [110, 111]. Activation of TLRs has become a target for cancer immunotherapy [112–117].

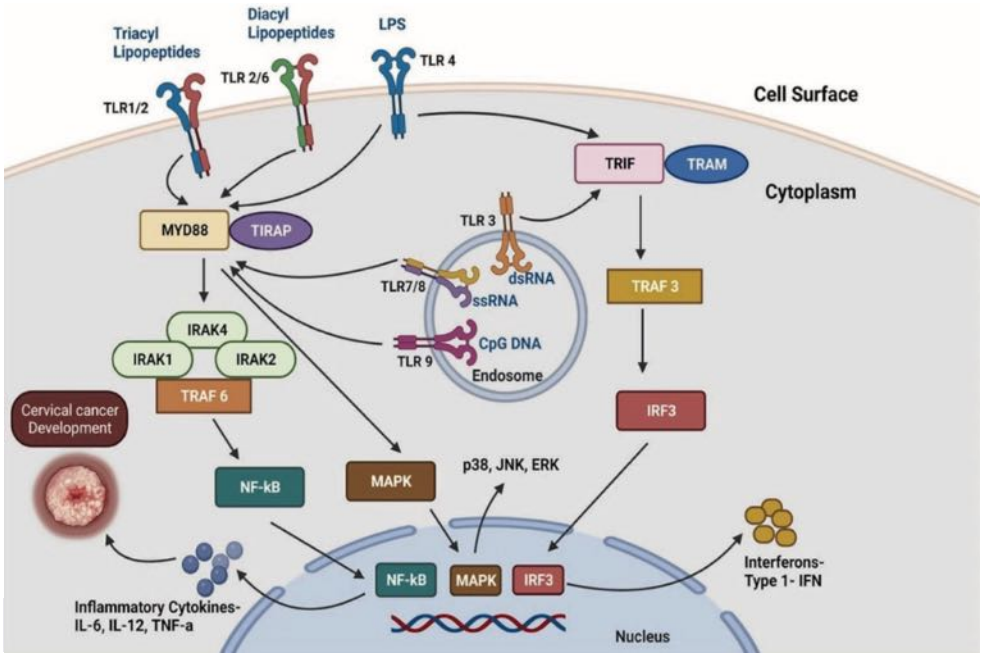

Figure 3. *Toll-like receptor pathways in cervical cancer development.*

TLRs 1, 2, 4, and 6 are membrane-bound, while TLRs 3, 7, 8, and 9 are found in endosomes. These receptors detect various PAMPs and, upon activation, form dimers that engage MyD88 or TRIF signaling pathways. TLR4 uniquely activates both MyD88 (via TIRAP) and TRIF (via TRAM). The activation of IRF3 leads to type I IFN production, while NF-kB and MAPK pathways drive inflammatory and immune responses, highlighting the potential of TLRs in cancer therapy research. (Figure reproduced from Agarwal et al. (2024) created using BioRender.com.) [5].

1.5.2. *TLR4* gene and its role in disease pathogenesis, cancer progression, and HPV-positive cervical cancer

The *TLR4* gene, which consists of three exons and is localized on chromosome 9q33.1, is one of the most studied TLRs. Mutations in the *TLR4* gene have been shown to induce resistance of pathogens to lipopolysaccharides in mice [118]. *TLR4* mutations are associated with endotoxin hyporesponsiveness in humans [119]. The TLR4 receptor is likely associated with a number of diseases because of the range of ligands (both pathogen-related and endogenous) identified as agonists of TLR4 [120]. Multiple published studies suggest that *TLR4* is linked to a range of diseases, including infectious disease [121–124], atherosclerosis [125–128], asthma [129–131], chronic obstructive pulmonary disease [132], cardiac disease [133–136], inflammatory bowel disease [137–139], liver disease [140–143], renal disease [144, 145], diabetes [146–148], rheumatoid arthritis [149, 150], Alzheimer's disease [151–154],

Parkinson's disease [155–158], neurovascular function [159], multiple sclerosis [160, 161], and bipolar disorder [162], with potential treatments targeting the *TLR4* pathway.

TLR4 is expressed on a variety of immune and tumor cells, but its activation can have opposing effects. While TLR4 activation can promote antitumor immunity, it can also, conversely, result in increased tumor growth and immunosuppression [163]. Changes in TLR4 gene expression are involved in carcinogenesis and tumor progression through chronic inflammation and the formation of a tumor microenvironment. Activated *TLR4* increases inflammatory cytokines and enhances cell proliferation, migration, invasion, and survival. While these functions in normal cells are essential for host defense and tissue repair, overexpression of TLR4 in malignant cells promotes tumor growth and metastasis [164]. High expression of TLR4 is likely associated with poor survival outcomes in patients with solid cancers [165].

TLR4 expression is closely associated with the tumorigenesis and growth of HPV-positive cervical cancer. TLR4 promotes HPV-positive cervical tumor growth and facilitates the formation of a local immunosuppressive microenvironment, potentially leading to cervical cancer (CC) development [166]. TLR4 expression correlates with histopathological grade in human papillomavirus (HPV)-infected cervical cells; it is higher in invasive cervical cancers (ICC) compared to cervical intraepithelial neoplasia (CIN) and low in normal cervical tissues. Moreover, higher TLR4 expression is observed in HPV-positive cervical cancer cell lines SiHa and HeLa compared to the HPV-negative cell line C33A, suggesting a role for HPV infection in *TLR4* regulation [167]. Increased TLR4 expression is linked to the severity of cervical lesions and closely associated with FIGO stage, lymph node metastases, and tumor size in CC. Higher TLR4 expression levels are observed in advanced FIGO stages with larger tumor sizes [168]. Various factors influencing the development of CC have been identified, with cervical tumorigenesis often initiated by high-risk HPV [169]. Scientific publications indicate a close relationship between TLRs, especially TLR4, and HPV infection in CC. TLRs have been found to regulate the local immune microenvironment in CC caused by high-risk HPV infection [170].

Single nucleotide polymorphism (SNP) is likely to affect cancer susceptibility. The influence of polymorphisms of the TLR4 gene on various cancerous diseases was investigated [171–174]. To comprehensively analyze the impact of germinal polymorphisms on the course of the disease, the main components influencing the spread of cancer are investigated. A review of the global literature focused on the influence of TLR4 gene polymorphisms and expression on the course of various cancers (tumor proliferation, differentiation, metastases, prognosis, and patient survival). An association between

TLR4 polymorphisms and a risk of hypersensitivity to HPV16/18 infection in women and increased risk of cervicitis, the precancerous lesion has been identified [175–179]. However there are very few studies on the impact of TLR4 gene polymorphisms on the pathomorphological features or course of cervical cancer.

1.6. Cervical cancer metastasis

Cancer is a leading cause of death characterized by abnormal cell growth, invasiveness, and metastasis. Many patients experience metastasis after diagnosis or treatment, contributing to high mortality and healthcare costs.

Metastasis stands as a paramount and intricate phenomenon in the domain of oncologic diseases. Earlier investigations have ascertained the noteworthy impact of the genetic context in which tumors originate on their proclivity for metastasis. Predictive human gene expression profiles associated with metastasis exhibit their presence not solely in mouse tumors featuring varying metastatic capacities but also display a discernible correlation with the inherent genetic backdrop. It is suggested that the genesis of human metastasis-predictive gene expression signatures may be markedly propelled by the genetic background, eclipsing the influence of acquired somatic mutations [180–185]. The capacity to discern individuals at an elevated risk of disseminated disease precisely during the clinical manifestation of primary cancer holds the potential for a substantial paradigm shift in cancer management.

The most common sites for cervical cancer metastasis are the lungs, bones, liver, and brain [186, 187].

1.6.1. The role of the RRP family

Some common ribosomal proteins that belong to the RRP family have been implicated in cancer biology: RPL (ribosomal protein large subunit) family, RPS (ribosomal protein small subunit) family. RPL5 has been implicated in cancer development and progression in various tumor types, but specific research on its role in cervical cancer appears limited. However, dysregulated expression of RPL5 has been reported in other gynecological cancers, suggesting poten tial relevance in cervical cancer as well [188–190]. RPL11 has been identified as a tumor suppressor protein that regulates the p53 pathway and cell cycle progression. While research specifically focusing on RPL11 in cervical cancer is limited, dysregulated expression of RPL11 has been reported in other cancer types, including ovarian and breast cancers [191–194]. RPL23 is involved in ribosome biogenesis and protein synthesis,

but its specific role in cervical cancer remains poorly understood [195–198]. RPL26 plays a role in ribosome assembly and protein translation. However, dysregulated expression of RPL26 has been associated with tumorigenesis and metastasis in other cancer types, suggesting possible relevance in cervical cancer as well [199, 200]. RPL36 is involved in ribosome assembly and protein synthesis, but its specific role in cervical cancer is not extensively studied [201, 202].

1.6.2. The role of the *RRP1B* gene in modulating metastasis and prognostic gene expression in cancer

Employing a meticulously characterized transgenic model of mouse mammary tumorigenesis, the ribosomal RNA processing 1 homolog B (*RRP1B/KIAA0179)* gene has been pinpointed as a potential modifier QTL gene impacting metastasis efficiency [8, 203, 204]. RRP1B is primarily identified as a nucleolar protein and is also nuclear membrane-associated protein, although it has been reported in multiple cellular locations. The *RRP1B* gene is located on chromosome 21q22.3, and the protein contains 758 amino acids. Previous investigations have revealed that *RRP1B* forms a binding alliance with the metastasis-modulating factor GTPase activator *SIPA1* [205, 206]. Simultaneously, *in vitro*, using mouse and human metastasis gene expression data, *RRP1B* expression was found to be associated with extracellular matrix genes (ECM) expression and to be a germline regulator of ECM genes, which are recognized as metastasis predictive components with different regulation in metastasis-prone tumors. Ectopic expression of RRP1B inhibited tumor growth and metastasis in the highly metastatic mouse mammary tumor cell line. The significance of *RRP1B* was underscored by the discovery that germline polymorphisms (SNPs) within the human *RRP1B* consistently correlate with clinical breast cancer outcomes and survival [8, 207].

RRP1B upregulation is associated with metastasis suppression. *RRP1B* physically interacts with many nucleosome binding factors. The primary outcome of transcriptional repression is RRP1B binding to chromatin, and occupies loci with decreased gene expression. *RRP1B* orchestrates the regulation of metastasis-associated gene expression through its interaction with the transcriptional corepressors tripartite motif-containing protein 28 (TRIM28) and heterochromatin protein 1-α (HP1α) by recruiting chromatin-modifying enzymes. RRP1B influences histone methylation changes [207,208]. RRP1B suppresses metastatic progression also modulating the expression of alternative mRNA isoforms through interactions with the splicing regulator and oncoprotein SRSF1 [209]. Further experimentation demonstrated that RRP1B

interacts with protein phosphatase 1 (PP1), whose functions are implicated in tumorigenesis, the tumor microenvironment, and the metastatic cascade, and regulates nucleolar phosphorylation signaling [210–212]. *RRP1B* enhances DNA damage-induced apoptosis by functioning as a transcriptional coactivator for proapoptotic target genes under the regulation of the transcriptional activator E2F1 [213].

RRP1B associates with the nucleolar phosphoprotein NPM1, participating in cellular proliferation, growth-suppression pathways, and the apoptotic response to oncogenic stimuli such as DNA damage and hypoxia. NPM1 is implicated in tumorigenesis [207, 214, 215]. Furthermore, RRP1B interacts with the protein BRD4, a transcriptional and epigenetic regulator that holds a pivotal role in cancer development [216–218]. *RRP1B* can upregulate the expression of claudin-1 by depleting DOCK1, and increase cell viability and motility of claudin-low breast cancer cells [219]. It is proposed that *RRP1B* is targeted by miR-320a and contributes to cancer survival [220].

Various studies, underscore the multifaceted nature of how *RRP1B* governs both transcription and metastasis. The dysregulation of *RRP1B* exerts a net effect on multiple pathways and biological processes, underscoring the complexity of its influence on metastasis and prognostic gene expression.

While the molecular understanding of *RRP1B* as a potential modifier of metastasis is present, there is a scarcity of reports concerning the impact of host genetic factors on various cancers progression and metastasis.

1.6.3. The role of the *SIPA1* gene in cancer metastasis and prognosis

Cancer metastasis, a major cause of mortality, involves complex processes. Signal Induced Proliferation Associated Protein 1 (*SIPA1*), a Rap-GTPase-activating protein, is mapped to chromosome 11q13.1 and spans 12.8 kb. This mitogen-inducible gene is implicated in metastasis and is associated with poor prognosis. *SIPA1* is a crucial gene implicated in effective tumor metastasis. The *SIPA1* gene, also called *SPA1* (suppressor of phyA-105), was initially identified in 1995 from a murine lymphoid cell line, LFD 14, following IL-2 stimulation. In mice, *SIPA1* was characterized as a 3,518 bp gene with a lengthy open reading frame (ORF) spanning from position 1,199 to 3,280, along with several shorter ORFs at the 5'-end. The same research team later isolated human *SIPA1* cDNA from peripheral blood lymphocytes (PBL) after stimulation with phytohemagglutinin and TPA [221, 222]. SIPA1 was initially thought to specifically act as a GAP for Rap1, Rap2, Rsr1, and nuclear Ran. However, recent findings suggest that SIPA1 does not function as a GAP for Ran or other small GTPases. Overexpression of SIPA1 leads to cell rounding and detachment from the extracellular matrix by inhibiting Rap1 activation,

indicating that SIPA1 negatively regulates cell adhesion through its effect on Rap1 signaling [221, 222]. SIPA1 protein exhibits variable expression levels and localization across different human tissues and cell types. It is most abundantly expressed in lymphohematopoietic tissues, including the spleen, bone marrow, and thymus. Depending on the cell type and its interactions with other proteins, SIPA1 can be found in the cytoskeleton, plasma membranes, or nuclei [223].

SIPA1 influences cancer development and metastasis through different mechanisms depending on the tumor type [9]. Mutations in SIPA1 are linked to juvenile myelomonocytic leukemia and MDS/MPN. SIPA1 deficiency in mice disrupts bone marrow (BM) stromal cells and accelerates MPN development. SIPA1 is downregulated in BM from MPN/MDS patients, leading to inflammatory cytokine changes and dysregulated genes. These alterations suggest SIPA1 is crucial for maintaining BM niche stability, and targeting SIPA1 or its pathways could be a potential MPN therapy [224]. Mice lacking SIPA1 rarely develop chronic myelogenous leukemia (CML) when transplanted with Bcr-Abl-expressing progenitor cells. SIPA1−/− mesenchymal stroma cells (MSCs) show increased activation and migration toward Bcr-Abl+ cells, producing Cxcl9 that recruits SIPA1−/− memory T cells with enhanced chemotactic activity [225].

SIPA1 was demonstrated to regulate the transcription of multiple genes involved in signal transduction, DNA synthesis, cell adhesion, and cell migration [226]. In HeLa cells, Brd4 binds directly to SIPA1's GRD domain, primarily within the nuclear region, enhancing SIPA1's RapGTPase activity for Rap1 and Rap2. This SIPA1-Brd4 interaction accelerates the cell cycle from M to G1 phases, potentially influencing cancer progression [227, 228]. It comprises domains like RapGTPase-activating protein (GRD), PDZ, and Leucine zipper-like (LZ). SIPA1 interacts with various proteins, including integrin β1, afadin (AF6), aquaporin-2 (AQP2), and bromodomain protein Brd4. These interactions regulate cell adhesion, tight junctions, and intracellular transport, influencing cancer metastasis. SIPA1 expression varies across tissues, influencing cellular functions [9]. Co-immunoprecipitation in 293T cells revealed that SIPA1 specifically interacts with AF6, binding at the GRD domain of SIPA1 and the PDZ domain of AF6. AF6 is localized at cell adhesion sites and associates with the tight junction protein ZO-1. SIPA1 also co-localizes with AF6 at these sites, indicating a potential role for SIPA1 in regulating tight junctions via AF6, though this connection is not yet fully established [229].

Nuclear SIPA1 interacts with several proteins and activates the integrin β1 promoter in breast cancer cells. Knockdown of SIPA1 in MDA-MB-231 cells significantly reduced its mRNA levels. Chromatin immunoprecipitation

showed that SIPA1 binds to the ITGB1 promoter, enhancing its transcription and altering integrin-mediated FAK/Akt signaling, which impacts cancer cell adhesion and invasion [230]. SIPA1also acts as a transcription factor (TF). SIPA1 binds to DNA, specifically recognizing a TGAGTCAB motif, and regulates the transcription of genes involved in signal transduction, DNA synthesis, cell adhesion, and migration. Notably, SIPA1 controls fibronectin 1 transcription, crucial for cell migration in triple-negative breast cancer (TNBC). Single-cell transcriptome analysis from a metastatic TNBC patient showed high SIPA1 expression in metastatic cells, highlighting SIPA1's role in promoting TNBC migration, invasion, and metastasis [226]. SIPA1 induces the expression of CD44 in breast cancer cells. The expression of SIPA1 is associated with the survival and prognosis of breast cancer, underscoring its potential significance in the disease's progression [231]. The expression of SIPA1 in breast cancer is positively correlated with the number of infiltrated macrophages in invasive breast ductal carcinoma tissues and MDA-MB-231 xenograft tumors [232]. SIPA1 drives aerobic glycolysis in metastatic breast cancer cells by upregulating EPAS1 and glycolysis-related genes, shifting ATP production from oxidative phosphorylation to glycolysis. Blocking SIPA1 or glycolysis reduced tumor metastasis *in vitro* and *in vivo*. SIPA1's role in glycolysis under normal oxygen conditions suggests it as a potential therapeutic target for aggressive breast cancer [233]. SIPA1 enhances breast cancer cell stemness by promoting tumorsphere formation and CD44 expression, and by upregulating SMAD2 and SMAD3. Blocking SMAD3 phosphorylation with SIS3 reduces these stemness features and increases chemotherapy sensitivity [234]. SIPA1 expression in invasive breast ductal carcinoma and MDA-MB-231 xenografts was linked to increased macrophage infiltration. EVs from MDA-MB-231 cells (231-EVs) boosted macrophage migration compared to those from SIPA1-knockdown cells (231/si-EVs). SIPA1 upregulated MYH9, increasing myosin-9 in cells and EVs. Blocking myosin-9, either by SIPA1 knockdown or blebbistatin, reduced macrophage infiltration. High SIPA1 and MYH9 levels were associated with worse relapse-free survival. SIPA1 thus enhances macrophage infiltration through myosin-9-enriched EVs, potentially worsening breast cancer [231].

Genetic mapping identified SIPA1 as a potential modifier of Mtes1, a metastasis suppressor gene located on mouse chromosome 19 and related to the human BRMS1 gene. In mice, SIPA1 is linked to Mtes1. Kidney cancer cells with the SIPA1 variant carrying alanine (SIPA1/741A) at position 741 exhibited greater metastatic potential compared to those with threonine (SIPA1/741T) at the same position. This increased metastasis is associated with higher Rap1GAP activation in SIPA1/741A cells [235]. In renal collecting ducts, SIPA1 binds directly to aquaporin-2 (AQP2) and regulates its

transport to the apical membrane. AQP2, which interacts with PDZ domain-containing proteins, associates with SIPA1 via its PDZ domain. SIPA1, by modulating the Rap1 signaling pathway, influences AQP2 trafficking through direct and indirect mechanisms [236].

SPA-1 expression positively correlates with disease progression and metastasis in prostate cancer (CaP) patients. LNCaP cells, which seldom metastasize, had low SPA-1 levels, while highly metastatic PC3 cells had high SPA-1 levels. Introducing SIPA1 into LNCaP cells increased metastasis without affecting primary tumor size, while SIPA1 knockdown or active Rap1 expression in PC3 cells reduced metastasis. SPA-1 also impaired ECM adhesion and decreased nuclear Brd4 levels, indicating that SPA-1 regulates metastasis by modifying ECM interactions [237].

SIPA1 significantly impacts lung adenocarcinoma by regulating HGF-mediated tight junctions (TJs) and enhancing tumor aggressiveness. Elevated SIPA1 expression in lung tumors correlates with advanced disease stages and poorer outcomes. Reducing SIPA1 levels in A549 cells led to decreased invasion, proliferation, and improved barrier function, along with changes in TJ component expression [238]. Rap1-GTPase is crucial for lymphocyte trafficking. Loss of Rasa3 and Sipa1 in T cells led to uncontrolled Rap1 activation, causing the cells to become trapped in the lungs. While these mutant T cells migrated normally within lymph nodes and showed heightened responses to chemokines, they had impaired exit from lymph nodes. This highlights the essential role of Rap1 inactivation for proper lymphocyte trafficking [239]. Analysis of human lung tumor samples and *in vitro* assays revealed that higher SIPA1 expression correlates with poorer prognosis. Knockdown of SIPA1 in lung cancer cells reduced invasiveness, proliferation, and TJ barrier function. SIPA1 knockdown also led to decreased MET protein levels and impaired MET internalization and recycling. SIPA1's role in regulating HGF/MET signaling and TJs highlights its potential as a diagnostic and prognostic biomarker, as well as a therapeutic target for non-small cell lung cancer (NSCLC) with aberrant MET expression and drug resistance [240].

SIPA1 positivity was associated with poorer tumor differentiation, increased lymph node metastases, and higher microvessel density, while negatively correlating with VEGF-A levels, indicating that low SIPA1 expression may drive gastric cancer progression by enhancing VEGF-A and vascular density [241].

In colorectal cancer, SIPA1 was significantly upregulated in tumor tissues compared to normal tissues. Lower SIPA1 expression was linked to poorly differentiated tumors and lymphatic metastasis. Knockdown of SIPA1 reduced cell growth but increased invasion and migration. Lower SIPA1 levels

were found in more advanced stages and poorly differentiated tumors, but no clear link to tumor size or TNM staging was observed. Higher SIPA1 expression was associated with shorter survival, though it did not significantly impact recurrence or metastasis outcomes [242]. Analysis of The Cancer Genome Atlas (TCGA) data showed that SIPA1 mRNA is elevated in colorectal cancer (CRC) and correlates with epithelial-mesenchymal transition (EMT) and STAT3 signaling. SIPA1 knockdown reduced CRC cell proliferation and migration, while SIPA1 activation increased STAT3 signaling and its nuclear translocation. Co-treatment with a STAT3 inhibitor confirmed that SIPA1 regulates EMT through STAT3 [243]. In colorectal cancer, AF6 regulates the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), while SIPA1, a kinase involved in tight junctions and the cell cycle, also affects tight junctions. Both proteins showed abnormal expression in tumors compared to normal tissues and were significantly correlated. High levels of AF6 and SIPA1 were linked to poorer overall and relapse-free survival. Their combined expression pattern enhanced survival predictions and was an independent prognostic factor for overall survival, though it was less significant for disease-free survival [244].

SIPA1 is significantly upregulated in oral squamous cell carcinoma (OSCC) and correlates with lymph node metastasis. Knockdown of SIPA1 reduces cell invasiveness and migration while maintaining high adhesion levels. It also increases ITGB1 expression and decreases MMP7 expression. These findings suggest that SIPA1 promotes OSCC metastasis by regulating ITGB1 and MMP7 [245].

To investigate the role of SIPA-1 in regulating bladder cancer invasion and metastasis, BIU-87 and T24 cells were transfected with the SIPA-1 gene or shRNA. The results showed that increased SIPA-1 levels reduced E-cadherin and ZO-1 expression, promoting cell motility and invasion *in vitro* and leading to more tumors *in vivo*. Conversely, SIPA-1 knockdown had the opposite effect. These findings suggest that SIPA-1 promotes bladder cancer metastasis by downregulating E-cadherin and ZO-1 [246].

Analysis of 32 melanoma cell lines identified two subgroups with distinct growth aggressiveness in mice. Genomic alterations, rather than gene expression profiles, distinguished these subgroups. The Rap1GAP SIPA1 was found to be deregulated in more aggressive melanoma models, and its knockdown influenced critical cancer characteristics. These results indicate that changes in gene dosage, including SIPA1, contribute to melanoma aggressiveness [247].

SIPA1 levels were higher in glioma tissues compared to normal tissues. In A172 cells, SIPA1 knockdown reduced migration and proliferation, increased apoptosis, and decreased the S phase cell ratio. SIPA1 knockdown

also downregulated cell cycle proteins. Conversely, SIPA1 overexpression elevated phosphorylated FAK levels, enhancing malignant traits in glioma cells [248].

1.6.4. The *SRSF1* gene

1.6.4.1. The role of the *SRSF1* gene

Alternative splicing plays a crucial role in gene expression regulation, contributing to the proteomic diversity in higher eukaryotes. This process is primarily controlled by the binding of protein factors to enhancers and silencers on the pre-mRNA [249–252].

SR proteins are a well-known family of splicing factors involved in both constitutive and alternative splicing [253]. SR proteins possess a modular structure comprising one or two N-terminal RNA recognition motifs (RRMs) that determine their RNA-binding specificity, along with a C-terminal domain [254]. The role of SR proteins in alternative splicing is influenced by the location of their binding sites; they generally enhance splicing when attached to exons and suppress splicing when bound to introns [255, 256]. The research has highlighted the involvement of SR proteins and their natural antagonists, hnRNP proteins, in the disruption of alternative splicing during cancer progression [257].

SRSF1, or serine and arginine rich splicing factor 1, emerges as a fundamental proto-oncogene with pervasive elevation in cancer. SRSF1 has been found to have multiple functions beyond splicing, such as influencing mRNA transcription, stability, nuclear export, nonsense-mediated decay (NMD), translation, and protein sumoylation. Furthermore, it was the first member of the SR protein family identified as a proto-oncogene, emphasizing the significant role of alternative splicing in cancer development [258–260]. Among the SR protein family, was the first member identified and is regarded as the prototype of the group. Initially recognized as a splicing factor, SRSF1 has since been shown to play a crucial role in virtually all stages of the mRNA lifecycle, including transcription, nonsense-mediated decay (NMD), mRNA export, and translation [261, 262]. SRSF1 levels are modulated during EMT/MET through alternative splicing linked to the nonsense-mediated mRNA decay (AS-NMD) pathway, a process regulated by the splicing factor Sam68 [263].

A cellular defense mechanism has been described to counteract the oncogenic effects of increased SRSF1 expression. This mechanism involves SRSF1 stabilizing the tumor suppressor protein p53 by preventing its degradation through the MDM2 proteasome pathway, which in turn leads to oncogene-induced senescence [264]. SRSF1 can serve as mRNA export adaptors

by interacting with the cellular export factor TAP [265]. SRSF1 enhances translation by facilitating the initiation of bound mRNAs through the suppression of 4E-BP, which inhibits cap-dependent translation. This effect is achieved through SRSF1's interactions with elements of the mTOR signaling pathway [266]. SRSF1 functions as an adaptor protein that recruits signaling molecules involved in the regulation of cap-dependent translation for specific mRNAs [267].

Several properties of SR proteins suggest that they act as adaptors for NXF1-dependent mRNA export and potentially couple the completion of splicing to mRNA export. SRSF1, bind directly to NXF1 only in their hypophosphorylated state, implying that binding occurs after splicing is completed. Although SRSF1 interacts directly with NXF1 *in vitro*, the sensitivity to RNase suggests that RNA binding may be crucial for stabilizing the complex *in vivo* [268–270]. NXF1–SRSF1 interactions persisted well beyond the degradation of rRNAs and the loss of the nuclear polyA-binding protein PABPN1 from mRNPs. This suggests that the binding of NXF1 to SRSF1, and likely other SR proteins, is stabilized through their association with mRNA [261]. SRSF1 binds to mRNAs of pro-inflammatory cytokines (Cxcl1, Tnf, and Cxcl2), sequestering them in the nucleus and creating a reserve of transcripts that can be quickly mobilized in response to external stimuli and signaling cascades [271].

It was identified that approximately 1500 mRNAs are translational targets of SRSF1, including those involved in cell cycle regulation and mitotic progression. The study highlights that SRSF1 is essential for normal mitotic progression and couples pre-mRNA splicing with translation, revealing its complex role in gene expression regulation and its implications for cancer [267].

SRSF1 is regulated by various posttranslational modifications that affect its localization and function. These include phosphorylation of Ser residues in the RS domain by Clk/Sty and SRPK kinases, as well as topoisomerase 1, and dephosphorylation by phosphatases PP1 and PP2A [272–275]. SRSF1 autoregulates its expression through several mechanisms. It modulates its own splicing to promote production of PTC-containing isoforms targeted for NMD, reduces translation efficiency by shifting its mRNA from polysomes to monosomes, and is silenced by miR7, creating a negative-feedback loop [276, 277].

Another role of SRSF1 involves its stage-specific association with chromatin during the cell cycle and its contribution to maintaining genomic stability [278]. The diverse functions of SRSF1 and other SR proteins highlight splicing as a key regulator of gene expression and cellular homeostasis [262]. A significant and unexpected role of SRSF1, especially its splice variant

SRSF1-3, is its crucial involvement in facilitating the activation-induced cytidine deaminase (AID) to act specifically on its natural substrate during somatic hypermutation (SHM) of immunoglobulin genes [279, 280].

1.6.4.2. Implications of *SRSF1* in cancers

SRSF1 resides on Chromosome 17q23, a locus that is amplified in some tumors, accounting for some instances of SRSF1 overexpression. Despite the regulatory mechanisms designed to keep SRSF1 levels in check, it is frequently overexpressed in various cancer types. Overexpression of SRSF1 in immortalized rodent fibroblasts or human mammary epithelial cells results in oncogenic transformation, characterized by enhanced cell proliferation and resistance to apoptosis [258, 259, 281]. SRSF1 facilitates Drosha cleavage of pri-miR-29b, which regulates helper T cell differentiation and represses target genes like IFN-γ. Downregulation of miR-29b in tumor-associated dendritic cells impairs immune response and supports a tumor-friendly environment, highlighting SRSF1's role in immune regulation and tumorigenesis [282, 283].

SRSF1 regulates miR-10b, linked to cancer metastasis and autoimmune disease. In cancer cells, miR-10b overexpression reduces MICB, a ligand for the NK cell receptor NKG2D, aiding tumor immune escape and enhancing metastasis [284].

The oncogene MYC, often overexpressed in cancers, is positively correlated with SRSF1 expression in lung and breast tumors and is responsible for driving SRSF1 overexpression in at least some lung tumors [285, 286]. Overexpression of SRSF1 in lung adenocarcinoma cells leads to a more aggressive phenotype and induces resistance to anticancer drugs such as carboplatin and paclitaxel. It was found that SRSF1 overexpression correlates with advanced stages (III/IV) and metastases (M+) in non-small cell lung cancer (NSCLC), especially in adenocarcinoma (ADC) [287]. An *in vitro* study showed that alternative splicing of Caspase 9, influenced by SRSF1, increases resistance to cisplatin, daunorubicin, and paclitaxel in NSCLC. SRSF1 interacts with the C9-I6/ISE enhancer element, leading to overexpression of the resistant Caspase 9b isoform, suggesting it mediates chemoresistance in lung cancer [288]. SRSF1 regulates aberrant alternative splicing of the BIN1 (Bridging Integrator 1) protein in NSCLC, particularly by promoting the BIN1+12a isoform through exon 12a inclusion. This isoform inhibits NSCLC cell apoptosis and invasion, contributing to neoplasia-free progression and immune escape [289]. SRSF1 regulates the translation and stability of DNA ligase 1 (LIG1) through mTOR activation. LIG1, a DNA repair gene, was identified as a marker of poor prognosis in NSCLC, correlating with shorter

disease-free survival (DFS) and overall survival (OS). Additionally, LIG1 was found to be an independent prognostic factor for NSCLC [290]. In a whole-transcriptome analysis of lung cancer (LC) and adjacent normal tissue, SRSF1 was found to regulate alternative splicing of HIF (Hypoxia-inducible factors), shifting from HIF-1αL to HIF-1αS. *In vitro* studies showed that the HIF-1αS isoform has higher metastatic potential in LC cells. The findings suggest that SRSF1 may enhance metastatic activities in LC [291].

A study combining *in vitro*, *in vivo* xenograft models, and clinical research identified several connections between SRSF1 and colon cancer. Researchers analyzed the ratio of DBF4B FL/S mRNA in 60 paired colorectal cancer and adjacent non-cancerous tissues. They discovered elevated levels of both SRSF1 and DBF4B-FL in cancerous tissues. This overexpression was associated with higher tumor grade and poorer survival outcomes, with SRSF1 and DBF4B-FL identified as independent prognostic markers for colon cancer [292]. A bioinformatics analysis of colorectal cancer identified SRSF1 as linked to genes important for cancer progression, such as those involved in the cell cycle, spliceosome activity, ubiquitin-mediated proteolysis, nucleotide excision repair, the p53 pathway, DNA replication, and RNA degradation. The study highlighted that SRSF1 is overexpressed in cancerous tissues compared to normal tissues, and from 2678 SRSF1-related genes, 468 were found to overlap with 3625 genes associated with colorectal cancer [293]. SRSF1 was confirmed as an independent prognostic factor in colorectal cancer, comparable to TNM classification. The research also linked SRSF1 to alternative splicing events involving MAPK and Mnk2, a key protein in the MAPK pathway that influences tumor growth and proliferation [294].

SRSF1 regulates miR-7 through a feedback loop where it binds pri-miR-7 to enhance Drosha cleavage, leading to miR-7-mediated repression of SRSF1 translation. Inhibition of miR-7 impairs CTL-mediated lysis of breast cancer cells [295]. SRSF1 directly influences oncogenic pathways; a 2021 study demonstrated its role in breast cancer by facilitating the alternative splicing that leads to overexpression of PTPMT1, which is linked to tumorigenesis via the P-AKT/C-MYC pathway [296]. SRSF1 functions beyond alternative splicing, significantly impacting IRES-mediated translation. In ER-negative breast cancer cells, SRSF1 stays in the nucleus under normal conditions, influencing splicing and translation regulation, but translocates to the cytoplasm under stress, where it acts as an ITAF, enhancing translation of MYC and other mRNAs. Conversely, in ER-positive breast cancer cells, SRSF1's role varies with the cell cycle: it modulates translation and splicing in G0/G1 phases and, during mitosis, translocates to the cytoplasm to regulate translation and mitotic processes [297]. SRSF1 plays a critical clinical role in breast cancer metastasis by promoting the alternative splicing of oncogenes like

C-MYC and DCUN1D5. Specifically, SRSF1 stimulates the inclusion of exon 4 in DCUN1D5, which stabilizes the protein and contributes to metastatic progression. Comparative analysis of DCUN1D5 expression in metastatic *vs.* non-metastatic breast cancer, along with 5-year survival rates, revealed that high levels of DCUN1D5-exon-4, regulated by SRSF1, are associated with advanced metastatic stages and poorer prognosis [298].

In a study with cell cultures and xenografts, lower HSD17B2 levels in prostate cancer (PCa) tissues indicated its role in inhibiting PCa progression. High HSD17B2 reduced dihydrotestosterone (DHT) production, while knocking it down increased DHT synthesis. Higher SRSF1 levels were observed in PCa cells with lower HSD17B2, alongside shorter HSD17B2 isoforms that degrade the functional one. This suggests SRSF1 supports PCa progression by regulating HSD17B2 splicing [299]. It was found that in castration-resistant prostate cancer (mCRPC) patients, loss of genes on 17q22, including SRSF1 and RNF43, was linked to Enzalutamide resistance and poor overall survival. Analysis revealed that this gene loss enhances the activity of key tumorigenesis-related proteins like PLK1 and AKT1 [300]. A study using TMA blocks from 368 prostate cancer (PCa) and benign prostate hyperplasia (BPH) patients revealed that SRSF1 and microvascular density (MVD) were higher in PCa cases and correlated with poorer outcomes. Immunohistochemistry showed SRSF1+ patients had worse biochemical recurrence rates at 5 and 9 years compared to SRSF1− patients, and similar results were seen for MVD+ patients. Among PCa patients with diabetes, SRSF1+ was associated with higher Ki-67 and MVD. SRSF1 expression was linked to MVD, Ki-67, androgen receptor, insulin receptors, IGF1-R, and PSMA, highlighting its role in PCa progression and recurrence [301]. Studies have shown increased VEGF expression in prostate cancer (PCa) cells. In 2016, research highlighted elevated levels of SRSF1 and SRPK1 (Serine Arginine Protein Kinase-1) in malignant PCa cells. It was also found that SRPK1 influences VEGF splicing to produce the more angiogenic VEGF165b isoform, accelerating cancer growth. SRSF1, a key regulator of VEGF splicing, is itself regulated by SRPK1-mediated phosphorylation [302,303].

Recent research on glioblastoma multiforme (GBM) has highlighted the role of circSMARCA5, a circular RNA abundant in the brain, in interacting with the splicing factor SRSF1. Studies found that circSMARCA5 is downregulated in GBM compared to adjacent healthy tissues, correlating with increased malignancy and decreased cell migration. This downregulation leads to elevated SRSF1 and VEGF levels, which enhance angiogenesis and cancer progression. Clinical data show that higher SRSF1, VEGF, and microvascular density (MVD) are associated with poorer overall survival (OS) and progression-free survival (PFS). The “GAUGAA” motif in circSMARCA5,

responsible for binding SRSF1, plays a critical role in GBM's angiogenic and migratory traits, presenting potential targets for new therapies [304–306]. SRSF1 expression was linked to glioma grading and subtypes. It was highly sensitive for diagnosing GBM and WHO grade 3 astrocytoma but less specific. Pilocytic astrocytomas lacked SRSF1. High SRSF1 levels correlated with worse prognosis and promoted glioma cell growth and invasion. SRSF1 could be a prognostic marker and contribute to glioma progression [307].

SRSF1 is upregulated in osteosarcoma (OS), where its knockdown reduces cell growth and promotes apoptosis, while overexpression enhances growth and migration. SRSF1 disrupts protein targeting, extracellular matrix processes, and key pathways such as PI3K-AKT, Wnt, and HIPPO. It also influences alternative splicing, impacting mRNA processing, including the splicing of genes like SRRM2, DMKN, and SCAT1 [308].

1.6.4.3. Understanding *SRSF1* in cervical cancer

Research highlights the oncogenic role of SRSF1, with recent studies linking it to cervical cancer. Elevated SRSF1 levels were found in cervical cancer cells, and activation of the SRSF1 gene promoter by the high-risk HPV16 E2 protein was observed, affecting SRSF1 levels in both the nucleus and cytoplasm. Alterations in SRSF1 hinder alternative splicing and drive genomic instability, promoting cervical cancer progression. Elevated levels of SRSF1 in the cytoplasm are associated with the early stages of tumor development [309].

SRSF1 interacts with long non-coding RNAs to regulate keratin 17 expression through alternative splicing, with elevated keratin 17 levels observed in cervical cancer cells [310]. In cervical cancer, alternative splicing events are prevalent and closely linked to both diagnosis and prognosis, with key splicing factors driving malignancy by increasing the production of HPV mRNAs and oncoproteins that facilitate cancer progression [311].

Cervical cancer cells exhibit elevated levels of hnRNPs, with hnRNPA1 being notably overexpressed. This protein disrupts cancer-related genes and induces alternative splicing of pyruvate kinase mRNA, promoting aerobic glycolysis and unchecked cell proliferation. Conversely, hnRNPA1 downregulation triggers cancer-specific apoptosis, making it a valuable biomarker for diagnosing cervical cancer [312]. Investigating hnRNPA1's role in alternative splicing in cervical cancer is vital due to its significant impact. During HPV infection and cervical epithelial cell differentiation, hnRNPA1 levels increase, facilitating oncoviral protein production. Furthermore, harmful mutations in hnRNPA1 can disrupt its normal function, leading to altered splicing, mRNA processing, and translation [314].

Analysis of two microarray datasets (GSE6791 and GSE63514) revealed that MIR155HG was significantly upregulated in cervical cancer compared to adjacent normal tissues. Knockdown of MIR155HG in SiHa and HeLa cells reduced proliferation, induced apoptosis, and decreased invasion. *In vivo*, MIR155HG knockdown also significantly inhibited tumor growth in xenografts. SRSF1 was identified as a binding partner of MIR155HG. These findings suggest that MIR155HG promotes cervical cancer progression through its interaction with SRSF1, highlighting its potential as a novel therapeutic target [315].

Radiotherapy is essential for managing advanced cervical cancer, though radioresistance can compromise its efficacy. In cervical cancer, a specific splice variant of nucleophosmin (NPM), produced through alternative splicing, is linked to radioresistance. NPM plays roles in mRNA processing, genome stability, and apoptosis, with the NPM2 variant enhancing radioprotection. Reducing NPM2 levels has been demonstrated to lower radioresistance in cervical cancer cells, with effects varying by dose [316].

Changes in splicing regulators, including mutations and reduced activity, can cause abnormal alternative splicing, which may drive tumor development and contribute to resistance to treatment. Nonetheless, alternative splicing biomarkers are being explored as promising targets for new therapeutic strategies [317].

Data from the Cancer Genome Atlas (TCGA) project suggest the expression level of SRSF1 is a prognostic factor in cervical cancer survival analysis (https://www.proteinatlas.org/ENSG00000136450-SRSF1/pathology/cervical+cancer).

1.7. Long non-coding RNAs

1.7.1. Long non-coding RNAs: biogenesis and gene regulation

Human physiological complexity cannot be fully explained by the limited number of protein-coding genes. Instead, a significant portion of the genome (70%–90%) transcribes non-coding RNAs (ncRNAs), which do not produce proteins but generate functional RNA molecules [318–321]. Long non-coding RNAs (lncRNAs), a subset of ncRNAs, are usually more than 200 nucleotides long and transcribed by RNA polymerase II. LncRNAs can fold into diverse secondary structures that enable interactions with DNA, RNA, and proteins. LncRNAs are versatile molecules that play roles in a wide range of biological processes. They are involved in almost every step of gene regulation, from transcription to translation, and their dysfunction can contribute to various diseases. LncRNAs are essential regulators of gene expression through various mechanisms, including chromatin remodeling,

mRNA modulation, and direct DNA interactions. They play critical roles in transcriptional and post-transcriptional regulation within cells. At the transcriptional level, lncRNAs can regulate gene expression through mechanisms such as epigenetic silencing and chromatin remodeling. Research highlights lncRNAs as key regulators of gene expression and cell differentiation, often interacting with chromatin-modifying proteins like PRC2. Many lncRNAs recruit chromatin-modifying proteins to specific genomic sites, thereby influencing gene expression by regulating chromatin states. For instance, ANRIL, an antisense lncRNA, interacts with the polycomb repressive complex 1 (PRC1) to silence the INK4b/ARF/INK4a locus, which encodes important tumor suppressor genes. Dysregulation of ANRIL can lead to cancer by disrupting this silencing mechanism. Another example is HOTAIR, which remodels the chromatin landscape, particularly in cancer, by interacting with PRC2 and LSD1 complexes to repress metastasis-suppressor genes, facilitating cancer progression [322–329].

An essential feature of lncRNAs is their potential to form hybrid structures with DNA, influencing chromatin accessibility. These interactions can manifest as triple helices (triplexes) or R-loops. Despite detection challenges *in vivo*, these structures are likely widespread and crucial for many lncRNAs' regulatory functions. RNA–DNA–DNA triplexes illustrate non-coding RNA–DNA interplay in gene regulation. The ability to form triplexes depends on the RNA sequence [330–332]. TrIP-seq (targeted RNA immunoprecipitation sequencing) has been developed to study these sequences *in vivo* [333]. For instance, the lncRNA KHPS1 forms a triplex upstream of the SPHK1 enhancer, recruiting chromatin modifiers that activate SPHK1 expression. Swapping KHPS1's triplex-forming region with that of MEG3 switches its specificity to the MEG3 target gene [332, 334]. R-loops, once considered threats to genome stability, are now seen as crucial regulatory hubs. They play roles in gene expression regulation and DNA repair [335–337]. In mouse embryonic stem cells (mESCs), the lncRNA TARID forms an R-loop at the CpG-rich promoter of TCF21. This R-loop is recognized by GADD45A, which recruits TET1, leading to TCF21 activation [338]. R-loops can also act in trans; for example, the lncRNA APOLO forms R-loops in Arabidopsis thaliana to regulate auxin-responsive genes [339]. Endogenous ncRNAs are involved in DNA repair mechanisms like HDR and NHEJ in yeast and mammalian cells [340–343]. Transcription of lncRNAs recruits DNA repair proteins, although whether Pol II alone or the nascent lncRNA is responsible remains unclear [343, 344]. Damage-induced long non-coding RNAs (dilncRNAs) are generated at DNA double-strand breaks, processed into small DNA damage response RNAs (DDRNAs) by endoribonucleases Drosha and Dicer, and help recruit repair proteins [344].

Several lncRNAs are activated during genotoxic stress to regulate DNA repair pathways, either p53-dependently or independently [345–347]. For example, NORAD decoys Pumilio proteins, affecting DNA replication and repair factors, and aids in maintaining genomic stability through topoisomerase 1 [348–350]. CONCR, expressed in an MYC-dependent manner, recruits DDX11 to replication forks, enhancing replication and sister chromatid cohesion [351]. These findings underscore the important roles of lncRNAs in genome integrity and their potential as therapeutic targets.

The positioning of lncRNAs relative to their neighboring genes is crucial for their regulatory interactions. The evolutionarily conserved nature of antisense and bidirectional lncRNA transcription indicates an adaptive mechanism for context-specific gene regulation. The genomic configuration of divergent lncRNAs is essential for cis gene regulation, either by the lncRNA transcript influencing nearby loci or through its transcription or splicing process affecting the chromatin state or creating steric hindrance [352–354].

A primary mechanism for lncRNA-mediated gene repression involves gene-dosage compensation, exemplified by the lncRNA XIST, responsible for X chromosome inactivation in female mammals. During embryonic development, XIST spreads over one X chromosome, silencing many genes [355, 356]. XIST can silence extensive chromosomal regions, even when expressed from a different chromosome, through interactions with various proteins [357–360]. XIST's ability to exploit 3D chromatin organization allows it to spread across the X chromosome and modify chromatin structure, playing a role in epigenetic memory maintenance even in its absence [361–363].

At other loci, cis-acting lncRNAs promote inactive chromatin states by interacting with nearby chromatin. For instance, the lncRNA ANRASSF1 forms an R-loop that directs PRCs to their targets [364–366]. In Arabidopsis thaliana, the lncRNA COOLAIR, induced by cold, remains at its transcription site to promote PRC2-dependent H3K27me3 at the FLOWERING locus [367, 368].

lncRNAs can also suppress gene expression by interfering with the transcription machinery, affecting transcription factor or Pol II recruitment, altering histone modifications, and reducing chromatin accessibility. The mouse imprinted Airn lncRNA, for instance, displaces Pol II from the overlapping Igf2r promoter during mESC differentiation, causing transcription pausing and gene silencing. Another example is the lncRNA CHASERR, which, located upstream of the CHD2 gene, regulates CHD2 levels by creating a feedback loop where CHD2 binds nascent RNAs, including CHASERR, promoting their expression and controlling chromatin accessibility at multiple promoters [369–373].

Active enhancers transcribe two main types of non-coding RNAs: eRNAs and enhancer-associated lncRNAs (elncRNAs). eRNAs are short, bidirec-

tional, unspliced, non-polyadenylated, and unstable, while elncRNAs are unidirectional, polyadenylated, and spliced. Although the correlation between enhancer activity and eRNA expression is established, the functionality of eRNA transcripts remains debated. Some eRNAs facilitate chromatin looping by interacting with scaffold proteins like Mediator or cohesin, creating regulatory contacts between enhancers and promoters [374–377].

Enhancer loci also produce elncRNAs, which correlate with enhancer activity. elncRNA splicing is associated with enhancer activity and the abundance of neighboring genes [378–380]. elncRNAs modulate chromatin structure with chromatin-regulating proteins [381]. Gene-activating mechanisms of eRNAs also apply to elncRNAs.

Gene activation by elncRNAs can result in disease-related phenotypes [382, 383]. For example, SWINGN, promotes interactions with chromatin remodeling complexes, contributing to its pro-oncogenic role [384]. Similarly, lncRNAs like ELEANORs promote genomic domain formation [385].

lncRNAs can also activate genes independently of their transcripts. Functional DNA elements within lncRNA loci can activate neighboring genes. For instance, Bendr regulates BEND4 in cis through enhancer elements. Other lncRNAs have similar enhancer activation roles [386–389]. The functions of lncRNA transcripts and their loci can be uncoupled, promoting opposite outcomes. For instance, the locus of HOXA upstream non-coding transcript (Haunt) contains enhancers activating HOXA genes, while the Haunt transcript inhibits HOXA expression [390].

Post-transcriptionally, lncRNAs influence splicing, RNA decay, and stability. lncRNAs control gene expression through interactions with proteins and nucleic acids. Some even translate into functional peptides, but primarily, they form lncRNA-protein complexes (lncRNPs) to regulate mRNA splicing, turnover, and signaling pathways [391]. Abundant lncRNAs like sno-lncRNAs and SPAs in the PWS region contain motifs that sequester splicing factors such as RBFOX2, TDP43, and PTBP1, thus suppressing the splicing of pre-mRNAs with the same motifs [392–394]. They also modulate splicing through post-translational modifications of splicing factors and chromatin remodeling [395]. In the cytosol, NORAD, expressed following DNA damage, maintains genomic stability by sequestering Pumilio proteins, which otherwise bind mRNA 3′ UTR motifs to promote decay. Each NORAD molecule can sequester multiple Pumilio proteins, thus protecting target mRNAs involved in genomic stability [350, 396].

lncRNAs can also form structures interacting with proteins in key signaling pathways. For example, FAST, essential for hESC pluripotency, interacts with β-TrCP to prevent β-catenin degradation, enabling its nuclear translocation and activation of WNT-dependent genes. Similarly, NKILA binds to p65 and

IκB to modulate NF-κB activity and T cell activation-induced cell death [397, 398]. Some lncRNAs pair with other RNAs to recruit proteins for mRNA degradation. For instance, lncRNAs with Alu elements promote Staufen-mediated mRNA decay by recruiting STAU1 to mRNAs with complementary sequences. Conversely, TINCR pairs with differentiation mRNAs and recruits STAU1 to stabilize them during epidermal differentiation [399–401]. Additionally, AS-Uchl1, a nuclear lncRNA, shuttles to the cytoplasm under stress, where it enhances Uchl1 mRNA translation by base pairing with its 5′ end [402].

Abundant lncRNAs with miRNA-complementary sites can act as competitive endogenous RNAs or "sponges", reducing miRNA availability and thus regulating gene expression [403]. The stoichiometric relationship between these lncRNAs and miRNAs is crucial for affecting target mRNA expression [404, 405]. In tumors, the lncRNA-PNUTS, generated by alternative splicing and mediated by hnRNPE1, contains seven binding sites for miR-205. This sponging of miR-205 by lncRNA-PNUTS upregulates ZEB1 and ZEB2, promoting epithelial-mesenchymal transition and breast cancer cell migration and invasion [398].

Many lncRNAs localize to specific organelles like exosomes and mitochondria. Exosome-localized lncRNAs, which can be secreted into recipient cells, are involved in epigenetic regulation, cell-type reprogramming, and genomic instability [406]. Mitochondria-localized lncRNAs, encoded by both nuclear and mitochondrial DNA, often associate with mitochondrial metabolism and apoptosis [407]. For example, the nuclear-encoded SAMMSON lncRNA regulates mitochondrial homeostasis and polypeptide expression [408].

lncRNAs are crucial post-transcriptional regulators, and future studies should focus on the molecular basis of lncRNA-protein interactions and commonalities among different lncRNPs. The main lncRNA mechanisms of action are illustrated in Figures 4–6 [409].

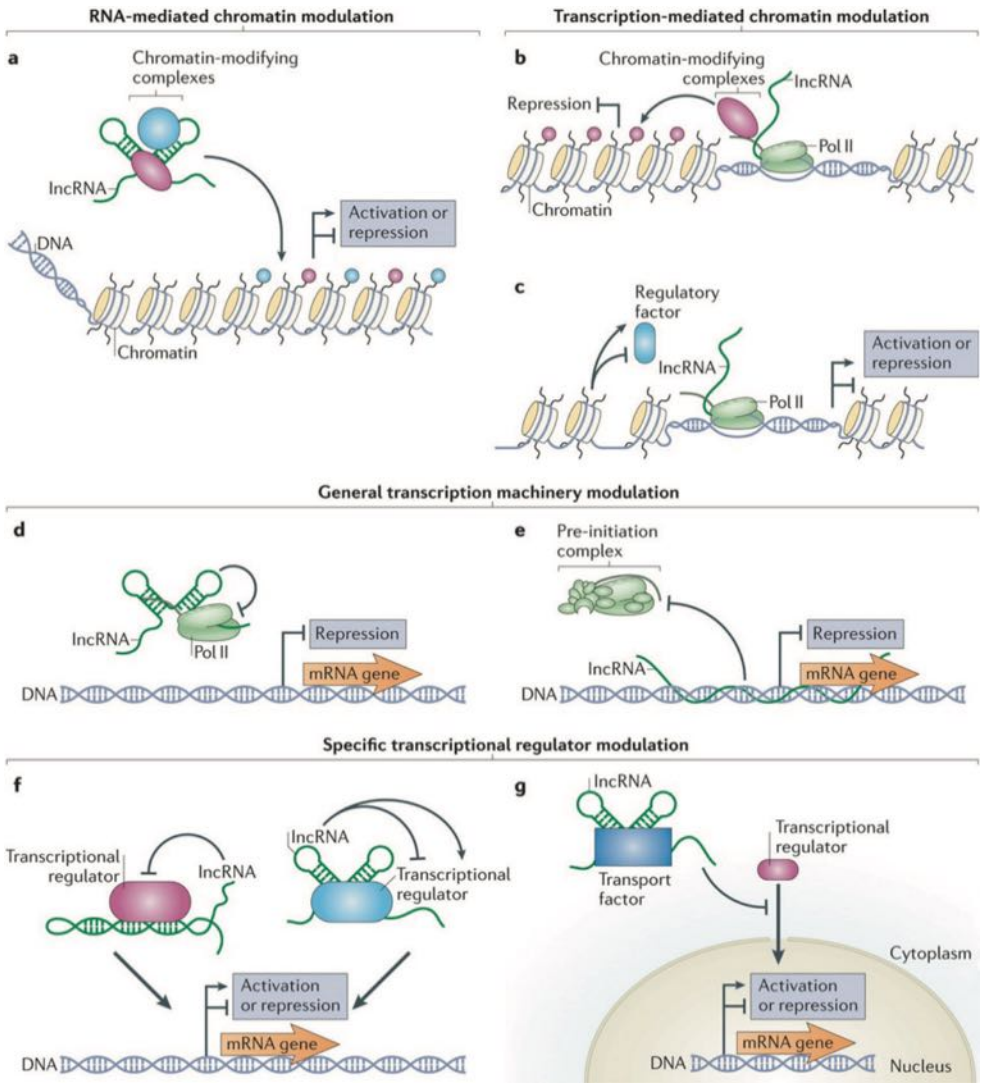

Figure 4. *Long non-coding RNAs (lncRNAs) modulate chromatin through various mechanisms.*

(a) lncRNAs bind chromatin-modifying complexes and target them to specific DNA loci, influencing gene expression; **(b)** They can also modify chromatin during transcription by interacting with RNA polymerase II; **(c)** lncRNA transcription may lead to chromatin remodeling that affects the binding of regulatory factors and gene expression; **(d)** lncRNAs can directly bind RNA polymerase II, inhibiting transcription; **(e)** They may form lncRNA-DNA triplex structures that block pre-initiation complex assembly; **(f)** lncRNAs can mimic DNA-binding sites, impacting specific transcription factors; **(g)** They can also regulate gene expression by binding transport factors, affecting the nuclear localization of transcription factors [409].

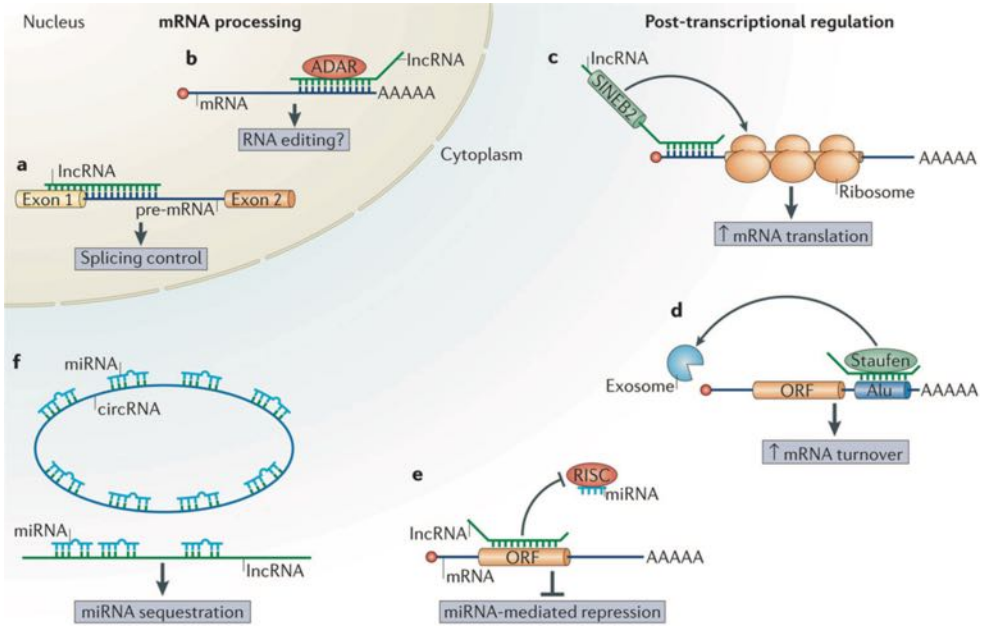

Figure 5. *lncRNAs affect mRNA processing and post-transcriptional regulation.*

(a, b) Long non-coding RNAs (lncRNAs) influence mRNA processing. lncRNAs can affect splicing patterns by binding to pre-mRNA (part a), such as preventing splicing of neuroblastoma MYC mRNA's first intron. They can also guide mRNA editing through interactions with ADAR enzymes (part b); **(c–f)** lncRNAs affect post-transcriptional regulation. They can enhance translation by binding to the 5′ region of mRNA (part c) or induce decay through interactions with Alu elements in the 3′ UTR (part d). lncRNAs can block miRNA silencing by masking miRNA-binding sites (part e) or act as miRNA decoys (part f) [409].

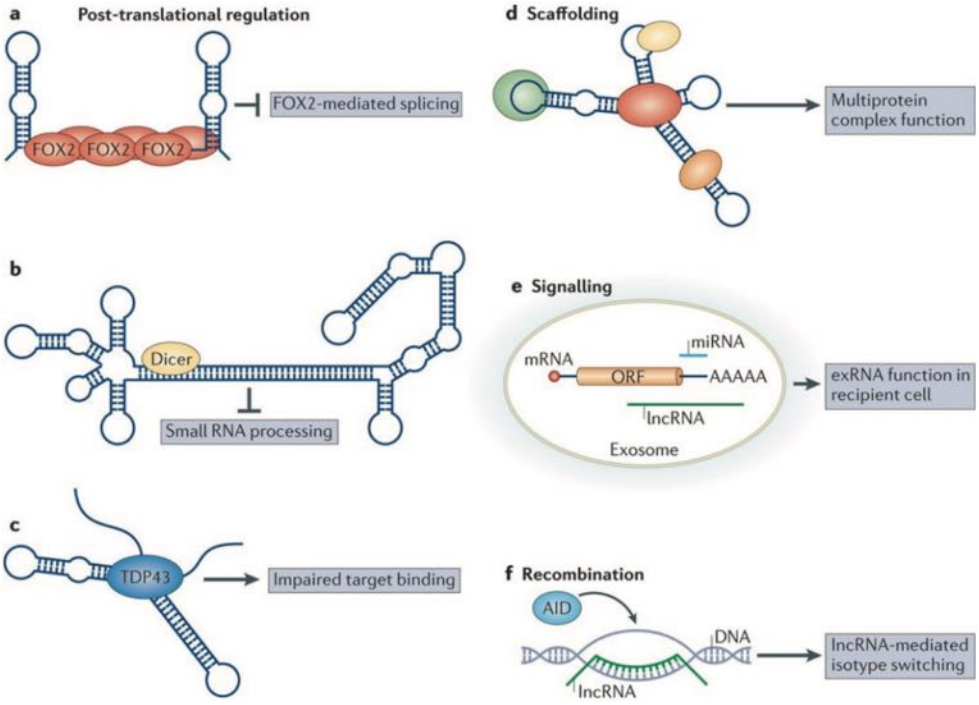

Figure 6. *lncRNAs play roles in diverse cellular processes.*

(a) Small nucleolar lncRNAs (sno-lncRNAs) from the 15q11-q13 locus can inhibit FOX2-mediated splicing by modulating FOX2 activity; **(b)** The structured rnc-s1 lncRNA binds Dicer to block small RNA processing. (c) The gadd7 lncRNA regulates TDP43's ability to process specific mRNAs; **(d)** lncRNAs can serve as scaffolds to organize multiple complexes; **(e)** Exosomal shuttle RNAs (exRNAs) may act as signaling molecules in cell-cell communication, carrying mRNAs, microRNAs (miRNAs), and lncRNAs; **(f)** lncRNAs from antibody switch regions form R-loops to guide class switch recombination via AID recruitment [409].

1.7.2. Observable phenotypes of lncRNAs and diseases

Most lncRNAs have not been identified in genetic screens due to the historical emphasis on protein-coding mutations and the subtle effects of regulatory mutations on quantitative traits. Identifying causal mutations in the many non-coding sequence variations is challenging, as most variations influencing human traits and disorders are found in lncRNA-rich regions [410, 411]. However, some lncRNAs, like roX1 and roX2 in fruitflies [412] and H19, Air, and Kcnq1ot1 in mice [413–415], have been identified genetically. In Arabidopsis, specific intronic SNPs in lncRNAs affect flowering-time adaptation by altering splicing [416]. Functional studies of lncRNAs typically involve silencing or deletion, although interpreting these experiments can be difficult [417, 418]. Advances in high-throughput genetic screens and CRISPR

technology are now rapidly identifying lncRNAs crucial for various biological processes [419–423]. Beyond these roles, lncRNAs regulate cell differentiation, development [319, 419, 424, 425], and numerous physiological processes such as DNA damage response [426], immune functions [424, 427], metabolism [428, 429], and stress responses in plants [425, 430], with emerging roles in association with cell membranes [431] and ribozymes [432].

Long non-coding RNAs (lncRNAs) play a critical role in maintaining genomic stability, and their mutations or dysregulation can lead to various diseases, including cancer [433–437]. Studies examining the expression of lncRNAs in tumors have identified specific lncRNAs associated with cancer subtypes and prognosis [438]. Abnormal levels of lncRNAs can significantly impact cancer prognosis, metastasis, and recurrence, with some lncRNAs influencing cell growth and proliferation [436]. Overexpression of proto-oncogenic lncRNAs has been shown to enhance tumor growth and invasion [436, 437], while others promote tumor-cell proliferation and metastasis through mechanisms such as chromatin looping [438]. LncRNAs are increasingly recognized for their roles in cancer progression, affecting cellular processes such as proliferation, survival, and metabolism [420, 439]. Many lncRNAs are regulated by key oncogenes and tumor suppressors, such as p53 and MYC, and contribute to the behavior of cancer cells [426, 440, 441]. For instance, the lncRNA MEG3, which activates p53, is often downregulated in cancers, underscoring its tumor-suppressive functions [442, 443].

In addition to their roles in cancer, lncRNAs are crucial for regulating cell differentiation, growth, and stress responses across various systems, including the nervous, muscular [325, 444], cardiovascular [445], adipose [446], and immune systems [447], with broad implications for numerous diseases. For example, lncRNAs are involved in the complex gene regulation necessary for the central nervous system, particularly in neuronal differentiation and regeneration [448, 449]. Dysregulation of certain lncRNAs has been linked to neurological disorders, such as Alzheimer's disease, where BACE1-AS promotes the accumulation of neurotoxic amyloid plaques [450–452].

In haematopoiesis and immune regulation, lncRNAs coordinate with transcription factors to control gene expression [453]. The lncRNA UMLILO, for example, primes immune gene activation in trained immunity by modulating chromatin structure [454]. Additionally, lncRNAs play a role in regulating the innate immune response to viral infections, with some lncRNAs promoting viral replication by inhibiting antiviral gene expression [455–457].

lncRNAs, due to their high tissue-specificity and role in cellular regulation, are promising therapeutic targets with potentially fewer side effects compared to protein targeting. Their lack of translation, fast turnover, and low

expression levels could also allow for quicker effects with lower doses [458, 459]. The most advanced therapies involve antisense oligonucleotides (ASOs), designed to downregulate nuclear lncRNAs by inducing RNA cleavage, though challenges remain with *in vivo* toxicity and delivery [458, 459]. Some ASOs targeting lncRNAs are already in development, with mRNA-targeting ASOs approved by regulatory agencies [460]. Small molecules targeting lncRNAs are less developed, requiring the identification of specific RNA structures for effective binding [461]. CRISPR–Cas systems also offer precise modulation of lncRNAs, though their therapeutic application is still emerging [462, 463].

1.7.3. Challenges in understanding lncRNAs

Modern transcriptome analyses, supported since 2004 by the National Human Genome Research Institute (NHGRI) through the ENCODE project, have challenged the earlier belief that 90% of our genome is "junk DNA", revealing that much of it is transcribed into non-coding RNAs (ncRNAs), including both small and long forms [464] (Figure 7).

Estimates of the number of human lncRNA genes vary significantly. GENCODE suggests there are over 16,000 lncRNA genes, but other estimates exceed 100,000 [465, 466]. Well over 100,000 human lncRNAs have been recorded, with many being specific to the primate lineage [337, 465]. This number is likely an underestimate due to the limited analysis across different cell types and developmental stages. Currently, hundreds of thousands of lncRNAs have been cataloged, and numerous databases exist to curate this information [467–469].

Non-coding RNAs exhibit diverse functions, complicating their classification. Some act locally, others distally, and many cytosolic lncRNAs encode small peptides [470–472]. Protein-coding genes can also produce lncRNAs via alternative splicing, with ~17% of major transcripts from these loci being non-coding [473, 474]. Both lncRNAs and mRNAs can generate various functional transcripts, including unspliced, spliced, circular, and intronic RNAs [475]. Transposable elements are frequently involved in gene expression and structure [476], making them unreliable as indicators of neutral evolution. Despite advances in transcriptome characterization, understanding lncRNA functions remains challenging due to their complex expression and processing. While some lncRNA roles are known, many remain unexplored, with emerging research focusing on their interactions and implications in diseases [477, 478]. A recently published Consensus Statement provides recommendations for advancing the understanding of long non-coding RNAs (lncRNAs) in development, cell biology, and disease, emphasizing standar-

dized nomenclature, research priorities, technological advancements, interdisciplinary approaches, clinical applications, data sharing, and educational initiatives [479].

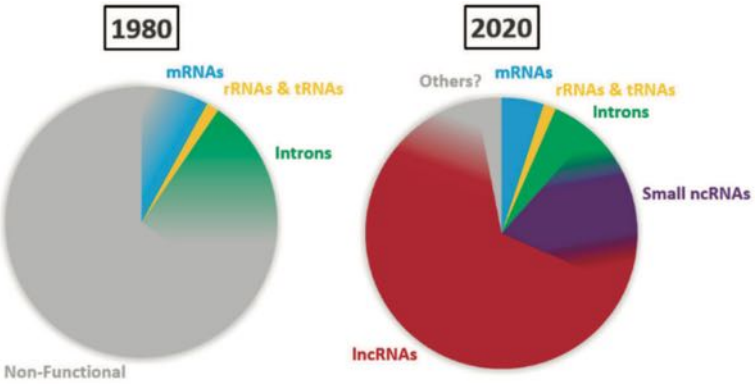

Figure 7. *Comparison of the information content of the human genome approximately 20 years before (left) [155] and after (right) [156].*

1.7.4. LncRNA HOTAIR

1.7.4.1. LncRNA HOTAIR: functions, expression, pathways, and molecular mechanisms

HOTAIR, a long non-coding RNA introduced by Rinn et al., is a spliced and polyadenylated RNA with 2,158 nucleotides and 6 exons, transcribed from the antisense strand of the HoxC gene on chromosome 12q13.13 [480]. It lacks features of pre-miRNA and is preferentially expressed in the body's posterior and distal regions [480]. HOTAIR has evolved rapidly compared to adjacent HoxC genes, showing poor sequence conservation but highly conserved structures [481].

HOTAIR acts as a trans-acting lncRNA, targeting various loci such as HOXD4. It interacts with the polycomb repressive complex 2 (PRC2), a histone methyltransferase essential for epigenetic silencing, including in cancer [482]. HOTAIR guides PRC2 across the genome to trimethylate histone H3 at lysine-27, involving key subunits like EZH2, SUZ12, and EED [483, 484]. PRC2’s binding to target genes is modulated by RNA, with JARID2 playing a critical role in activating EZH2's function [485–487]. HOTAIR serves as a molecular scaffold, interacting not only with PRC2 but also with the LSD1 complex, which demethylates histone H3 at lysine-4 [488, 489]. These interactions coordinate chromatin modifications and influence the expression of multiple genes involved in various cellular functions [483].

HOTAIR regulation is influenced by various pathways and factors. DNA methylation, particularly in downstream intergenic CpG islands, significantly impacts HOTAIR expression [490]. Post-synthetic methylation of specific cytosines within or near key HOTAIR regions also plays a crucial role in its regulation [321]. HOTAIR's function can be inhibited by the argonaute2 (Ago2) complex in the presence of microRNA-141 (miR-141), which acts as a tumor suppressor by binding to and suppressing HOTAIR [491]. Osteopontin (OPN), an extracellular matrix protein, transcriptionally activates HOTAIR expression, with its regulator, CD44, influencing this process. Conversely, interferon regulatory factor 1 (IRF1) binds to HOTAIR's promoter and decreases its expression, with OPN regulating IRF1 and thus indirectly activating HOTAIR [492]. The oncoprotein c-Myc also regulates HOTAIR by binding to an E-box element in its promoter, enhancing HOTAIR expression. c-Myc knockdown reduces HOTAIR levels, while its upregulation increases both HOTAIR expression and promoter activity [493]. Additionally, diethylstilbestrol and bisphenol-A can upregulate HOTAIR in breast cancer cells via estrogen response elements in its promoter [494]. TGF-β, involved in epithelial-mesenchymal transition (EMT), also induces HOTAIR expression, contributing to cancer stem cell (CSC) maintenance [321]. Type I collagen (Col-1), abundant in the tumor microenvironment, has been shown to upregulate HOTAIR in lung cancer cells, further highlighting its role in non-small cell lung cancer [495, 496].

HOTAIR is an oncogenic factor and serves as a prognostic biomarker across various cancer types. It plays a crucial role in both the initiation and progression of different cancers. LncRNA HOTAIR is involved in various carcinogenic processes, including cell mobility, proliferation, apoptosis, invasion, aggression, and metastasis [497]. HOTAIR complexes disrupt various cellular processes in carcinogenesis, primarily by deregulating multiple oncogenic signaling pathways [498]. HOTAIR regulates cell proliferation, alters gene expression, and enhances tumor cell invasion and migration. However, its precise molecular mechanisms are not yet fully understood [499]. HOTAIR regulates cell cycle progression, tumor proliferation, epithelial-to-mesenchymal transition (EMT), and tumor migration and invasion by modulating gene expression [500]. Patients with high HOTAIR expression levels exhibited a higher incidence of cancer compared to those with low HOTAIR expression levels [501–505]. HOTAIR overexpression has significant implications across various types of cancer, often leading to more aggressive disease and poorer patient outcomes [506]. HOTAIR were elevated in other disease, such as Parkinson's disease, rheumatoid arthritis [507, 508]. lncRNA HOTAIR is upregulated in sepsis and has been linked to septic cardiomyopathy [509].

Epithelial-to-mesenchymal transition (EMT) involves the transformation of epithelial cells into a mesenchymal cell type, enabling them to lose their epithelial characteristics and migrate to surrounding and distant tissues, a process crucial for embryonic development, organogenesis, and tumor invasion and metastasis [510, 511]. The transforming growth factor β (TGF-β) signaling pathway involves TGFβ subtypes, bone morphogenetic proteins, activin, and growth factors, crucial for cell proliferation and metastasis [512]. TGF-β regulates epithelial-to-mesenchymal transition (EMT) through transcription factors such as Twist, Snail, ZEB1, ZEB2, and FOXC2, which inhibit E-cadherin expression [513, 514]. E-cadherin is vital for maintaining epithelial cell adhesion and suppressing tumor progression [515, 516]. HOTAIR is implicated in regulating EMT through the TGF-β pathway, functioning as a competitive endogenous RNA (ceRNA) that modulates key EMT-related genes like E-cadherin, N-cadherin, and Snail [517]. HOTAIR also influences gene transcription via epigenetic mechanisms [483, 500].

Studies have shown that silencing HOTAIR enhances cell invasion, proliferation, and migration while promoting apoptosis in various cancer cells [518]. For instance, the interaction between miR-203 and HOTAIR regulates metastatic genes in renal cell carcinoma [519]. HOTAIR downregulation also affects migration and epithelial marker expression in RCC cells by modulating miR-217 [520]. Downregulation of HOTAIR reduces colorectal cancer cell proliferation and invasion by upregulating UPP1 through EZH2 recruitment, suggesting the HOTAIR/EZH2/UPP1 axis as a potential therapeutic target in CRC [521]. In breast cancer, HOTAIR regulates SETDB1 expression and EMT through sponging miR-7 [522], and in liver cancer, HOTAIR affects proliferation and migration by interacting with miR-217-5p [523]. HOTAIR sponges miR-601, and its silencing reverses miR-601's effects on breast cancer progression. It regulates ZEB1 through miR-601, with HOTAIR interference reducing tumor growth *in vivo* [524]. HOTAIR drives EMT through epigenetic regulation, such as by modulating HOXC8 expression in esophageal cancer [525]. In oral squamous cell carcinoma, HOTAIR, in conjunction with EZH2, inhibits E-cadherin expression, promoting metastasis [526]. Similarly, HOTAIR's interaction with TGF-β1 influences breast cancer progression [527] and gastric cancer by negatively regulating E-cadherin [528]. It also affects non-small cell lung cancer (NSCLC) by regulating the cell cycle [529] and cervical cancer by modulating EMT transcription factors [530]. In urothelial bladder cancer, a lack of HOTAIR expression decreases migration and invasion by regulating EMT-related genes [531].

The PI3K/Akt signalling pathway influences cell proliferation, apoptosis, and metastasis [532, 533], with HOTAIR playing a key role in tumour metastasis by affecting upstream and downstream gene expression [534]. Over-

expression of miR-203 suppresses HOTAIR, leading to increased PTEN expression, which then upregulates p21 and suppresses downstream pathways [519, 535]. HOTAIR also regulates the PI3K/Akt pathway through miR-34a, promoting tumour growth [536]. Knockdown of HOTAIR in MCF-7 cells increases p53 expression and decreases AKT and JNK levels, reducing cell migration and invasion [535]. Additionally, HOTAIR silencing lowers p-mTOR, p-PI3K, and p-AKT levels, thus reducing osteosarcoma cell proliferation and breast cancer cell metastasis [537, 538]. The HOTAIR/miR-326/FUT6 axis affects colorectal cancer progression through the PI3K/Akt/mTOR pathway [539], and HOTAIR modulates cell proliferation and apoptosis in leukaemia and melanoma via interactions with miRNAs and PI3K/Akt sig nalling [540–542]. Silencing HOTAIR reduced PI3K, AKT, and mTOR levels in MCF-7 cells and decreased cell proliferation, indicating HOTAIR's role in promoting breast cancer via the PI3K/AKT/mTOR pathway [543]. Overall, the PI3K/Akt/MAPK pathway plays a crucial role in tumour metastasis by affecting cell migration, adhesion, and angiogenesis. HOTAIR also inhibits FGF1 expression, affecting cell proliferation and migration [544, 545].

The Wnt/β-catenin pathway plays a crucial role in cellular processes and angiogenesis, impacting oncogenesis [546]. Abnormal activation of this pathway is linked to various cancers, including ovarian and liver cancer [547]. HOTAIR promotes tumour progression by activating Wnt/β-catenin signalling [548]. In liver cancer, HOTAIR overexpression correlates with poor metastasis, enhancing proliferation, invasion, and tumorigenicity through this pathway [549]. HOTAIR also downregulates TET1 in HeLa cells, further activating Wnt/β-catenin by altering gene methylation patterns [550,551]. HOTAIR knockdown, combined with miR-203a-3p overexpression, suppresses Wnt/β-catenin, reducing tumour cell proliferation and invasion [552]. Additionally, HOTAIR triggers Wnt/β-catenin by methylating the WIF-1 promoter, leading to increased tumour invasion [553].

The vascular endothelial growth factor (VEGF) pathway is crucial for tumour angiogenesis, invasion, and migration [554]. HOTAIR promotes VEGFA transcription, enhancing tumour cell invasiveness [555]. It also influences the degradation of extracellular matrix proteins via MMPs, aiding cancer cell proliferation and migration [556]. In non-small cell lung cancer (NSCLC), HOTAIR downregulates HOXA5 and upregulates MMP-2 and MMP-9, facilitating invasion and migration [557]. Additionally, HOTAIR is linked to increased VEGF and MMP expression in various cancers, promoting metastasis [558, 559]. These findings suggest that HOTAIR drives tumour metastasis through VEGF-related proteins.

Epigenetic modification involves heritable changes in gene expression without altering DNA sequences, with chromatin remodeling playing a key

role [560]. HOTAIR acts as a molecular scaffold, linking PRC2 and LSD1 histone modification complexes to reprogram chromatin states, promoting cancer metastasis through epigenetic gene silencing [483]. It can also inhibit RBM38, an RNA-binding protein, to enhance HCC cell migration and invasion [561]. Additionally, HOTAIR is found in extracellular vesicles (EVs), contributing to tumor invasion and metastasis by altering gene expression in recipient cells [562]. The precise role of exosomal HOTAIR in cancer metastasis warrants further investigation [563].

HOTAIR plays a significant role in the tumor microenvironment, which is comprised of various cellular components (such as cancer-associated fibroblasts (CAFs), endothelial cells, and immune cells like T-cells, tumor-associated macrophages, dendritic cells, and mast cells) and non-cellular components (including cytokines, growth factors, and metabolites). This signaling impacts various cancer-related processes, such as metastasis and therapy resistance [564]. HOTAIR upregulates proteins in NF-κB, TNFα, and MAPK pathways, boosting inflammatory complexes and immune responses, including T-cell co-stimulation. It promotes NF-κB activation by inhibiting UBXN1, leading to increased NF-κB nuclear translocation, while reduced HOTAIR decreases PD-L1 expression, enhancing susceptibility to immune attack [565].

Early detection of HOTAIR in body fluids may support its use as a biomarker for diagnosing various carcinomas [566]. Serum exosomes containing HOTAIR have potential as diagnostic and prognostic markers for breast cancer, with high expression linked to treatment response [567]. HOTAIR is also significantly associated with clinical parameters in laryngeal squamous cell carcinoma (LSCC), making it a promising biomarker for LSCC screening and prognosis [568]. Similar associations have been found in lung, stomach, and liver cancers [569–571]. HOTAIR plays a multifaceted role in promoting chemotherapy resistance by modulating apoptosis, cell cycle, and EMT in various cancers [572].

1.7.4.2. LncRNA HOTAIR in cervical cancer

Numerous lncRNAs have been identified as key regulators in various biological processes, significantly contributing to the onset and progression of cervical cancer [11, 573]. It has been reported that the interaction between HPV16 E7 oncoprotein and lncRNA-HOTAIR is linked to cellular proliferation and metastasis in cervical cancer. This suggests a close relationship between HPV proteins and lncRNA in the progression of cervical cancer [574]. HOTAIR expression was elevated in cervical cancer tissues and was associated with FIGO stage, lymphatic metastasis, tumor size, and depth of

invasion, suggesting its role in cervical cancer progression. It may serve as a potential diagnostic marker and an independent indicator of overall survival [575]. HOTAIR levels were significantly higher in cancer tissue and bodily fluids compared to paracancerous tissue and were reduced after surgery. Diagnostic analysis showed HOTAIR in vaginal discharge had a higher accuracy (92.7%) compared to serum (89.3%), suggesting it as a promising marker for early detection and monitoring of cervical carcinoma [576]. Reducing HOTAIR expression diminished cell motility and invasiveness in cervical cancer *in vitro*. HOTAIR regulated the expression of VEGF, MMP-9, and EMT-related genes, which are crucial for cell migration and metastasis [558].

Further experiments confirmed that Notch1 and STAT3 are key players in the reduced migration and invasion seen after HOTAIR knockdown. Functional studies on VIM, a critical mesenchymal marker involved in EMT, showed that HOTAIR influences HeLa cell motility and invasion, partly by regulating VIM expression [577]. Another study investigated the effects of silencing HOTAIR in cervical cancer cells using siRNA. Following knockdown, a significant reduction in cell proliferation, migration, and invasion was observed, along with an increase in apoptosis. Molecular analyses revealed that silencing HOTAIR decreased the expression of Notch1, EpCAM, vimentin, and STAT3, while increasing E-cadherin expression [578]. Increased serum HOTAIR levels in cervical cancer patients correlated with larger tumors, lymphovascular invasion, and shorter survival times. *In vitro*, HOTAIR overexpression in cervical cancer cell lines enhanced proliferation and invasion, while HOTAIR knockdown reduced these effects and increased apoptosis. *In vivo* xenograft models showed HOTAIR's role in promoting tumor growth and modulating epithelial-mesenchymal transition and Notch-Wnt signaling pathways, highlighting HOTAIR as a potential therapeutic target [579]. Loss-of-function studies demonstrated that HOTAIR overexpression enhanced, while its silencing suppressed, the growth, invasion, and viability of cervical cancer cells both *in vitro* and *in vivo*. Additionally, HOTAIR influenced human leukocyte antigen-G expression by competitively binding to miR-148a, underscoring its oncogenic role in cervical cancer. These findings suggest that HOTAIR could serve as a biomarker and potential target for prognosis and therapeutic intervention in cervical cancer [580]. Elevated The luciferase reporter assay showed that miR-17-5p directly targets the 3'-UTR of HOTAIR, regulating cervical cancer growth. Consequently, inhibiting the tumor-promoting activity of HOTAIR in cervical cancer could offer a promising new therapeutic approach for future treatments [581].

HOTAIR promotes cervical cancer by targeting the miR-331-3p/RCC2 axis, with clinical tissues showing a negative correlation between miR-331-3p,

HOTAIR, and RCC2, indicating a potential therapeutic target for the disease [582]. HOTAIR is upregulated in cervical cancer and promotes tumor growth by acting as a sponge for miR-143-3p, which normally inhibits BCL2. This interaction enhances BCL2 expression, counteracting the tumor-suppressive effects of miR-143-3p and suggesting HOTAIR as a novel regulatory mechanism [583]. HOTAIR is upregulated and miR-214-3p is downregulated in HPV16-positive cervical cancer cells, with HOTAIR acting as a competitive endogenous RNA (ceRNA) that promotes cell proliferation and inhibits apoptosis by binding miR-214-3p. This interaction influences the Wnt/β-catenin signaling pathway, suggesting HOTAIR/miR-214-3p as potential biomarkers and therapeutic targets in cervical cancer [584]. Propofol, a widely used intravenous anesthetic, has been shown to have antitumor effects in various cancers, including cervical cancer. A study reported that propofol significantly reduced cell viability and increased apoptosis in CaCx cells, effects that were counteracted by HOTAIR overexpression. Additionally, exogenous HOTAIR expression reversed the propofol-induced suppression of mTOR/p70S6K, key Ser/Thr kinases involved in regulating cell growth, proliferation, survival, and metabolism. Similar findings were observed in an animal model, where propofol inhibited tumor growth and promoted apoptosis through HOTAIR-mediated inhibition of the mTOR/p70S6K pathway in cervical cancer [585]. Artesunate (ART), a derivative of artemisinin, exhibits anti-cancer effects across various solid tumors, including cervical cancer. ART's anti-cancer effects include inducing cell cycle arrest, promoting apoptosis, and inhibiting angiogenesis and metastasis. ART's anti-metastatic effects on cervical cancer cells show that it inhibits metastasis and reduces HOTAIR expression. HOTAIR overexpression partially counteracted ART's anti-metastatic effects by interacting with and upregulating COX-2. Additionally, COX-2 overexpression reversed the effects of HOTAIR knockdown on cell migration and invasion. These findings suggest ART may suppress cervical cancer metastasis by inhibiting HOTAIR, leading to decreased COX-2 expression [586].

Increased HOTAIR levels were found to confer radio-resistance in HeLa cells by inhibiting p21, while HOTAIR knockdown increased p21 levels, thereby enhancing radio-sensitivity in C33A cells *in vitro* and making cervical cancer more responsive to radiotherapy *in vivo*. This suggests that HOTAIR induces radiation resistance by downregulating p21 in cervical cancer cells [587]. Studies show that ART enhances radiosensitivity in HeLa cells and suppresses tumor growth in a cervical cancer xenograft model [588,589]. HOTAIR was found to interact with 348 proteins, including YBX1, which it promotes to the nucleus, thereby activating the PI3K/Akt and ERK/RSK pathways and influencing cell proliferation through regulation of YBX1

targets like PCK2 and PDGFRβ, highlighting the utility of novel imaging tools for studying lncRNA-protein interactions *in vivo* [590].

A Chinese study examining circulating HOTAIR expression in cervical cancer revealed significant upregulation of HOTAIR in patients compared to controls. The study included 118 cervical cancer patients and 100 age-matched healthy women and investigated the relationship between HOTAIR levels and various clinicopathological parameters. Elevated HOTAIR expression was found to be associated with advanced tumor stages, adenocarcinoma subtype, lymphatic invasion, and lymph node metastasis. Furthermore, high levels of HOTAIR correlated with tumor recurrence and reduced overall survival, highlighting its potential as a prognostic biomarker in cervical cancer [591].

1.7.5. LncRNA MALAT1

1.7.5.1. LncRNA MALAT1: biogenesis, expression, and cell cycle regulation

The lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also called nuclear-enriched transcript 2 (NEAT2), is likely one of the most prevalent lncRNAs in cultured cells. MALAT1 is a highly abundant and widely expressed long noncoding RNA, approximately 8,000 nucleotides in length [592–594]. The longest isoform, MALAT1-201, has a length of 10,434 base pairs. Previously described in many species, MALAT1 is located on chromosome 11 in humans. In 2003, it was first discovered through subtractive hybridization as a prognostic marker for patient survival in stage I lung adenocarcinoma and squamous cell carcinoma [592]. Subsequent research identified MALAT1 as a noncoding transcript predominantly found in the nucleus of human primary fibroblasts and transformed lymphoblasts [595]. It is specifically found in nuclear speckles [595, 596]. Although MALAT1 has a poly(A) tract in its genome, the post-transcriptional process removes this tail. RNase P cleaves the primary MALAT1 transcript, producing a mature MALAT1 transcript with a poly(A)-rich stretch and small tRNA-like molecules. The long MALAT1 transcript stays in nuclear speckles, while the small, triple-helix MALAT1 is found in the cytoplasm. This structure enhances stability and nuclear localization despite the lack of a poly(A) tail. MALAT1 is also known to be misregulated in many cancers [597–599].

MALAT1 is crucial for regulating pre-mRNA splicing and transcription [600–602]. Although MALAT1 interacts with numerous proteins, its depletion does not disrupt the formation of nuclear speckles but instead leads to alterations in their composition [603]. MALAT1 forms numerous long-range structures, which likely facilitate its multivalent interactions with various RBPs

and pre-mRNAs [604]. MALAT1 acts as a central RNA hub for numerous highly expressed RNAs. RIC-seq uncovered multiple interaction sites between U1 small nuclear RNA and MALAT1, a finding also supported by psoralen analysis of RNA interactions and structures [604, 605]. A high-confidence analysis of NEAT1-RNA interactions indicated that the 5′ region of NEAT1 interacts with MALAT1 in trans [605].

MALAT1 has multiple functions in regulating gene expression. Under oxidative stress conditions in endothelial cells exposed to H2O2, MALAT1 transcription was found to be induced by the p53 protein, a regulation previously suggested in mice [606]. A mouse study found that MALAT1 expression is induced by hypoxia and regulated through the CaMKK/AMPK/HIF-1α axis, which enhances the MALAT1 promoter in response to Ca2+ inputs [607, 608]. The expression of MALAT1 was found to correlate with the transcription factors Sp1 and Sp3, whose co-silencing reduced MALAT1 levels, indicating their role in its positive regulation [609]. PCDH10 overexpression in AN3CA and HEC-1-B cell lines significantly downregulated MALAT1, which correlated with decreased cell proliferation; this suppression was mediated by the Wnt/β-catenin signaling pathway [610]. A study on SOX17 in esophageal squamous cell carcinoma (ESCC) found that the MALAT1 promoter has an SRY element bound by SOX17, which significantly limits MALAT1 expression [611]. In neuroblastoma, MALAT1 expression is activated by oxytocin receptors through CREB binding to its promoter. In bladder cancer, MALAT1 is upregulated by TGF-β, enhancing tumor invasion and metastasis, while targeting MALAT1 reduces these effects [612]. In colorectal and gastric cancer cultures, silencing Yes-associated protein 1 (YAP1) significantly reduced MALAT1 expression [613]. In Ewing sarcoma research, MALAT1 transcription was found to depend on SYK signaling, with c-MYC enhancing SYK's binding to the MALAT1 promoter and boosting tumor proliferation [614]. In colorectal and gastric cancer cultures, silencing Yes-associated protein 1 (YAP1) reduced MALAT1 expression [613]. MALAT1 expression can be regulated post-transcriptionally by miR-101 and miR-217, leading to its silencing and reduced proliferation of ESCC cells by arresting the G2/M cell cycle [615]. MALAT1 was found to be involved in a positive feedback loop with NRF1 and NRF2, regulated by KEAP1, indicating that targeting MALAT1 could offer a new treatment option for multiple myeloma (MM). NRF1, a key regulator of the proteasome response, enhances sensitivity to proteasome inhibitors when inhibited [616, 617]. MALAT1 upregulation during liver regeneration was found to accelerate cell cycle progression and promote hepatocyte proliferation *in vitro* [618]. In L428 and U87MG cell lines, it was shown that miR-9 regulates MALAT1 expression through AGO2 [619]. In diabetic mice, high glucose levels stimulated MALAT1 expression

in the kidneys, correlating positively with serum creatinine and urinary albumin levels [620].

Many studies have explored how genes like MALAT1 affect cell cycle regulation and carcinogenesis, highlighting MALAT1's pro-proliferative effects. Elevated MALAT1 levels are notably observed during the G1/S and mitotic (M) phases [621, 622]. MALAT1 accelerates the liver cell cycle: knockout prolongs the G0/G1 phase, while overexpression increases cells in the replication phase and reduces those in G0/G1 [620]. MALAT1 interacts with the nuclear protein hnRNP C, aiding its transport to the cytoplasm and promoting the transition from G2 to M phase [623]. Silencing MALAT1 with miR-101 and miR-217 causes G2/M phase cell cycle arrest, likely by altering p21, p27, and B-MYB expression [615]. Knockout of MALAT1 in LNCaP cells delays the G1 to S phase transition and reduces cyclin D1 and CDK6 expression. MALAT1 depletion also lowers B-MYB levels, affecting genes expressed during M phase. Additionally, MALAT1's interaction with hnRNP C aids its movement from the nucleus to the cytoplasm, facilitating the G2 to M phase transition [623, 624]. Flow cytometry of MALAT1-knockout breast cancer cells showed more cells in G0/G1 and fewer in S phase. In esophageal cancer cells, MALAT1 knockout increased G2/M phase cells and activated the ATM-CHK2 pathway, which inhibits rapid tumor growth by controlling the G2/M phase [625, 626]. MALAT1 has been linked to chemotherapy resistance in chronic myeloid leukemia, head and neck squamous cell carcinoma, and hepatocellular carcinoma by enhancing DNA repair, evading cell cycle checkpoints, and regulating apoptosis, autophagy, and stemness [627]. MALAT1's impact on the cell cycle varies by cell type. In cancer cells, uncontrolled cycles result from mutations in tumor suppressor genes or oncogenes, unlike normal cells. Additionally, different cancers or genetic alterations may explain MALAT1's varying effects on the cell cycle.

1.7.5.2. LncRNA MALAT1: carcinogenesis, cancer and other diseases

MALAT1 plays a role in cancer progression and metastasis. In the MMTV-PyMT mouse model, genetic loss or systemic knockdown of MALAT1 using antisense oligonucleotides (ASOs) slows tumor growth, promotes cystic differentiation, and reduces metastasis. MALAT1 loss also decreases branching morphogenesis in tumor organoids, increases cell adhesion, and impairs migration. Molecularly, MALAT1 knockdown alters gene expression and splicing of genes involved in differentiation and cancer signaling [628]. MALAT1 is upregulated in hepatocellular carcinoma and acts as a proto-oncogene by activating the Wnt pathway and inducing the oncogenic splicing

factor SRSF1. This induction enhances the production of antiapoptotic splicing isoforms and activates the mTOR pathway through alternative splicing of S6K1. Inhibiting SRSF1 or mTOR eliminates MALAT1's oncogenic effects, indicating that SRSF1 induction and mTOR activation are crucial for MALAT1-driven cancer transformation [629]. Alternative splicing in cancer cells differs from that in healthy cells. Besides splicing factors like SR proteins and HnRNPs, lncRNAs such as DGCR5 and LINC01232 also influence splicing. MALAT1's abundance in nuclear speckles indicates its key role in splicing events by interacting with splicing factors to protect them, direct their transport, or maintain their interactions [630]. MALAT1, similar to other lncRNAs, influences alternative splicing by interacting with splicing factors to produce variants that support cancer progression, such as those with anti-apoptotic (BIM, BIN1) and pro-proliferative (TEAD1) properties. It is essential for the binding of splicing factors PTBP1 and PSF, and their interaction with MALAT1 is crucial for promoting malignant traits like cell growth, invasion, and migration in hepatocellular carcinoma cells [629, 631]. The MALAT1/mTOR/HIF-1α pathway increases VEGF and FGF2 levels, promoting angiogenesis and tumor growth [632]. MALAT1 enhances metastasis by activating the Wnt/β-catenin pathway through overexpression. Specifically, the 3′ end of MALAT1 directly interacts with EZH2's N-terminal, leading to increased EZH2 expression [633]. MALAT1 activates the Wnt/β-catenin pathway, upregulating metastasis-related genes such as c-Myc, cyclinD1, MMP-7, and CD44. Additionally, MALAT1 influences the PI3K-AKT pathway, regulating EMT markers like E-cadherin, N-cadherin, vimentin, snail42, MMP2, and MMP9. This effect is mediated by MALAT1 sponging miR-146a, which downregulates the pathway by targeting the 3′UTR of PI3K mRNA [634–636]. This mechanism also involves PI3K-AKT pathway activation and interactions with microRNAs such as miR-101 and miR-125b. MALAT1 binds to miR-140-5p, leading to upregulation of HDAC4, an epigenetic regulator that inhibits pro-apoptotic gene transcription through chromatin modifications [637, 638]. In bladder cancer, researchers have found that MALAT1 interacts with Suz12, a PRC2 component, rather than EZH2 [612]. Upregulated EZH2 promotes the downregulation of GSK-3β by tri-methylating lysine 27 on histone H3. This inhibits β-catenin ubiquitination, thereby activating the Wnt/β-catenin pathway [637]. In natural killer (NK) cell lymphomas, MALAT1 has been shown to interact with both components of the PRC2 complex [639]. In line with findings on MALAT1's oncogenic roles, its knockdown was shown to suppress tumor growth by reducing proliferation and invasion while increasing apoptosis in Hs578T TNBC cells. Among the predicted miRNAs targeted by MALAT1, miR-1 exhibited the most signifi-

cant change following MALAT1 silencing, a result consistent with observations in TNBC tissues [640].

MALAT1 has been shown to stimulate cancer cell proliferation in gastric cancer. Additionally, it inhibits the anti-oncogene PCDH10, further promoting stomach cancer cell growth and metastasis. In colorectal cancer, MALAT1 enhances proliferation, invasion, and migration through PRKA kinase anchor protein 9 (AKAP-9), which is linked to cancer progression and metastasis [641–643]. MALAT1 was found to interact with miR-218, promoting the proliferation of human choriocarcinoma cells by regulating the oncogenic F-box/WD repeat-containing protein 8 (Fbxw8) in phosphorylation-dependent ubiquitination [644]. In hilar cholangiocarcinoma (HCCA), increased MALAT1 expression was correlated with the tumor's pathological T stage (TNM classification), nerve invasion, and larger tumor size. Additionally, MALAT1 contributes to oncogenesis by regulating CXCR4 through a miR-204-dependent mechanism [645]. Knocking out MALAT1 was shown to suppress the proliferation and metastasis of human tongue cancer cells while elevating miR-124 levels, which in turn regulates jagged1 (JAG1), a factor that promotes cancer cell proliferation [646].

MALAT1 overexpression is associated with poor prognosis in various cancers, including breast cancer, non-small-cell lung cancer, and glioma [647–649]. HPV-positive cells were found to express MALAT1; however, tissues affected by vulvar squamous cell carcinoma (VSCC), which is associated with HPV, exhibited reduced MALAT1 expression levels [650, 651]. Recent research has shown that silencing MALAT1 in ovarian cancer cells leads to reduced cell proliferation, invasion, and migration. This evidence underscores MALAT1's role as a key oncogenic lncRNA that promotes the progression of ovarian cancer [652]. MALAT1 promotes tumorigenesis and metastatic traits in ovarian cancer cells – such as proliferation, migration, and invasion – by repressing miR-22 [653]. The identification of a negative correlation between MALAT1 and miR-1271-5p in cisplatin-resistant ovarian cancer tissues reveals that silencing MALAT1 or overexpressing miR-1271-5p increases resistance to cisplatin while decreasing proliferation, migration, and invasion, and promoting apoptosis. This suggests MALAT1 acts as an oncogene in cisplatin-resistant ovarian cancer. Furthermore, miR-1271-5p inhibits E2F5, a positive regulator of cancer development, and MALAT1 modulates this interaction. Thus, targeting the MALAT1/miR-1271-5p/E2F5 axis offers a promising approach for ovarian cancer therapy [654, 655]. An inverse relationship between MALAT1 and miR-506 was observed in ovarian cancer tissues. As miR-506 acts as a tumor suppressor, its upregulation notably reduced DNA synthesis in ovarian cancer cells, thereby inhibiting tumor growth. Additionally, miR-506 directly regulates iASPP expression [656, 657].

Elevated MALAT1 levels have been linked to increased recurrence and reduced overall survival in epithelial ovarian cancer (EOC) cells [658, 659]. Studies have shown that high MALAT1 expression promotes metastasis in epithelial ovarian cancer (EOC) by suppressing apoptosis and enhancing proliferation, migration, and invasion. MALAT1 knockdown significantly reduces EMT-related proteins (N-cadherin, vimentin, snail) and matrix metalloproteases (MMP2, MMP9) while increasing E-cadherin expression, thereby inhibiting tumorigenicity. Additionally, MALAT1 silencing decreases p-AKT levels without affecting total AKT, suggesting that MALAT1 influences EMT through the PI3K/AKT signaling pathway. This highlights MALAT1 as a potential target for EOC therapy and diagnosis [660, 661]. In epithelial ovarian cancer (EOC), elevated MALAT1 expression has been linked to distant metastases, with multiple studies suggesting that MALAT1 plays a crucial role in driving the metastatic process [651]. Elevated MALAT1 expression has been observed in both acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) [662]. MALAT1 upregulation has also been noted in other cancers, including bladder epithelial cancer [663].

In a study of lung cancer patients, lncRNA-MALAT1 expression was significantly higher in tumor samples compared to adjacent normal tissue. Elevated lncRNA-MALAT1 levels were also detected in the serum of NSCLC patients using fluorescent quantitative PCR [664]. To study lncRNA-MALAT1 in nasopharyngeal carcinoma, researchers used lentiviral vectors to either inhibit or activate lncRNA-MALAT1 in the CNE-1 cell line. They found that upregulation of lncRNA-MALAT1 enhanced cell proliferation, invasion, and metastasis in these cells [665]. MALAT1 is notably dysregulated in laryngeal squamous cell carcinoma (LSCC) specimens, suggesting its involvement in LSCC development [666].

It was found that MALAT1 levels were significantly upregulated under hypoxia, regulated by HIF-1α and HIF-2α, and predominantly located in the cytoplasm. MALAT1 acts as a miRNA sponge for miR-3064-5p, promoting breast cancer cell migration and proliferation, suggesting its potential as a therapeutic target in breast cancer [667]. In breast cancer, miR-26b levels were consistently low, while METTL3, MALAT1, and HMGA2 levels were high. METTL3 increased MALAT1 expression through m6A modification, and MALAT1 promoted HMGA2 expression by sponging miR-26b. Silencing METTL3 inhibited EMT and tumor invasion by suppressing MALAT1. Overall, METTL3 drives breast cancer progression via the MALAT1/miR-26b/HMGA2 axis [668]. METTL3 is known to contribute to various cancers by promoting translation, RNA stability, and miRNA maturation, affecting processes like cell proliferation and invasion. Both MALAT1 and METTL3 were found to be upregulated in BC cells. Silencing METTL3 led

to MALAT1 downregulation, reduced EMT markers, and decreased cell migration and invasion. Additionally, the low expression of miR-26b in BC cells suggests that MALAT1 may act as a ceRNA for miR-26b [669]. MALAT1 was found to be upregulated in both breast cancer (BC) patients and cell lines, and its high expression is linked to poor prognosis. MALAT1 overexpression promoted proliferation, migration, and aggressiveness of BC cells, while knockdown of MALAT1 reversed these effects. Mechanistically, MALAT1 negatively regulated miR-26a/26b, and its knockdown reduced the expression of ST8SIA4, a target of miR-26a/26b. Functional analyses showed that miR-26a/26b overexpression or MALAT1 knockdown inhibited BC cell progression, while miR-26a/26b silencing or MALAT1 upregulation had the opposite effect, highlighting the MALAT1/miR-26a/26b/ST8SIA4 axis in BC progression [670]. MALAT1 was found to act as a ceRNA for miR-124, with MALAT1 knockdown inhibiting proliferation, inducing cell cycle arrest, and reducing tumor growth by sponging miR-124. Additionally, miR-124 targets CDK4, and its upregulation by MALAT1 activates the CDK4/E2F1 pathway, promoting BC progression [671].

Elevated MALAT1 expression has been noted in blood and fibrovascular membranes in proliferative vitreoretinopathy [672]. MALAT1 overexpression leads to lipid accumulation, hepatic steatosis, and insulin resistance by increasing SREBP-1c and its target genes ACC1, ACLY, SCD1, and FAS [673]. MALAT1 regulates cytokines IL-6 and TNF-α by activating SAA3 in arterial hyperglycemia [674]. Increased MALAT1 expression is found in RF/6A hyperglycemic cells and in the aqueous humor and fibrovascular membranes of diabetic retinopathy patients [675]. MALAT1 expression in adipose tissue is linked to increased levels of FABP4 and LPL, which are involved in fat accumulation. This lncRNA regulates PPARγ, fatty acid metabolism, and insulin signaling, highlighting its potential as a target for developing obesity treatments in humans. In livestock, MALAT1 may also be useful as a marker for controlling fat content [676].

1.7.5.3. The role of MALAT1 in cervical cancer

To explore MALAT1's role in cervical cancer, short hairpin RNA was used to inhibit its expression in CaSki cells, and gene expression and cell behavior were assessed using reverse transcriptase–polymerase chain reaction. The results showed that MALAT1 regulates cell growth, cycle progression, and invasion by affecting genes such as caspase-3, -8, Bax, Bcl-2, and Bcl-xL. Specifically, MALAT1 downregulation increases caspase-3, -8, and Bax levels while decreasing Bcl-2 and Bcl-xL, leading to enhanced apoptosis, highlighting its potential as a therapeutic target in cervical cancer [677].

MALAT1 expression is elevated in cervical cancer cells and is believed to play a significant role in the progression of the disease. Elevated MALAT1 expression was found in HR-HPV (+) cervical cancer. MALAT1 knockdown reduced cell growth and invasion, increased apoptosis in HeLa and SiHa cells, and raised miR-124 levels. Conversely, miR-124 overexpression lowered MALAT1 levels. MALAT1 also modulates GRB2 expression indirectly through miR-124. GRB2 knockdown led to decreased invasion and increased apoptosis. Overall, MALAT1 enhances HR-HPV (+) cancer cell growth and invasion via the MALAT1-miR-124-GRB2 axis [678]. Silencing MALAT1 in cervical cancer cell lines results in decreased cell viability and metastasis, and promotes apoptosis. Additionally, MALAT1 has been found to target and downregulate miR-429 in these cells. Thus, the MALAT1/miR-429 axis plays a role in the progression of cervical cancer [679]. MALAT1 has been shown to promote cervical tumor cell proliferation by inhibiting miR-124 in HeLa, C-33A, CaSki, and SiHa cell lines. The expression of miR-124, which is negatively correlated with MALAT1, is reduced in cervical cancer cells, tissues, and mouse models. Increasing miR-124 levels inhibits cervical carcinoma proliferation and improves tumor weight, size, volume, and survival rates. These findings suggest that targeting MALAT1 could enhance clinical outcomes in cervical cancer treatment [680]. In the study exploring the relationship between MALAT1 and MAT2A gene expression in HPV-positive cervical cancer tissues, it was found that MALAT1 functions as a competing endogenous RNA (ceRNA), upregulating MAT2A by sponging miR-485-5p. Consequently, the MALAT1/miR-485-5p/MAT2A axis may serve as a promising therapeutic target for HPV-positive cervical cancer [679].

Periostin gene expression is elevated in cervical cancer tissues and cell lines. Knockdown of periostin in HeLa and SiHa cells reduces metastasis, epithelial-mesenchymal transition (EMT), and suppresses the AKT/mTOR signaling pathway. Additionally, periostin expression in cervical cancer correlates positively with MALAT1 levels and negatively with miR-202-3p levels. These findings suggest that the MALAT1/miR-202-3p/periostin axis plays a role in regulating these biological processes in cervical cancer [681]. MALAT1 knockdown in cervical cancer cells led to increased expression of epithelial markers E-cadherin and ZO-1, while decreasing mesenchymal markers such as β-catenin and Vimentin. Additionally, the EMT transcription factor Snail showed reduced expression after MALAT1 silencing. These results indicate that MALAT1 plays a role in promoting EMT and metastasis in cervical cancer cells [12].

MALAT1 expression was significantly higher in radio-resistant compared to radio-sensitive patients and contributes to radio-resistance in cervical cancer by suppressing miR-145 [682]. MALAT1 expression was significantly

higher in tumor tissue compared to adjacent normal tissue and was linked to tumor size, FIGO stage, vascular invasion, and lymphatic spread, serving as an independent prognostic factor in cervical cancer [683].

The study examined how Casiopeina II-gly (Cas-II-gly) affects the regulatory network involving MALAT1, miR-17-5p, and FZD2 in cervical carcinoma through the Wnt signaling pathway. *In vitro* results showed that treatment with Cas-II-gly reduced MALAT1 and FZD2 levels while increasing miR-17-5p expression in HeLa and CaSki cell lines [684].

Chemotherapeutic drugs, including cisplatin, are crucial in treating various cancers, such as cervical cancer. However, resistance to cisplatin can diminish its effectiveness. To understand the mechanisms behind cisplatin resistance, the expressions of MALAT1 and BRWD1 were examined in HeLa and C-33A cells. The results revealed that MALAT1 overexpression enhances cell proliferation and induces cisplatin resistance by interacting with apoptosis-regulating molecules like BRWD1. Additionally, MALAT1 upregulation activates the PI3K/AKT signaling pathway, affecting apoptosis regulation. Therefore, MALAT1 contributes to cisplatin resistance in cervical cancer cells by disrupting apoptotic control [685]. Recent studies suggest that targeting MALAT1 offers therapeutic potential in treating various human cancers [686, 687].

2. METHODS

2.1. Study design

The retrospective cohort study of adult patients with cervical cancer was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-10 and P1-BE-2-10/2014, Supplementary Picture 1). All the patients were investigated at the Hospital of Lithuanian University of Health Sciences Kauno klinikos in Kaunas, Lithuania, from October 2014 to August 2020. A total of 172 patients with stages I–IV cervical cancer were consecutively enrolled, with their diagnoses confirmed through clinical (gynecological and radiological examinations) and histological (cervical biopsies) assessments. The blood samples were collected from peripheral veins for further genetic testing. Cancer treatment was administered following institutional guidelines and in accordance with international standards. The follow-up period extended until November 2020. The research flow chart is presented in Figure 8.

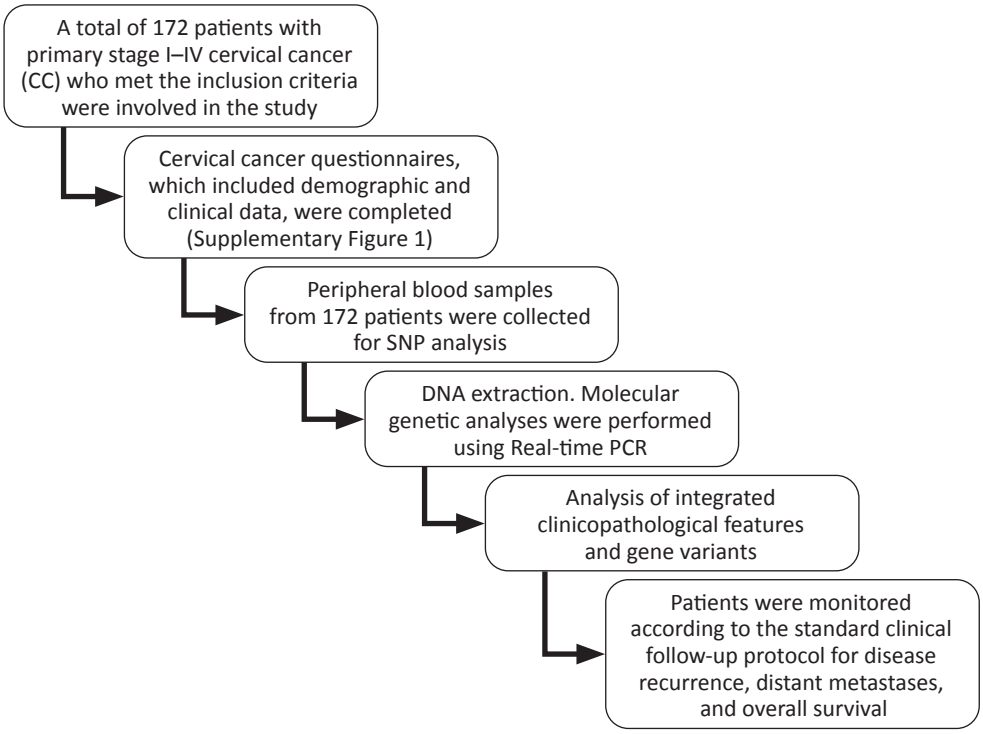

Figure 8. *Study flow chart.*

2.2. Patient inclusion and exclusion criteria

Inclusion criteria:

- Availability of complete data on clinicopathological characteristics.
- Patient's written consent to participate (Supplementary Figure 2).

Exclusion criteria:

- Presence of other malignancies.
- Significant comorbidities.
- Incomplete medical records, which were used to extract clinical and pathological features as well as details about the disease course.

2.3. SNP selection

Genotype information was derived from established online repositories, including The International HapMap Project (accessible at http://www.HapMap.org) and the 1000 Genomes Project (available at http://www.1000genomes.org). The criteria employed for the selection of Single Nucleotide Polymorphisms (SNPs) were comprehensive. These criteria involved the prerequisite that these SNPs had been previously identified in diverse populations, showcasing associations with the outcomes of various diseases as reported in scientific literature. Moreover, our analysis specifically targeted SNPs that had not been extensively investigated within the context of cervical cancer patients, thereby exploring new avenues of genetic inquiry. Additionally, SNPs under consideration were required to exhibit a minor allele frequency (MAF) of equal to or greater than 5% within the European population. This criterion was pivotal in ensuring that the selected SNPs had a sufficiently substantial presence to be statistically significant. Finally, we also considered the potential functional relevance of these SNPs, exploring whether they might be involved in regulating key biological processes. As a point of reference, Table 1 provides a comprehensive listing of the candidate SNPs, their locations, and MAF within the European population data from the 1000 Genomes Project Phase 3 database.

Table 1. *SNPs genomic region, minor allele frequency (MAF).*

Chromosome/gene	SNP	Genomic position in chromosome	Region/location	Minor allele frequency (MAF) (1000 Genomes)	Highest population MAF:
Chr9/*TLR4* Alias symbols: ARMD10, CD284, TOLL, hToll	rs10983755	117702392 (GRCh38) 120464670 (GRCh37)	Promoter, 5'-UTR, intergenic variant, regulatory region variant −2081	0.07 (A)	0.31
	rs10759932	117702866 (GRCh38) 120465144 (GRCh37)	Promoter, 5'-UTR, intergenic variant −1607	0.18 (C)	0.35
	rs11536865	117703745 (GRCh38) 120466023 (GRCh37)	Promoter, 5'-UTR, regulatory region variant −729	0.04 (C)	0.24
	rs4986790/ Asp299Gly	117713024 (GRCh38) 120475302 (GRCh37)	Exon, 3′-UTR, missense variant 896	0.06 (G)	0.14
	rs4986791/ Thr399Ile	117713324 (GRCh38) 120475602 (GRCh37)	Exon, 3′-UTR, missense variant 1196	0.04 (T)	0.17
	rs11536897	117717732 (GRCh38) 120480010 (GRCh37)	3′-UTR 3084	0.04 (A)	0.11
	rs1927906	117717837 (GRCh38) 120480115 (GRCh37)	3'-UTR 3189	0.21 (C)	0.49
	rs11536898	117717932 (GRCh38) 120480210 (GRCh37)	3'-UTR 3284	0.13 (A)	0.27
Chr21/*RRP1B* Alias symbols: KIAA0179, Nnp1, RRP1, PPP1R136	rs2838342	43657984 (GRCh38) 45077865 (GRCh37)	upstream transcript variant, intron variant	0.42 (G)	0.50
	rs7276633	43658919 (GRCh38) 45078800 (GRCh37)	upstream variant	0.42 (C)	0.49
	rs2051407	43659364 (GRCh38) 45079245 (GRCh37)	upstream variant	0.37 (T)	0.42
	rs9306160	43687681 (GRCh38) 45107562 (GRCh37)	missense variant	0.38 (T)	0.44
	rs762400	43693748 (GRCh38) 45113629 (GRCh37)	3′-UTR variant	0.37 (C)	0.50

Table 1. *Continued.*

Chromosome/gene	SNP	Genomic position in chromosome	Region/location	Minor allele frequency (MAF) (1000 Genomes)	Highest population MAF:
Chr11/*SIPA1* Alias symbols: SPA1	rs931127	65637829 (GRCh38) 65405300 (GRCh37)	upstream variant	0.45 (A)	0.49
	rs3741378	65641466 (GRCh38) 65408937 (GRCh37)	missense variant	0.21 (T)	0.38
	rs746429	65649963 (GRCh38) 65417434 (GRCh37)	synonymous variant	0.24 (A)	0.48
Chr17/*SRSF1* Alias symbols: ASF, MGC5228, SF2, SF2P33, SF2p33, SRp30a	rs8819	58001377 (GRCh38) 56078738 (GRCh37)	3′-UTR variant, non coding transcript variant	0.23 (T)	0.49
	rs34592492	58001676 (GRCh38) 56079037 (GRCh37)	3′-UTR variant, non coding transcript variant	0.07 (C)	0.20
	rs11654058	58002949 (GRCh38) 56080310 (GRCh37)	3′-UTR variant, non coding transcript variant	0.06 (C)	0.14
	rs2233908	58007896 (GRCh38) 56085257 (GRCh37)	upstream transcript variant, 2KB upstream variant	0.24 (A)	0.47
	rs2585828	58009002 (GRCh38) 56086363 (GRCh37)	2KB upstream variant, upstream transcript variant	0.23 (C)	0.49
Chr12/*HOTAIR* Alias symbols: HOXAS, HOXC-AS4, HOXC11-AS1, NCRNA00072	rs12826786	53961717 (GRCh38) 54355501 (GRCh37)	regulatory region variant	0.36 (T)	0.50
	rs7958904	53963768 (GRCh38) 54357552 (GRCh37)	non coding transcript exon variant	0.48 (C)	0.49
	rs920778	53966448 (GRCh38) 54360232 (GRCh37)	intron variant	0.44 (G)	0.49

Table 1. *Continued.*

Chromosome/gene	SNP	Genomic position in chromosome	Region/location	Minor allele frequency (MAF) (1000 Genomes)	Highest population MAF:
Chr11/*MALAT1* Alias symbols: HCN, LINC00047, MALAT-1, NCRNA00047, NEAT2, PRO1073, PRO2853, mascRNA	rs619586	65498698 (GRCh38) 65266169 (GRCh37)	non coding transcript exon variant	0.07 (G)	0.20
	rs664589	65501878 (GRCh38) 65269349 (GRCh37)	non coding transcript exon variant	0.07 (G)	0.20
	rs3200401	65504361 (GRCh38) 65271832 (GRCh37)	splice donor region variant	0.14 (T)	0.26

GRCh37 and GRCh38 are human genome assemblies versions by the Genome Reference Consortium.

2.4. SNP genotyping

The DNA extraction process involved the isolation of genetic material from leukocytes in peripheral venous blood samples, which were initially collected in ethylenediaminetetraacetate (EDTA) vacuum tubes and subsequently stored in a laboratory biobank at –20 ºC. Genomic DNA extraction was carried out utilizing a genomic DNA purification kit provided by Thermo Fisher Scientific Baltics, based in Vilnius, Lithuania. Genotyping of selected SNPs within the *TLR4*, *RRP1B, SIPA1, SRSF1, HOTAIR, and MALAT1* genes was conducted at the Institute of Oncology, Lithuanian University of Health Sciences. This was achieved using TaqMan® probe SNP Genotyping Assays, also sourced from Thermo Fisher Scientific in Lithuania.

Molecular genetic analyses were conducted using the Real-time polymerase chain reaction (RT-PCR) method, designed to amplify specific DNA segments according to the established protocol.

Reactions were performed into a total volume of 12 µL, including: 6.125 µL of TaqMan Universal Master Mix, 0.625 µL of TaqMan SNP Genotyping Assays, 4.25 µL of nuclease-free water, 1 µL of DNA. For negative control nuclease-free H2O was used. The genotyping was performed with a QuantStudio 3 RealTime PCR System (Thermo Fisher Scientific, Cat. #A28137) with the following conditions: 95 °C for 10 minutes, 95 °C for 15 seconds, 60 °C for 1 minute for 45 cycles. The results were analyzed using an Allelic discrimination plot according to VIC and FAM fluorescence intensity on the QuantStudio 3 Real-Time PCR System.

2.5. Assessment of clinicopathological features and investigated associations

All carcinoma cases were staged according to the guidelines set forth by the International Federation of Gynecology and Obstetrics (FIGO). Tumor grading was determined based on architectural and cytologic (nuclear) criteria. This analysis incorporated clinicopathological features, including age at the time of diagnosis, tumor size (T), lymph node involvement (N), metastasis spread (M), stage, degree of differentiation (G), response to treatment, presence of disease progression, and patient mortality.

In our comprehensive investigation, we delved into the potential interconnections between SNPs in the genotype and allelic models, and the intricate landscape of tumor clinicopathological features. These attributes encompass the patient's age (categorized into age ≤ 50 and age > 50), the tumor's size (distinguished as T1–T2 and T3–T4), the status of pathological regional lymph nodes (delineated as N0 and N1), the presence of distant metastasis

(defined by M0 or M1), the tumor's grade (G1 + G2 or G3), the disease stage (categorized as stage I–II and stage III–IV), and the overall disease prognosis (specifically, the worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2). Furthermore, the study extended its scrutiny to encompass clinical outcomes, specifically progression-free survival (PFS) and overall survival (OS). Within the patient cohort, PFS was computed commencing from the date of diagnosis until the point of local disease spread or the occurrence of distant metastasis/metastasis spread. In parallel, OS was calculated from the date of diagnosis to the date of the patient's demise.

2.6. Statistical analysis and software utilization

The identified SNPs were subsequently integrated into a comprehensive statistical analysis, encompassing both genotype and allelic models. The statistical evaluation was carried out using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). To investigate the associations between genotypes, alleles, and tumor characteristics, statistical tests, including Pearson's Chi-square and Fisher's Exact tests, were employed. In order to present a robust analysis, both univariate and multivariate models were adopted, with adjustments for age at the time of diagnosis and various cancer clinicopathological features. These models enabled the calculation of odds ratios along with their corresponding 95% confidence intervals (CIs) and *p*-values, using logistic regression. The analysis of differences in PFS and OS involved the performing of hazard ratios (HRs) derived from univariate and multivariate Cox proportional hazard models. The survival curves were constructed and assessed employing the log-rank test, and the Kaplan-Meier method was used for generating these curves. Throughout the entirety of the analysis, a *p*-value less than 0.05 was deemed statistically significant.

Haploview v4.1 software was utilized to assess linkage disequilibrium (LD) among SNPs and generate LD plots (available at http://www.broad.mit.edu/mpg/haploview/). Haplotypes were inferred from the analyzed SNPs using Bayesian methods through the Phase software v2.1 (Department of Statistics, University of Washington, Seattle, WA, USA) [688, 689]. Finally, we analyzed the associations of haplotypes with clinical manifestations of the disease and survival outcomes. These findings are instrumental in our quest to elucidate the potential genetic factors that may exert influence over these pivotal facets of the disease's clinical intricacies.

3. RESULTS

3.1. Clinical characteristics

In the course of our investigation, the study cohort primarily comprised Lithuanian nationals, constituting 90.1% of the participants, with the remaining individuals originating from others European countries. The demographic profile of the subjects exhibited a broad spectrum of ages, spanning a considerable range from 22 to 83 years. When the participants were diagnosed, their mean age stood at 55.4 years, with a standard deviation of 13.5 years, indicating the spectrum of ages represented in this study. An in-depth analysis of the tumor size dimensions unveiled a noteworthy predominance of the T2 category, constituting 48.8% of the cases. Lymph node involvement was documented in 44.8% of the patient cohort. Furthermore, the study uncovered that metastasis to paraaortic lymph nodes was documented in 5.2% of the cases. Distant metastasis was detected in 10 cases, constituting 5.8% of the total. Cancer staging indicated that IIB and IIIC1 were the prevailing stages, representing 32.0% and 31.0% of the cases, respectively. This stratification also revealed that lower stages (I–II) accounted for 44.2% of the participants, while the more advanced stages (III–IV) encompassed 55.8% of the study population. Further scrutiny unveiled a distribution of tumor differentiation, with 7.6% classified as well-differentiated (G1), 65.7% as intermediate (G2), and 26.7% as poorly differentiated, thereby illustrating the heterogeneity of tumor grades within the study cohort. Regarding treatment, a significant majority of patients, amounting to 69.2%, undergoing standard chemoradiation therapy. The remaining participants underwent surgery followed by radiotherapy or systemic treatment. Importantly, a substantial 70.3% of the patients exhibited a complete response to treatment, while 21.5% showed a partial response. A smaller segment, comprising 8.2%, exhibited either stable disease or progressive disease. Within the context of progression, the median progression-free survival (PFS) was calculated at 13 months, exhibiting a range spanning from a minimum of 1 month to an maximum of 201 months. Over the course of the follow-up period, disease progression was confirmed in 52 cases, impacting 30.2% of the cohort. A substantial majority of those experiencing progression exhibited localized advancement and metastasis in regional lymph nodes, affecting 51 patients, while an additional 18 cases demonstrated progression in paraaortic lymph nodes. The disease also metastasized in sixteen patients. Regrettably, 40 events of death occurred during the follow-up period, accounting for 23.3% of the cohort. The median overall survival (OS) spanned from 1 to 201 months, with the midpoint recorded at 16.5 months.

Notably, 45.9% of the patients had concurrent chronic diseases, yet the underlying cause of death in all cases was the relentless progression of cancer. Table 2 offers a comprehensive breakdown of clinicopathological features.

Table 2. *General clinicopathological characteristics and frequencies of 172 study participants.*

Variables	Subgroups	Frequencies (count/%)
Age groups (years) (mean ± SD: 55.4 ± 13.5)	≥ 50	123/71.5%
	< 50	49/28.5%
Histology	Squamous	157/92.3%
	Non-squamous	15/8.7%
Tumor size (T)	T1A	1/0.6%
	T1B	25/14.6%
	T2A	4/2.3%
	T2B	80/46.5%
	T3A	13/7.6%
	T3B	38/22.1%
	T3C	4/2.3%
	T4A	4/2.3%
	T4B	3/1.7%
Tumor size (groups)	T1–T2	110/63.9%
	T3–T4	62/36.1%
Pathological regional lymph nodes status	N0	95/55.2%
	N1	77/44.8%
Paraaortic lymph node metastasis present	No	163/94.8%
	Yes	9/5.2%
Distant metastasis	M0	162/94.2%
	M1	10/5.8%
Stage	IA	1/0.6%
	IB	15/8.7%
	IIA	5/2.9%
	IIB	55/32.0%
	IIIA	9/5.2%
	IIIB	12/7.0%
	IIIC1	53/31.0%
	IIIC2	9/5.2%
	IVA	3/1.7%
	IVB	10/5.8%
Stage (groups)	I–II	76/44.2%
	III–IV	96/55.8%
Grade	1	13/7.6%
	2	113/65.7%
	3	46/26.7%

Table 2. *Continued.*

Variables	Subgroups	Frequencies (count/%)
Disease progression	No	120/69.8%
	Yes	52/30.2%
Death	No	132/76.7%
	Yes	40/23.3%

T1 + T2 – smaller tumor size; T3 + T4 – larger tumor size; N0 – no regional lymph node metastasis; N1 – positive regional lymph node metastasis; M0 – no distant metastasis; M1 – positive distant metastasis; G1–G2 – well and moderately differentiated tumor; G3 – poorly differentiated tumor; stage I–II – lower stages; stage III–IV – advanced stages.

3.2. SNP frequencies

In our study, a total of 172 patients underwent genotyping for a set of eight SNPs in the *TLR4* gene (rs10983755, rs10759932, rs11536865, rs4986790, rs4986791, rs11536897, rs1927906, and rs11536898), five in *RRP1B* (rs2838342, rs7276633, rs2051407, rs9306160, and rs762400), three in *SIPA1* (rs746429, rs931127, and rs3741378), five in *SRSF1* (rs8819, rs34592492, rs11654058, rs2233908, and rs2585828), three in *HOTAIR* (rs12826786, rs7958904, and rs920778), and three in *MALAT1* (rs619586, rs664589, and rs3200401). Among these, the *RRP1B* SNP rs9306160 was identified in 169 cases, with three cases excluded due to non-amplification. It is noteworthy that almost all of the SNPs examined were found to be in accordance with Hardy-Weinberg equilibrium, as indicated by *p*-values exceeding 0.05. However, *TLR4* rs619586 showed a strong deviation from the expected frequencies under Hardy-Weinberg equilibrium, particularly with a complete absence of the C allele. However, we decided to include those SNPs in association analyses.

Upon comparing the allele frequencies determined within our cohort to those of the European population data from the 1000 Genomes Project, we detected slight, yet statistically not significant, disparities in the minor allele frequencies (MAF) for twenty-six of the SNPs, with *p*-values > 0.05. It should be noted that the allele distribution in our sample differs significantly from the European population data in the case of *SRSF1* SNP rs746429 ($p < 0.05$), likely due to our sample consisting of cancer patients. Comprehensive details regarding genotype and allele frequencies can be found in Table 3.

Table 3. *The distribution of genotypes and alleles.*

Gene	SNP	Sample size N (study cohort)	Geno-types	Genotypes frequencies		Alleles	Alleles frequencies (study cohort)	Sample size N (1000 Genomes)	Alleles frequencies (1000 Genomes)	MAF *p*-value[a]
TLR4	rs10983755 G > A	172	GG GA AA	159/92.4% 13/7.56% 0/0%	0.924 0.076 0	G A	0.962 0.038	1006	0.959 0.041	0.854
	rs10759932 T > C	172	TT TC CC	121/70.4% 47/27.3% 4/2.3%	0.704 0.273 0.023	T C	0.841 0.159	1006	0.847 0.153	0.931
	rs11536865 G > C	172	GG GC CC	172/100% 0/0% 0/0%	1.000 0 0	G C	1.000 0.000	1006	0.998 0.002	1.0
	rs4986790 A > G	172	AA AG GG	148/86.0% 23/13.4% 1/0.6%	0.860 0.134 0.060	A G	0.927 0.073	1006	0.943 0.057	0.521
	rs4986791 C > T	172	CC CT TT	147/85.4% 24/14.0% 1/0.6%	0.854 0.140 0.006	C T	0.924 0.076	1006	0.942 0.058	0.459
	rs11536897 G > A	172	GG GA AA	165/95.9% 7/4.1% 0/0%	0.959 0.041 0	G A	0.979 0.021	1006	0.948 0.052	0.077
	rs1927906 T > C	172	TT TC CC	133/77.3% 38/22.1% 1/0.6%	0.773 0.221 0.006	T C	0.883 0.117	1006	0.899 0.101	0.616
	rs11536898 C > A	172	CC CA AA	129/75.0% 38/22.1% 5/2.9%	0.750 0.221 0.029	C A	0.861 0.139	1006	0.889 0.111	0.350

Table 3. *Continued.*

Gene	SNP	Sample size N (study cohort)	Geno-types	Genotypes frequencies		Alleles	Alleles frequencies (study cohort)	Sample size N (1000 Genomes)	Alleles frequencies (1000 Genomes)	MAF *p*-value[a]
RRP1B	rs2838342 A>G	172	AA AG GG	58/33.7% 85/49.4% 29/16.9%	0.337 0.494 0.169	A G	0.584 0.416	1006	0.589 0.420	0.791
	rs7276633 T>C	172	TT TC CC	59/34.3% 84/48.8% 29/16.9%	0.343 0.488 0.169	T C	0.587 0.413	1006	0.581 0.420	0.676
	rs2051407 C>T	172	CC CT TT	63/36.6% 84/48.8% 25/14.5%	0.366 0.489 0.145	C T	0.610 0.390	1006	0.634 0.366	0.282
	rs9306160 * C>T	169	CC CT TT	55/32.5% 92/54.4% 22/13.0%	0.325 0.545 0.130	C T	0.598 0.402	1006	0.617 0.383	0.450
	rs762400 G>C	172	GG GC CC	63/26.6% 88/51.2% 21/12.2%	0.366 0.512 0.122	G C	0.622 0.378	1006	0.626 0.374	0.804
SIPA1	rs746429 G>A	172	GG GA AA	45/26.2% 89/51.7% 38/22.1%	0.262 0.517 0.221	G A	0.520 0.480	1006	0.643 0.357	0.021
	rs931127 A>G	172	AA AG GG	65/37.8% 86/50.0% 21/12.2%	0.378 0.500 0.122	A G	0.628 0.372	1006	0.563 0.437	0.111
	rs3741378 C>T	172	CC CT TT	138/80.2% 30/17.4% 4/2.3%	0.802 0.175 0.023	C T	0.890 0.110	1006	0.869 0.131	0.523

Table 3. *Continued.*

Gene	SNP	Sample size N (study cohort)	Geno-types	Genotypes frequencies		Alleles	Alleles frequencies (study cohort)	Sample size N (1000 Genomes)	Alleles frequencies (1000 Genomes)	MAF *p*-value[a]
SRSF1	rs8819 C>T	172	CC CT TT	124/72.1% 43/25.0% 5/2.9%	0.721 0.250 0.029	C T	0.846 0.154	1006	0.839 0.161	0.905
	rs34592492 G>C	172	GG GC CC	155/90.1% 16/9.3% 1/0.6%	0.901 0.093 0.006	G C	0.948 0.052	1006	0.944 0.056	0.975
	rs11654058 T>C	172	TT TC CC	141/82% 28/16.3% 3/1.7%	0.820 0.163 0.017	T C	0.901 0.099	1006	0.902 0.098	1.0
	rs2233908 G>A	172	GG GA AA	124/72.1% 43/25.0% 5/2.9%	0.721 0.250 0.029	G A	0.846 0.154	1006	0.840 0.160	0.842
	rs2585828 A>G	172	AA AG GG	124/72.1% 43/25.0% 5/2.9%	0.721 0.250 0.029	A G	0.846 0.154	1006	0.840 0.160	0.842
HOTAIR	rs12826786 C>T	172	CC CT TT	74/43.0% 77/44.8% 21/12.2%	0.430 0.448 0.122	C T	0.654 0.346	1006	0.705 0.295	0.178
	rs7958904 G>C	172	GG GC CC	62/36.0% 88/51.2% 22/12.8%	0.360 0.512 0.128	G C	0.616 0.384	1006	0.697 0.303	0.055
	rs920778 A>G	172	AA GA GG	62/36.0% 88/51.2% 22/12.8%	0.360 0.512 0.128	A G	0.616 0.384	1006	0.697 0.303	0.055

Table 3. *Continued.*

Gene	SNP	Sample size N (study cohort)	Geno-types	Genotypes frequencies		Alleles	Alleles frequencies (study cohort)	Sample size N (1000 Genomes)	Alleles frequencies (1000 Genomes)	MAF *p*-value[a]
MALAT1	rs619586 A>G	172	AA AG GG	164/95.3% 8/4.7% 0/0%	0.953 0.046 0	A G	0.978 0.022	1006	0.971 0.029	0.638
	rs664589 C>G	172	CC CG GG	164/95.3% 7/4.1% 1/0.6%	0.953 0.407 0.058	C G	0.974 0.026	1006	0.971 0.029	0.955
	rs3200401 C>T	172	CC CT TT	132/76.7% 38/22.1% 2/1.2%	0.767 0.221 0.012	C T	0.878 0.122	1006	0.798 0.202	0.122

*Genotypes were determined among 169 patients due to non-amplification in three cases. a A chi-squared test for independence analysis for the number of each minor allele in cases and controls.

3.3. Linkage disequilibrium and haplotypes distribution

In our analysis of linkage disequilibrium (LD) among the SNPs in the *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR*, and *MALAT1* genes, we calculated two commonly used measures: D' and r^2.

3.3.1. *TLR4* gene

The combination of numerical data and color coding in Figure 9 provides a comprehensive overview of the LD structure among TLR4 SNPs. This analysis is crucial for understanding the genetic architecture of the TLR4 gene, as well as its potential implications in disease susceptibility and phenotypic variation. LD measure D' (a): D' values range from 0 to 1, with values closer to 1 indicating a higher level of linkage disequilibrium due to minimal recombination between the SNPs. The color gradient used in the figure for D' provides a visual assessment of the recombination frequency between SNP pairs. Darker shades indicate higher D' values, suggesting that the SNPs are closely linked and likely to be inherited together. Note that D' values of 1.0 are not depicted in the figure; the corresponding boxes are left empty. LD measure r^2 (b): The r^2 values also range from 0 to 1, where a value closer to 1 indicates a stronger correlation between alleles at two loci. In the figure, darker shades represent higher r^2 values, highlighting SNP pairs with the strongest linkage disequilibrium. This visualization helps identify regions where alleles are more likely to be inherited together, which could be significant for understanding genetic linkage and identifying potential areas of interest for further genetic studies. Figure 9 also provides insight into LD Block 1 within the TLR4 gene. The algorithm used to identify LD blocks is based on the method described by Gabriel et al. [690]. This approach generates 95% confidence bounds on D' values, classifying each comparison as 'strong LD' when the confidence bounds suggest minimal recombination between SNPs. LD blocks are defined as regions where SNPs exhibit high linkage disequilibrium with each other, indicating that these regions are likely inherited together due to low recombination rates. D' values within this block are consistently high, ranging from 0.95 to 1.0, suggesting minimal recombination. Additionally, r^2 values within this block range from 0.6 to 0.95, reflecting moderate to strong linkage disequilibrium.

Table 4 presents the frequencies of different TLR4 haplotypes based on the SNPs rs4986790, rs4986791, and rs1927906. The "ACT" haplotype was the most common, observed in 304 chromosomes, accounting for 88.37% of the total haplotypes. Its high frequency suggests that "ACT" is the predominant haplotype in the studied population. The "ACT" haplotype was less

common, with 14 occurrences, representing 4.07% of the total. The “ATC” haplotype was extremely rare, observed only once, constituting 0.29% of the total. Its rarity indicates it has a minimal impact on the overall genetic makeup of the population. The “GTC” haplotype was present in 25 chromosomes, making up 7.27% of the total haplotypes. Although less common than “ACT”, it is more frequent than “ATC” and “ACC”. For further analysis of associations, we focused on the most common haplotype observed in our study, namely “ACT”.

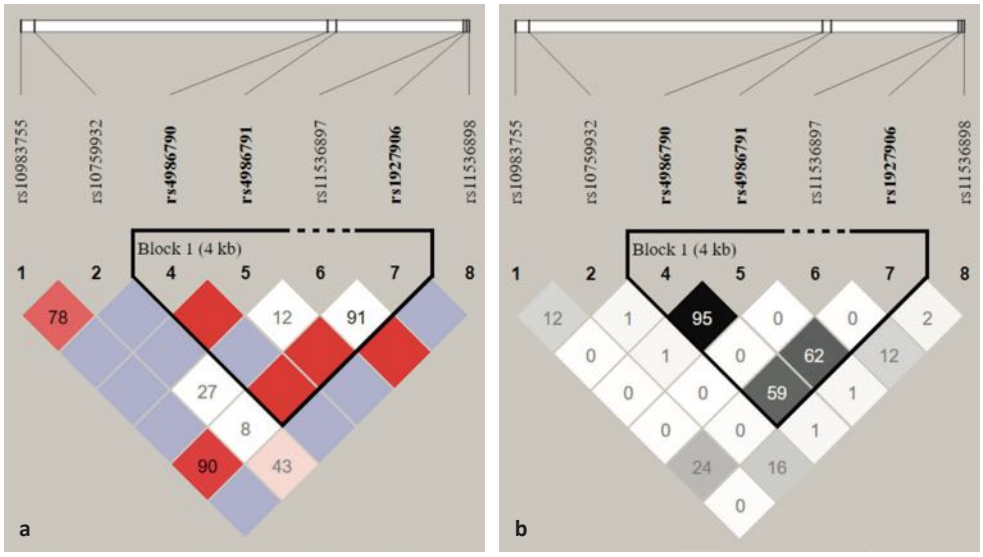

Figure 9. *The LD data for TLR4 single nucleotide polymorphisms includes numerical values and color coding for both r^2 and D', providing insights into the linkage disequilibrium.*

Pairwise linkage disequilibrium (LD) pattern for *TLR4* polymorphisms in cervical cancer patients. **(a)** The color LD plot indicates the strength of D', with dark red representing strong LD; **(b)** The color LD plot indicates the strength of r^2, with dark grey representing strong LD.

Table 4. *TLR4 haplotypes and their frequencies.*

Haplotype number	rs4986790 rs4986791 rs1927906 haplotypes	Chromosomes (counts)	Frequencies (%)
1	ACT	304	88.37
2	ACC	14	4.07
3	ATC	1	0.29
4	GTC	25	7.27

Four haplotypes were generated from the analyzed SNPs hrough the Phase software v2.1.

3.3.2. *RRP1B* gene

For D', the mean value was approximately 0.949 ± 0.037, indicating a relatively strong LD on average. The range of D' values varied from a minimum of 0.907 to a maximum of 0.987. Similarly, for r^2, the mean value was approximately 0.802 ± 0.065, suggesting a moderate to high degree of LD on average. The range of r^2 values spanned from a minimum of 0.761 to a maximum of 0.953 (Figure 10). These findings provide insights into the patterns of LD within the *RRP1B* gene, highlighting regions of potential genetic linkage and association. Based on the calculated mean values and the range of D' and r^2 values, it appears that there is a significant level of linkage disequilibrium (LD) among the SNPs in the *RRP1B* gene. The mean values for both D' and linkage disequilibrium r^2 indicate a relatively strong LD on average, and the range of values suggests consistency in LD across the analyzed SNPs. Therefore, it would be reasonable to conclude that LD between these SNPs in the *RRP1B* gene is indeed strong. Given the observed strong linkage disequilibrium (LD) among the SNPs within the *RRP1B* gene, it was decided to include all five SNPs in haplotype analysis. This decision was based on the premise that SNPs in strong LD tend to be inherited together as haplotype blocks, allowing for a more comprehensive understanding of the genetic variations within this genomic region. By analyzing haplotypes constructed from these SNPs, we aimed to capture the collective influence of genetic variations on phenotypic traits or disease susceptibility, thereby enhancing the depth of our genetic investigation.

The results revealed a variety of haplotypes present among the tested individuals. Thirteen haplotypes were identified (Table 5). Among the identified haplotypes, the most prevalent was “ATCCG”, accounting for approximately 55% of the total haplotypes observed. Following closely behind, “GCTTC” constituted around 36% of the haplotypes. Other haplotypes, such as “ATCTG”, “ATCTC”, “GCCTG”, etc., were observed at lower frequencies, each comprising less than 10% of the total haplotypes. The diversity in haplotype composition suggests genetic variability within the *RRP1B* gene region among the studied population. Understanding the distribution of these haplotypes can provide valuable insights into genetic susceptibility, disease association, and population genetics within the context of our research objectives.

For further analysis of associations, we focused on the two most common haplotypes observed in our study, namely “ATCCG” and “GCTTC”.

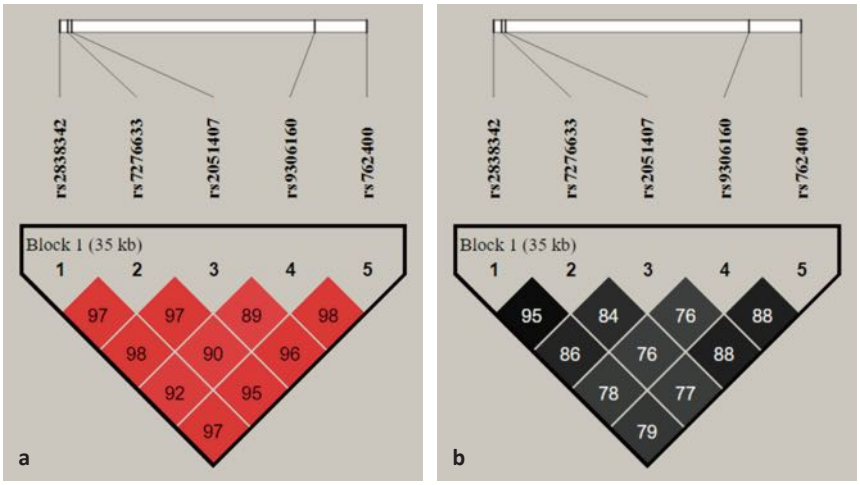

Figure 10. *The LD data for RRP1B single nucleotide polymorphisms includes numerical values and color coding for both r^2 and D', providing insights into the linkage disequilibrium.*

Pairwise linkage disequilibrium (LD) pattern for *RRP1B* polymorphisms in cervical cancer patients. **(a)** The color LD plot indicates the strength of D′, with dark red representing strong LD; **(b)** The color LD plot indicates the strength of r^2, with dark grey representing strong LD.

Table 5. *RRP1B haplotypes and their frequencies.*

Haplotype number	rs2838342 rs7276633 rs2051407 rs9306160 rs762400 haplotypes	Chromosomes (counts)	Frequencies (%)
1	ATCCG	190	55.23
2	ATCTG	5	1.45
3	ATCTC	2	0.58
4	ATTCG	1	0.29
5	ACCCG	2	0.58
6	GTCCG	1	0.29
7	GTTTC	1	0.29
8	GCCCG	4	1.16
9	GCCTG	5	1.45
10	GCCTC	1	0.29
11	GCTCG	6	1.74
12	GCTCC	2	0.58
13	GCTTC	124	36.05

Thirteen haplotypes were generated from the analyzed SNPs through the Phase software v2.1.

3.3.3. *SIPA1* gene

Figure 11 shows that D' values ranged from a minimum of 0.774 to a maximum of 1.0. In contrast, r^2 values spanned from 0.331 to 0.953. A high D' with a low r^2 may suggest that, although the alleles are strongly associated, the overall variance in allele frequencies is low, resulting in a weak correlation. Blocks with haplotypes were not identified and were not used for further analysis.

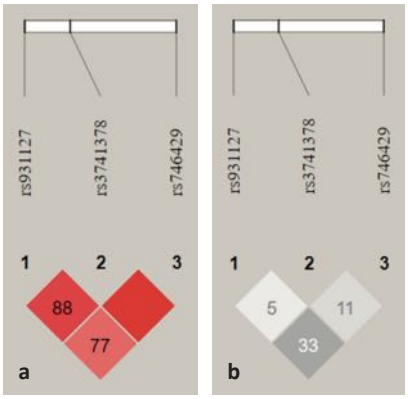

Figure 11. *The LD data for SIPA1 single nucleotide polymorphisms includes numerical values and color coding for both r^2 and D', providing insights into the linkage disequilibrium.*

Pairwise linkage disequilibrium (LD) pattern for *SIPA1* polymorphisms in cervical cancer patients. **(a)** The color LD plot indicates the strength of D', with shades of red representing moderate to strong linkage disequilibrium (LD); **(b)** The color LD plot indicates the strength of r^2, with light grey representing weak linkage disequilibrium (LD).

3.3.4. *SRSF1* gene

Figure 12 shows that D' values varied from 0.922 to 1.0, while r^2 values ranged from 0.005 to 1.0. The block was composed of four SNPs (rs8819, rs11654058, rs2233908, rs2585828). Table 6 reveals that five distinct haplotypes were detected for the SRSF1 gene based on these SNPs. Among these haplotypes, one was notably prevalent.

Haplotype “CTGA” was the most common, observed in 290 chromosomes, which accounts for 84.30% of the total haplotypes. Its high frequency indicates that “CTGA” is the predominant haplotype in the population. Haplotype “TCAG” was less frequent, found in 34 chromosomes, representing 9.89% of the total. Although less common than “CTGA”, “TCAG” still comprises a significant portion of the genetic makeup.

The remaining haplotypes – “CTAG”, “TTGA”, and “TTAG” – were much less frequent, with “CTAG” and “TTGA” each occurring only once (0.29% of the total) and “TTAG” appearing in 18 chromosomes (5.23%). This distribution underscores the dominance of “CTGA” in the population, with a notable presence of “TCAG”, while the rarer haplotypes contribute minimally to the overall genetic diversity.

For further analysis, we used the most common haplotype, “CTGA”.

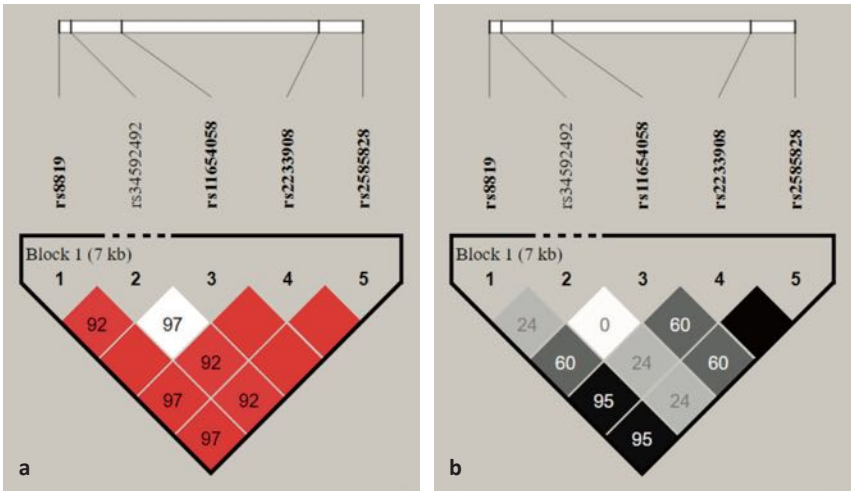

Figure 12. *The LD data for SRSF1 single nucleotide polymorphisms includes numerical values and color coding for both r^2 and D', providing insights into the linkage disequilibrium.*

Pairwise linkage disequilibrium (LD) pattern for *SRSF1* polymorphisms in cervical cancer patients. **(a)** The color LD plot indicates the strength of D', with dark red representing strong linkage disequilibrium (LD). White indicates a low logarithm of the odds (LOD < 2), suggesting weak evidence for linkage; **(b)** The color LD plot indicates the strength of r^2, with white representing weak linkage disequilibrium (LD) and shades of grey and black representing moderate to strong LD.

Table 6. *SRSF1 haplotypes and their frequencies.*

Haplotype number	rs8819 rs11654058 rs2233908 rs2585828 haplotypes	Chromosomes (counts)	Frequencies (%)
1	CTGA	290	84.30
2	CTAG	1	0.29
3	TTGA	1	0.29
4	TTAG	18	5.23
5	TCAG	34	9.89

Five haplotypes were generated from the analyzed SNPs hrough the Phase software v2.1.

3.3.5. *HOTAIR* gene

Analyzing LD between three HOTAIR gene SNPs (rs12826786, rs7958904, rs920778), strong linkage disequilibrium (LD) was detected. D' values varied from 0.972 to 1.0, and r^2 values ranged from 0.812 to 0.988. The block was composed of all three SNPs. LD plots are represented in Figure 13.

Table 7 presents the frequencies of different HOTAIR haplotypes based on the SNPs rs12826786, rs7958904, and rs920778. Among the seven identified haplotypes, two were notably prevalent. Haplotype "CGA" was the most common, observed in 123 chromosomes, accounting for 35.76% of the total haplotypes. Its high frequency suggests that "CGA" is the predominant haplotype in the population. The second most frequent haplotype, "CGG", was found in 88 chromosomes, representing 25.58% of the total. It also plays a significant role in the genetic makeup.

The remaining haplotypes – "TCA", "TCG", "CCA", "TGA", and "CCG" – were less frequent, varying from 0.29% to 21.51%, respectively.

This distribution underscores the dominance of "CGA" and "CGG" haplotypes in the population, with the other haplotypes contributing to the overall genetic diversity. These haplotypes were utilized in further analysis.

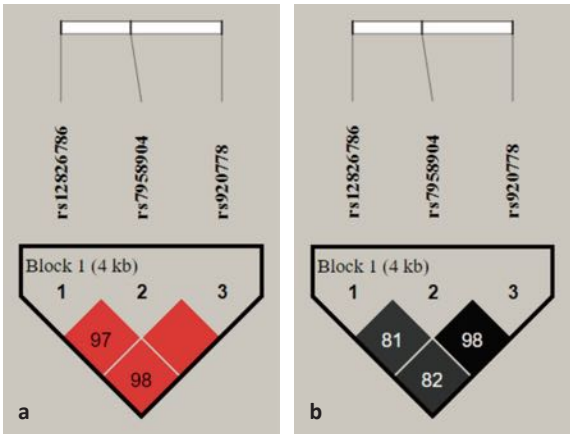

Figure 13. *The LD data for HOTAIR single nucleotide polymorphisms includes numerical values and color coding for both r^2 and D', providing insights into the linkage disequilibrium.*

Pairwise linkage disequilibrium (LD) pattern for *HOTAIR* polymorphisms in cervical cancer patients. **(a)** The color LD plot indicates the strength of D′, with dark red representing strong LD; **(b)** The color LD plot indicates the strength of r^2, with dark grey representing strong LD.

Table 7. *HOTAIR haplotypes and their frequencies.*

Haplotype number	rs12826786 rs7958904 rs920778 haplotypes	Chromosomes (counts)	Frequencies (%)
1	CGG	88	25.58
2	CGA	123	35.76
3	CCG	1	0.29
4	CCA	13	3.78
5	TGA	2	0.58
6	TCG	43	12.50
7	TCA	74	21.51

Seven haplotypes were generated from the analyzed SNPs hrough the Phase software v2.1.

3.3.6. *MALAT1* gene

Figure 14 represents LD between three SNPs in the MALAT1 gene, which were identified as weak. D' values ranged from 0.653 to 1.0, while r^2 values ranged from 0.004 to 0.383. Haplotype blocks were not identified and, therefore, were not used for further analysis.

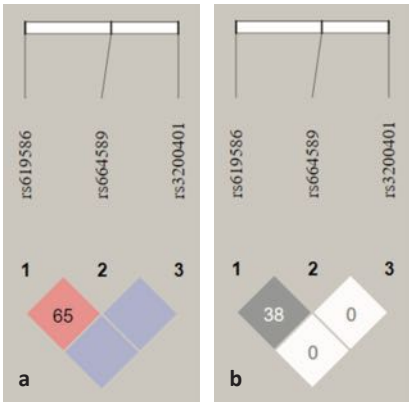

Figure 14. *The LD data for MALAT1 single nucleotide polymorphisms includes numerical values and color coding for both r^2 and D', providing insights into the linkage disequilibrium.*

Pairwise linkage disequilibrium (LD) pattern for *MALAT1* polymorphisms in cervical cancer patients. **(a)** The color LD plot indicates the strength of D', with light red representing moderate linkage disequilibrium (LD). Bright blue indicates a low logarithm of the odds (LOD < 2), suggesting weak evidence for linkage; **(b)** The color LD plot indicates the strength of r^2, with white and bright grey representing week LD.

3.4. Association analysis

3.4.1. *TLR4* gene

3.4.1.1. *TLR4* gene: focus on SNPs rs10759932, rs1927906, rs11536865, rs10983755, rs4986790, rs4986791, and rs11536897

In our study, we analyzed potential interactions between SNPs and tumor clinicopathological features. However, no statistically significant correlations were found between rs10759932, rs1927906, rs11536865, rs10983755, rs4986790, rs4986791, rs11536897, and tumor size, node status, metastases, tumor cell differentiation, stage, or prognosis using logistic regression. Additionally, none of the analyzed polymorphisms were related to the patients' age at diagnosis ($p > 0.05$). All the results represented in Supplementary Table 1 and Supplementary Table 2.

3.4.2.1. *TLR4* gene: focus on SNP rs11536898

However, no statistically significant correlations between rs11536898 and tumor size, nodal status, tumor cell differentiation, stage, prognosis, or patient age were found using logistic regression. Nevertheless, we detected a significant association between SNP rs11536898 and metastasis (M). Carrying the A allele significantly increased the likelihood of having metastasis (OR = 5.068, 95% CI: 1.357–18.918, $p = 0.008$). This finding was partially corroborated by the genotype model, as patients with the CA genotype had a 4.735 times higher risk of distal metastases compared to patients with the CC genotype (95% CI: 1.204–18.626, $p = 0.026$). It is important to note that only five patients with the AA genotype were identified in our study, which may have influenced the *p*-value in this comparison.

Furthermore, the multivariate logistic regression analysis confirmed the significant link between rs11536898 and metastasis. In the multivariate analysis (Model No. 1), the CA genotype significantly increased the risk of metastasis (OR = 4.609, 95% CI: 1.166–18.212, $p = 0.029$), and the A allele increased the risk of metastasis (OR = 5.044, 95% CI: 1.346–18.899, $p = 0.016$) when adjusted for patient age at diagnosis. This relationship remained statistically significant when adjusted for both age at diagnosis and tumor differentiation (G) (Model No. 2): the CA genotype significantly increased the risk of metastasis (OR = 4.419, 95% CI: 1.111–17.576, $p = 0.035$), and the A allele increased the risk of metastasis (OR = 4.884, 95% CI: 1.297–18.392, $p = 0.019$).

We did not include tumor size and nodal status in the multivariate models due to complete data separation. All results are detailed in Table 8, Supplementary Table 1, and Supplementary Table 2.

Table 8. *Univariate and multivariate logistic regression analyses were conducted for rs11536898, adjusting for genotypes, alleles, and clinicopathological characteristics, with a focus on metastasis.*

SNP	Dependent	Covariates	Univariate			Multivariate					
						Model No. 1			Model No. 2		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs11536898	Positive M	CA *vs.* CC	4.735	1.204–18.626	**0.026**	4.609	1.166–18.212	**0.029**	4.419	1.111–17.576	**0.035**
		AA *vs.* CC	7.812	0.704–86.710	0.094	9.452	0.803–111.217	0.074	9.871	0.827–117.76	0.070
		Age (years)				0.977	0.928–1.028	0.370	0.977	0.928–1.029	0.376
		Possitive G3 *vs.* G1 + G2							1.729	0.445–6.716	0.429
rs11536898	Positive M	A allele+ *vs.* A–	5.068	1.357–18.918	**0.008**	5.044	1.346–18.899	**0.016**	4.884	1.297–18.392	**0.019**
		Age (years)				0.979	0.931–1.030	0.415	0.980	0.932–1.030	0.426
		Positive G3 *vs.* G1 + G2							1.670	0.433–6.439	0.456

Bolded *p*-values indicate statistical significance ($p < 0.05$).

3.4.2. *RRP1B* gene

All the examined polymorphisms exhibited statistically significant associations with the clinical manifestations of cervical cancer. However, we did not find any statistically significant associations between SNPs and nodal involvement or tumor differentiation. On the other hand, all the polymorphisms were linked to tumor size or metastasis. Furthermore, some of them appeared to influence cancer stage and prognosis. The tabulated results furnish us with a trove of statistical insights. This meticulous analysis unveils the intriguing associations between specific SNPs and an array of vital tumor characteristics, offering a multifaceted perspective on the clinical attributes of cervical.

3.4.2.1. *RRP1B* gene: focus on SNP rs2838342

The analysis of SNP rs2838342 yielded noteworthy results. According to the univariate logistic regression analysis, individuals with the presence of the A allele (A allele+) exhibited a significantly lower odds ratio (OR) of 0.281 (95% CI: 0.122–0.643, $p = 0.002$) for advanced tumor size (T3–T4) when compared to those with its absence (A−), indicating a significantly reduced likelihood of advanced tumor size (T3–T4). In the multivariate logistic regression analyses across four models, the A allele was consistently associated with significantly lower odds of larger tumor size, with an OR of 0.280 (95% CI: 0.122–0.643, $p = 0.003$) in the presence of patient age at diagnosis (Model No. 1). Model No. 2 introduced additional covariates (age at diagnosis and tumor differentiation grade) and continued to demonstrate a consistent association between rs2838342 and the tumor size (OR = 0.299, 95% CI: 0.129–0.692, $p = 0.005$). However, a possible trend emerged where G3 was associated with higher odds of larger tumor size (OR = 1.991, 95% CI: 0.978–4.051, $p = 0.058$). Model No. 3 expanded the analysis to include the presence of regional lymph node involvement (N1 *vs.* N0). In this model, rs2838342 remained associated with tumor size (OR = 0.244, 95% CI: 0.096–0.619, $p = 0.003$), but the addition of N1 as a covariate substantially increased the odds of larger tumor size (OR = 7.367, 95% CI: 3.347–16.217, $p < 0.001$). Model No. 4 further extended the analysis to consider the presence of distant metastasis (M1 *vs.* M0), further supporting a significant relationship (OR = 0.266, 95% CI: 0.102–0.691, $p = 0.007$). Throughout all these models, the association between rs2838342 and tumor size persisted. In summary, these multivariate logistic regression analyses, while adjusting for covariates, reveal a robust and consistent association between the presence of at least one A allele and a lower risk of larger tumor size. In genotypic models, the GG

genotype showed an increased odds ratio of 2.160 (95% CI: 0.867–5.380). Nevertheless, this difference did not reach statistical significance ($p = 0.098$).

The univariate logistic regression analysis revealed that the A allele significantly reduced the odds of having distant metastasis (OR = 0.274, 95% CI: 0.072–1.040, $p = 0.044$). Conversely, when comparing the presence of at least one G allele to having none (G allele+ *vs.* G–), the OR was 1.199, indicating slightly higher odds of having distant metastasis. However, this association was not statistically significant ($p = 0.798$). Across all multivariate models for the A allele+ *vs.* A− comparison, when adjusting for age, G, N, and tumor size, the OR suggests a potential protective effect of the A allele in reducing the risk of distant metastasis. Unfortunately, statistical significance was limited (all cases p-value > 0.05). These results provide preliminary evidence that the A allele of rs2838342 might play a protective role against the development of distant metastasis.

In the analysis, focused on a worse prognosis group, characterized by T3–T4 tumor stages and the G3 tumor grade, the A allele of rs2838342 significantly reduces the likelihood of a worse prognosis (T3–T4 + G3) compared to those with its absence (A–), with an OR of 0.182. The 95% CI spans from 0.061 to 0.538, and the p-value is a strikingly low 0.002. The GG genotype of rs2838342 presents a notably high OR (3.000) for a worse prognosis (T3–T4 + G3) when compared to the AA genotype. Although the p-value (0.071) suggests a potential association, it did not reach conventional significance levels. Similarly, the G allele of rs2838342 does not significantly impact the likelihood of a worse prognosis, as reflected in the wide 95% CI from 0.274 to 1.847 and a p-value of 0.485.

While the genotypes did not show a significant association in the comparison of positive stages III–IV *vs.* stages I–II, allelic comparisons provided additional insights. The A allele demonstrated a substantially lower odds ratio (OR = 0.341, 95% CI = 0.137–0.849, $p = 0.017$), suggesting a potential protective effect in the context of advanced cancer stages. Conversely, the G allele did not exhibit a statistically significant association ($p = 0.239$).

When considering age as a dichotomous variable (≤ 50 *vs.* > 50 years), those carrying the AG genotype had a reduced risk of developing cervical cancer before the age of 50 (OR = 0.471, 95% CI: 0.226–0.983, $p = 0.045$).

These findings underscore the potential relevance of the rs2838342 SNP in influencing the progression and severity of cervical cancer, particularly in the transition from early to advanced stages. The protective effect associated with specific genotypes and alleles implies a potential role for rs2838342 as a prognostic marker in cervical cancer patients. All the results are presented in Tables 9, Table 10, Supplementary Table 3, and Supplementary Table 4.

Table 9. *Univariate and multivariate logistic regression analyses were conducted for rs2838342, adjusting for alleles, and clinicopathological characteristics, with a focus on tumor size and metastasis.*

SNP	Dependent	Covariates	Univariate			Multivariate											
						Model No. 1			Model No. 2			Model No. 3			Model No. 4		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs2838342	Positive T3–T4	A allele+ *vs.* A–	0.281	0.122–0.643	**0.002**	0.280	0.122–0.643	**0.003**	0.299	0.129–0.692	**0.005**	0.244	0.096–0.619	**0.003**	0.266	0.102–0.691	**0.007**
		Age (years)				1.001	0.978–1.025	0.909	1.002	0.979–1.027	0.842	1.028	1.000–1.057	0.054	1.027	0.999–1.056	0.059
		Positive G3 *vs.* G1 + G2							1.991	0.978–4.051	0.058	1.798	0.826–3.914	0.140	1.687	0.762–3.732	0.197
		Positive N1 *vs.* N0										7.367	3.347–16.217	< 0.001	6.161	2.756–13.771	< 0.001
		Positive M1 *vs.* M0													5.977	0.690–51.748	0.105
rs2838342	Positive M	A allele+ *vs.* A–	0.274	0.072–1.040	**0.044**	0.272	0.071–1.140	0.057	0.291	0.075–1.127	0.074	0.267	0.063–1.136	0.074	0.521	0.124–2.190	0.374
		Age (years)				0.979	0.932–1.028	0.395	0.980	0.933–1.029	0.417	1.009	0.960–1.061	0.714	0.978	0.927–1.031	0.401
		Positive G3 *vs.* G1 + G2							1.603	0.416–6.170	0.493	1.293	0.315–5.308	0.721	1.130	0.276–4.633	0.865
		Positive N1 *vs.* N0										0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4													15.623	1.853–131.722	0.012

Bolded *p*-values indicate statistical significance ($p < 0.05$).

Table 10. *Univariate logistic regression analysis: assessing odds ratios for the relationships between RRP1B SNPs rs2838342, rs7276633, rs2051407, and patients' age, cancer stage groups, and disease prognosis.*

SNP	Genotype, alleles	Age (groups): ≤ 50 *vs.* > 50			Positive stage III–IV *vs.* stage I–II			Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2		
		OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
rs2838342	AG *vs.* AA	0.471	0.226–0.983	**0.045**						
	GG *vs.* AA									
	A allele+ *vs.* A–				0.341	0.137–0.849	**0.017**	0.182	0.061–0.538	**0.002**
	G allele+ *vs.* G–									
rs7276633	TC *vs.* TT									
	CC *vs.* TT									
	T allele + *vs.* T–				0.341	0.137–0.849	**0.021**	0.182	0.061–0.538	**0.002**
	C allele + *vs.* C–	2.138	1.080–4.230	**0.029**						
rs2051407	CT *vs.* CC									
	TT *vs.* CC									
	C allele + *vs.* C–							0.267	0.087–0.823	**0.021**
	T allele + *vs.* T–									

Bolded *p*-values indicate statistical significance ($p < 0.05$).

3.4.2.2. *RRP1B* gene: focus on SNP rs7276633

The carriers of the T allele in rs7276633 were significantly associated with a decreased risk of falling into the higher tumor size category (T3–T4), with an odds ratio (OR) of 0.281 (95% CI = 0.122–0.643, $p = 0.003$). Moving on to the multivariate logistic regression analysis, the findings remain consistent across all four models. In multivariate Model No. 1, the presence of the T allele (+) is significantly associated with a reduced risk of having a higher tumor size compared to the absence of the T allele (−). This association is statistically significant with an odds ratio (OR) of 0.280 and a 95% confidence interval (CI) of 0.122–0.643 ($p = 0.003$). This association persists in Model No. 2, demonstrating a significant reduction in the odds of higher tumor size (OR = 0.299, 95% CI: 0.129–0.692, $p = 0.005$). Models No. 3 and 4 also support this finding, with ORs of 0.277 and 0.264 (95% CI: 0.125–0.708, $p = 0.003$; 95% CI: 0.147–0.893, $p = 0.007$, respectively). Conversely, the C allele of rs7276633 did not exhibit a significant association with tumor size ($p = 0.145$). Otherwise, the trend of the CC genotype compared to the TT genotype showing an increased risk of higher tumor size remained consistent across all models, although this association was non-significant.

Patients with the presence of the T allele (+) were significantly associated with a reduced risk of higher tumor stage (III–IV) (OR = 0.341, 95% CI: 0.137–0.849, $p = 0.021$) and worse prognosis (T3–T4 + G3) (OR = 0.182, 95% CI: 0.061–0.538, $p = 0.002$), while the C allele did not exhibit significant associations with the parameters studied. In conclusion, these findings imply that the T allele of rs7276633 might confer a protective effect against advanced tumor size and prognosis. Moreover, carriers of the C allele were at a higher risk of developing the disease at an age younger than 50 years (OR = 2.138, 95% CI: 1.080–4.230, $p = 0.029$). The results are presented in Tables 10 and 11, Supplementary Table 3, and Supplementary Table 4.

Table 11. *Univariate and multivariate logistic regression analyses were conducted for rs7276633, adjusting for alleles and clinicopathological characteristics, with a focus on tumor size.*

SNP	Dependent	Covariates	Univariate			Multivariate											
						Model No. 1			Model No. 2			Model No. 3			Model No. 4		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs7276633	Positive T3–T4	T allele+ *vs.* T–	0.281	0.122–0.643	**0.003**	0.280	0.122–0.643	**0.003**	0.299	0.129–0.692	**0.005**	0.277	0.125–0.708	**0.003**	0.264	0.147–0.893	**0.007**
		Age (years)				1.001	0.978–1.025	0.909	1.002	0.979–1.027	0.842	1.028	1.000–1.057	0.054	1.027	0.999–1.056	0.059
		Positive G3 *vs.* G1 + G2							1.991	0.978–4.051	0.058	1.798	0.826–3.914	0.140	1.687	0.762–3.732	0.197
		Positive N1 *vs.* N0										7.367	3.347–16.217	< 0.001	6.161	2.756–13.771	< 0.001
		Positive M1 *vs.* M0													5.977	0.690–51.748	0.105

Bolded *p*-values indicate statistical significance ($p < 0.05$).

3.4.2.3. *RRP1B* gene: focus on SNP rs2051407

There were no significant associations between genotypes and clinicopathomorphological features. However, the presence of the C allele (+) was associated with a decreased risk of having a larger tumor (T3–T4) compared to those without the C allele (C–) (OR 0.393, 95% CI: 0.166–0.929, p = 0.033). This association was consistent and statistically significant across three multivariate analysis models, when the adjustment of age and tumor clinicopthatological features was made (Model No. 1: OR 0.392, 95% CI: 0.166–0.928, p = 0.033; Model No. 2: OR 0.414, 95% CI: 0.173–0.992, p = 0.048; Model No. 3: OR 0.354, 95% CI: 0.134–0.930, p = 0.035, respectively). But Model No.4 shows that the association did not reach statistical significance (OR 0.409, 95% CI: 0.149–1.123, p = 0.083).

In the univariate logistic regression analysis, investigating the association between alleles and metastasis, carrying the C allele significantly decreased the chance of having metastasis, with an OR of 0.223 (95% CI: 0.058–0.858) and a p-value of 0.019. These findings suggest that the presence of the C allele may serve as a protective factor against metastasis. In multivariate analysis, Models No. 1, 2, and 3 showed a consistent association between the presence of the C allele and a reduced risk of metastasis (p = 0.030, p = 0.038, p = 0.037, respectively). However, Model No. 4 did not yield significant results for this polymorphism, when the adjustment of age, G, N, and T was made. Tumor stage (T3–T4) was consistently identified as a significant predictor of metastasis in all models.

The presence of the C allele was associated with a significantly reduced risk of transitioning to a worse prognosis disease (T3–T4 + G3), as evidenced by an OR of 0.267 (95% CI: 0.087–0.823, p = 0.021), suggesting that it may serve as a protective factor. These findings highlight the potential influence of this SNP on the expected prognosis of the disease. The results are presented in Tables 10 and 12, Supplementary Table 3, and Supplementary Table 4.

Table 12. *Univariate and multivariate logistic regression analyses were conducted for rs2051407, adjusting for alleles and clinicopathological characteristics, with a focus on tumor size and metastasis.*

SNP	Dependent	Covariates	Univariate			Multivariate											
						Model No. 1			Model No. 2			Model No. 3			Model No. 4		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs2051407	Positive T3–T4	C allele+ *vs.* C–	0.393	0.166–0.929	**0.033**	0.392	0.166–0.928	**0.033**	0.414	0.173–0.992	**0.048**	0.354	0.134–0.930	**0.035**	0.409	0.149–1.123	0.083
		Age (years)				1.002	0.978–1.025	0.890	1.003	0.979–1.027	0.820	1.028	1.000–1.057	0.052	1.027	0.000–1.055	0.060
		Positive G3 *vs.* G1 + G2							2.067	1.027–4.161	0.042	1.885	0.877–4.050	0.104	1.803	0.827–3.928	0.138
		Positive N1 *vs.* N0										6.993	3.233–15.125	< 0.001	5.795	2.639–12.726	< 0.001
		Positive M1 *vs.* M0													6.116	0.713–52.493	0.099
rs2051407	Positive M	C allele + *vs.* C–	0.223	0.058–0.858	**0.019**	0.223	0.058–0.863	**0.030**	0.236	0.060–0.920	**0.038**	0.209	0.048–0.913	**0.037**	0.355	0.083–1.510	0.355
		Age (years)				0.979	0.931–1.029	0.403	0.980	0.932–1.031	0.432	1.007	0.957–1.059	0.801	0.979	0.926–1.034	0.443
		Positive G3 *vs.* G1 + G2							1.627	0.421–6.285	0.480	1.307	0.315–5.429	0.712	1.088	0.262–4.514	0.908
		Positive N1 *vs.* N0										0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4													15.475	1.852–129.314	0.011

Bolded *p*-values indicate statistical significance ($p < 0.05$).

3.4.2.4. *RRP1B* gene: focus on SNP rs9306160

The analysis suggests that the rs9306160 SNP may have a significant protective effect against metastasis, as indicated by the statistically significant result for the C allele (+) (OR = 0.179, 95% CI: 0.044–0.721, $p = 0.008$). There was no significant association between the CT genotype and the presence of metastasis. However, for the TT genotype compared to CC, the OR was 5.889 (95% CI: 0.993–34.906) with a p-value close to the significance threshold at 0.051. This implies a potential trend towards an increased risk of metastasis for the TT genotype. In the multivariate logistic regression analysis for metastasis (M), Model No. 1 showed that the presence of the C allele (+) significantly reduced risk of metastasis (OR = 0.187, 95% CI: 0.046–0.760, $p = 0.019$). Model No. 2 continued to show a protective effect the C allele (+) with an OR of 0.166 (95% CI: 0.039–0.702, $p = 0.015$). In Model No. 3 the C allele (+) still exhibited a protective effect (OR = 0.151, 95% CI: 0.032–0.717, $p = 0.017$). This confirms the significantly reduced risk of metastasis associated with the C allele. However, in Model No. 4, the protective effect is not statistically significant, while there is a protective trend. The results are presented in Table 13, Supplementary Table 3, and Supplementary Table 4.

Table 13. *Univariate and multivariate logistic regression analyses were conducted for rs9306160, adjusting for alleles and clinicopathological characteristics, with a focus on metastasis.*

SNP	Dependent	Covariates	Univariate			Multivariate											
						Model No. 1			Model No. 2			Model No. 3			Model No. 4		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs9306160	Positive M	C allele+ *vs.* C–	0.179	0.044–0.721	**0.008**	0.187	0.046–0.760	**0.019**	0.166	0.039–0.702	**0.015**	0.151	0.032–0.717	**0.017**	0.262	0.059–1.170	0.079
		Age (years)				0.979	0.929–1.032	0.430	0.981	0.931–1.034	0.479	1.002	0.949–1.059	0.932	0.977	0.921–1.036	0.437
		Positive G3 *vs.* G1 + G2							2.623	0.629–10.932	0.186	2.193	0.482–9.992	0.310	1.581	0.363–6.897	0.542
		Positive N1 *vs.* N0										0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4													12.411	1.442–106.842	0.002

Bolded *p*-values indicate statistical significance ($p < 0.05$).

3.4.2.5. *RRP1B* gene: focus on SNP rs762400

This SNP also showed significant results. The G allele was significant for a reduced risk of advanced tumor size (T3–T4) compared to the absence of the G allele (G−) (OR = 0.383, 95% CI: 0.151–0.967, $p = 0.037$). Based on multivariate logistic regression analysis, taking into account age, tumor grade, nodal involvement, and distant metastasis, the association maitains significance in the initial models: Model No. 1 (OR = 0.383, 95% CI: 0.151–0.968, $p = 0.042$), Model No. 2 (OR = 0.378, 95% CI: 0.148–0.970, $p = 0.043$), and Model No. 3 (OR = 0.330, 95% CI: 0.115–0.946, $p = 0.039$). However, in Model No. 4, the association was not statistically significant ($p = 0.106$). Thus, the results indicated that the role of other covariates is more important with regard to the impact of the G allele.

Moreover, the univariate logistic regression suggests that individuals carrying the G allele (+) had a significantly lower risk of having metastasis (OR = 0.176, 95% CI: 0.045–0.686, $p = 0.006$). The multivariate analyses reinforce this association, with the presence of the G allele consistently linked to a reduced risk of metastasis. This significance holds in Models No. 1, 2, and 3 (OR = 0.165, 95% CI: 0.042–0.659, $p = 0.011$; OR = 0.168, 95% CI: 0.042–0.673, $p = 0.012$; OR = 0.149, 95% CI: 0.032–0.703, $p = 0.016$, respectively). In Model No. 4, while the association between the G allele and metastasis does not reach conventional statistical significance, it still suggests a notable trend towards a reduced risk of metastasis associated with the G allele. Importantly, age and other clinical factors did not demonstrate significant associations with metastasis (Table 14). The results are presented in Supplementary Table 3, and Supplementary Table 4.

Table 14. *Univariate and multivariate logistic regression analyses were conducted for rs762400, adjusting for alleles and clinicopathological characteristics, with a focus on tumor size and metastasis.*

SNP	Dependent	Covariates	Univariate			Multivariate											
						Model No. 1			Model No. 2			Model No. 3			Model No. 4		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs762400	Positive T3–T4	G allele+ *vs.* G–	0.383	0.151–0.967	**0.037**	0.383	0.151–0.968	**0.042**	0.378	0.148–0.970	**0.043**	0.330	0.115–0.946	**0.039**	0.401	0.132–1.216	0.106
		Age (years)				1.001	0.977–1.024	0.963	1.002	0.978–1.026	0.884	1.026	0.998–1.055	0.064	1.025	0.998–1.054	0.072
		Positive G3 *vs.* G1 + G2							2.173	1.080–4.372	0.029	2.008	0.933–4.320	0.074	1.906	0.877–4.143	0.103
		Positive N1 *vs.* N0										6.875	3.190–14.820	< 0.001	5.717	2.612–12.511	< 0.001
		Positive M1 *vs.* M0													5.895	0.682–50.958	0.107
rs762400	Positive M	G allele+ *vs.* G–	0.176	0.045–0.686	**0.006**	0.165	0.042–0.659	**0.011**	0.168	0.042–0.673	**0.012**	0.149	0.032–0.703	**0.016**	0.265	0.062–1.135	0.074
		Age (years)				0.974	0.924–1.027	0.327	0.976	0.926–1.029	0.370	0.999	0.946–1.054	0.957	0.977	0.924–1.034	
		Positive G3 *vs.* G1 + G2							1.796	0.460–7.017	0.400	1.332	0.314–5.647	0.697	1.196	0.289–4.951	0.805
		Positive N1 *vs.* N0										0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4													14.735	1.757–123.541	0.013

Bolded *p*-values indicate statistical significance ($p < 0.05$).

3.4.3. *SIPA1* gene: focus on SNPs rs931127, rs3741378, and rs746429

In the cases of rs931127 and rs3741378, no significant link between genotypes or alleles and tumor phenotype was detected. The results are presented in Supplementary Table 5, and Supplementary Table 6.

Table 15 presents the results of univariate and multivariate logistic regression analyses assessing the relationship between the SNP rs746429 and various clinicopathological characteristics, particularly focusing on tumor differentiation (G). In the univariate analysis, the odds ratio (OR) for the GA *vs.* GG genotype is 0.329 (95% CI: 0.147–0.736) with a *p*-value of 0.007, indicating a statistically significant reduction in the likelihood of having poorly differentiated tumors (G3) for patients with the GA genotype compared to those with the GG genotype. The OR for the AA *vs.* GG genotype is 0.692 (95% CI: 0.279–1.716) with a *p*-value of 0.427, suggesting a reduction in risk, although this reduction did not reach statistical significance. Additionally, the OR for the presence of the A allele is 0.424 (95% CI: 0.205–0.880) with a *p*-value of 0.019, showing a significant association with a lower risk of poor tumor differentiation.

In the multivariate analysis, four different models were employed, adjusting for age, nodal status (N1 *vs.* N0), metastasis (M1 *vs.* M0), and tumor size (T3–T4 *vs.* T1–T2). In Model No. 1, the adjusted OR for GA *vs.* GG remains significant at 0.330 (95% CI: 0.148-0.740, p = 0.007), and the adjusted OR for the A allele is 0.426 (95% CI: 0.205–0.884, p = 0.002). In Model No. 2, the adjusted OR for GA *vs.* GG is 0.312 (95% CI: 0.138–0.707, p = 0.005), and the adjusted OR for the A allele is 0.410 (95% CI: 0.196–0.858, p = 0.018). In Model No. 3, the adjusted OR for GA *vs.* GG is 0.312 (95% CI: 0.137–0.708, p = 0.005), and the adjusted OR for the A allele is 0.412 (95% CI: 0.197–0.863, p = 0.019). In Model No. 4, the adjusted OR for GA *vs.* GG is 0.320 (95% CI: 0.140–0.731, p = 0.007), and the adjusted OR for the A allele is 0.423 (95% CI: 0.200–0.891, p = 0.024). Additional variables in the multivariate analysis show that age is not a significant factor across all models. The odds ratios for nodal status (N1 *vs.* N0) and metastasis status (M1 *vs.* M0) are not statistically significant. The odds ratios for tumor size (T3–T4 *vs.* T1–T2) approach significance but do not reach statistical significance in the multivariate models.

In conclusion, the SNP rs746429, specifically the GA genotype and the presence of the A allele, is significantly associated with a lower risk of poor tumor differentiation (G3) in both univariate and multivariate analyses. These findings suggest that the rs746429 SNP may play a protective role against aggressive tumor characteristics.

Table 16 presents the results of univariate logistic regression analysis assessing the relationship between the SIPA1 SNP rs746429 and two clinical parameters: patients' age (categorized as ≤ 50 *vs.* > 50) and disease prognosis (categorized as T3–T4 + G3 *vs.* T1–T2 + G1–G2). For the GA *vs.* GG genotype, the OR is 0.435 (95% CI: 0.200–0.945) with a *p*-value of 0.036, indicating that individuals with the GA genotype have a significantly lower likelihood of being younger than 50 years compared to those with the GG genotype. In contrast, the AA *vs.* GG genotype shows an OR of 0.611 (95% CI: 0.243–1.534) with a *p*-value of 0.294, which is not statistically significant, suggesting no strong association between the AA genotype and age groups. When considering the presence of the G allele, the OR is 0.972 (95% CI: 0.439–2.154) with a *p*-value of 0.943, indicating no significant association. However, for the A allele, the OR is 0.484 (95% CI: 0.236–0.996) with a *p*-value of 0.049, demonstrating a statistically significant lower likelihood of being younger than 50 years for individuals carrying the A allele.

Examining the association with disease prognosis, the GA *vs.* GG genotype has an OR of 0.255 (95% CI: 0.088–0.739) with a *p*-value of 0.012, indicating a significantly lower risk of having a worse prognosis for individuals with the GA genotype compared to those with the GG genotype. For the presence of the G allele, the OR is 1.170 (95% CI: 0.352–3.892) with a *p*-value of 0.798, showing no significant association. However, the A allele presents an OR of 0.296 (95% CI: 0.114–0.769) with a *p*-value of 0.012, indicating a significantly lower risk of worse prognosis for individuals carrying the A allele.

The univariate logistic regression analysis reveals significant associations for the SNP rs746429 in the SIPA1 gene. Specifically, the GA genotype and the presence of the A allele are linked to a lower likelihood of being younger than 50 years and a reduced risk of having a worse prognosis. These findings suggest a potential protective role of the GA genotype and the A allele against aggressive tumor characteristics and adverse outcomes.

Table 15. *Univariate and multivariate logistic regression analyses were conducted for rs746429, adjusting for genotypes, alleles, and clinicopathological characteristics, with a focus on tumor differentiation (G).*

SNP	Dependent	Covariates	Univariate			Multivariate											
						Model No. 1			Model No. 2			Model No. 3			Model No. 4		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs746429	Positive G3	GA *vs.* GG	0.329	0.147–0.736	**0.007**	0.330	0.148–0.740	**0.007**	0.312	0.138–0.707	**0.005**	0.312	0.137–0.708	**0.005**	0.320	0.140–0.731	**0.007**
		AA *vs.* GG	0.692	0.279–1.716	0.427	0.694	0.280–1.721	0.430	0.687	0.275–1.720	0.423	0.700	0.279–1.757	0.448	0.718	0.284–1.815	0.484
		Age (years)				0.994	0.969–1.019	0.623	1.000	0.974–1.028	0.974	1.000	0.974–1.028	0.984	0.997	0.970–1.025	0.829
		Positive N1 *vs.* N0							1.852	0.881–3.850	0.104	1.737	0.808–3.733	0.157	1.374	0.604–3.127	0.449
		M1 *vs.* M0										1.538	0.372–6.361	0.552	1.231	0.287–5.276	0.780
		Positive T3–T4													1.871	0.849–4.125	0.120
rs746429	Positive G3	A allele+ *vs.* A–	0.424	0.205–0.880	**0.019**	0.426	0.205–0.884	**0.002**	0.410	0.196–0.858	**0.018**	0.412	0.197–0.863	**0.019**	0.423	0.200–0.891	**0.024**
		Age (years)				0.993	0.969–1.019	0.607	1.000	0.973–1.027	0.981	1.000	0.973–1.027	0.974	0.996	0.969–1.024	0.782
		Positive N1 *vs.* N0							1.768	0.854–3.661	0.125	1.686	0.791–3.592	0.176	1.330	0.589–3.003	0.492
		M1 *vs.* M0										1.399	0.347–5.645	0.637	1.123	0.269–4.689	0.874
		Positive T3–T4													1.875	0.856–4.104	0.116

Bolded *p*-values indicate statistical significance ($p < 0.05$).

Table 16. *Univariate logistic regression analysis: assessing odds ratios for the relationships between SIPA1 SNP rs746429 and patients' age, and disease prognosis.*

SNP	Genotype, alleles	Age (groups): ≤ 50 *vs.* > 50			Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs746429	GA *vs.* GG	0.435	0.200–0.945	**0.036**	0.255	0.088–0.739	**0.012**
	AA *vs.* GG	0.611	0.243–1.534	0.294	0.412	0.112–1.511	0.181
	G allele+ *vs.* G–	0.972	0.439–2.154	0.943	1.170	0.352–3.892	0.798
	A allele+ *vs.* A–	0.484	0.236–0.996	**0.049**	0.296	0.114–0.769	**0.012**

Bolded *p*-values indicate statistical significance ($p < 0.05$).

3.4.4. *SRSF1* gene: focus on SNPs rs8819, rs34592492, rs11654058, rs2233908, and rs2585828

For the SNPs rs8819, rs34592492, rs11654058, and rs2233908, no significant association was found between genotypes or alleles and tumor phenotypes, prognosis, or patient age.

The analysis shows a significant association between the SRSF1 rs34592492 G allele and metastasis (M). Carriers of the G allele are predominantly represented among those with metastasis, with statistical tests supporting a meaningful relationship. However, the exact significance tests suggest some caution in interpreting the strength of this association, likely due to the small number of non-carriers. Among non-carriers, there are 0 individuals without metastasis (M = 0) and 1 individual with metastasis (M = 1), representing 0% and 10% of non-carriers, respectively. Overall, non-carriers make up 0.6% of the total sample. In contrast, there are 162 individuals without metastasis (M = 0) and 9 individuals with metastasis (M = 1) among G allele carriers, representing 100% and 90% of carriers, respectively. Carriers make up 99.4% of the total sample. The chi-square tests, particularly the Pearson Chi-Square test with a p-value < 0.001, indicate a statistically significant association between the G allele and the presence of metastasis. This significant p-value suggests that the distribution of metastasis differs between carriers and non-carriers of the G allele. However, the exact significance values (Fisher's Exact Test) are on the borderline of conventional statistical significance ($p = 0.058$), indicating that while there is an association, it may not be as strong when considering the exact probabilities. This discrepancy can occur due to the small sample size or the distribution of cases. The risk estimate shows that individuals with the G allele are at a significantly higher risk (19 times) of having metastasis compared to non-carriers, with a 95% confidence interval ranging from 10.060 to 35.886. This wide confidence

interval reflects uncertainty in the exact risk estimate, likely due to the small number of non-carriers. While the initial chi-square test demonstrates a significant association between the G allele and metastasis, the small number of non-carriers (1 individual) limits the robustness of this finding. The borderline significance in tests accounting for small sample sizes suggests caution in interpreting these results. Therefore, although there is an observed association, the evidence is not strong enough to conclusively determine that the G allele directly influences metastasis due to the limitations in sample size and the resulting statistical power. This nuanced understanding explains why, despite significant *p*-values in some tests, the influence of the G allele on metastasis may be considered uncertain or not definitive.

The results are detaled in Supplementary Table 7, and Supplementary Table 8.

3.4.5. *HOTAIR* gene: focus on SNPs rs12826786, rs7958904, and rs920778

In the cases of rs12826786, rs7958904, and rs920778, no significant link between genotypes or alleles and tumor phenotype (tumor size, nodes, metastasis, tumor differentiation, stage, disease prognosis, and patient age) was detected. All the data is presented in Supplementary Tables 9 and 10.

3.4.6. *MALAT1* gene: focus on SNPs rs619586, rs664589, and rs3200401

For the cases of rs619586, rs664589, and rs3200401, no significant associations were found between the genotypes or alleles and various tumor phenotypes, including tumor size, lymph node involvement, metastasis, tumor differentiation, stage, disease prognosis, and patient age. Detailed data can be found in Supplementary Tables 11 and 12.

3.4.7. Haplotypes

TLR4 gene SNPs rs4986790, rs4986791, and rs1927906 did not show significant associations with tumor clinicopathological features as independent factors. Analyzing the most frequent haplotype based on these SNPs, the overall results did not find significant associations between *TLR4* diplotypes and the clinical characteristics. This suggests that the presence of the ACT haplotype, whether in homozygous or heterozygous form, does not significantly impact the severity of tumor characteristics, metastasis, or prognosis in this cohort. Table 17 presents the results of the univariate logistic regression analysis assessing the relationships between *TLR4* gene diplotypes and various clinical characteristics, including patients' age and tumor characte-

ristics. This analysis compared non-carriers and heterozygous carriers of the ACT haplotype against homozygous carriers (ACT/ACT).

Table 17. *Univariate logistic regression analysis: assessing odds ratios for the relationships between TLR4 gene diplotypes and patients' age, tumor characteristics.*

Cinical characteristics	*TLR4* rs4986790 rs4986791 rs1927906 diplotypes					
	ACT haplotype non-carriers *vs.* homozygous diplotype (ACT/ACT)			Heterozygous diplotype (ACT/alternative hap) *vs.* homozygous diplotype (ACT/ACT)		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Positive T3–T4 *vs.* T1–2	0.000	0.000	1.000	1.033	0.489–2.183	0.932
Positive N1 *vs.* N0	0.000	0.000	1.000	0.668	0.318–1.403	0.287
Positive M1 *vs.* M0	0.000	0.000	1.000	1.543	0.379–6.278	0.545
Positive G3 *vs.* G1 + G2	0.000	0.000	1.000	1.919	0.887–4.143	0.098
Age (groups): ≤ 50 *vs.* > 50	0.000	0.000	1.000	1.120	0.496–2.528	0.785
Positive stage III–IV *vs.* stage I–II	0.000	0.000	1.000	0.655	0.317–1.350	0.251
Positive worse prognosis: T3–T4+G3 *vs.* T1–T2+G1–G2	0.000	0.000	1.000	2.159	0.785–5.940	0.136

With the understanding that *RRP1B* gene SNPs rs2838342, rs7276633, rs2051407, rs9306160, and rs762400 may not act independently, we opt to explore haplotypes. By analyzing haplotypes, we aim to capture the combined effect of multiple SNPs within the gene, thus providing a more comprehensive understanding of the genetic landscape and its potential implications in our study. We meticulously analyzed the associations between diplotypes and various clinical characteristics. Specifically, we examined the heterozygous diplotype (ATCCG/alternative haplotype) *vs.* the homozygous diplotype (ATCCG/ATCCG), ATCCG haplotype non-carriers *vs.* the homozygous diplotype (ATCCG/ATCCG), heterozygous diplotype (GCTTC/alternative haplotype) *vs.* the homozygous diplotype (GCTTC/GCTTC), GCTTC haplotype non-carriers *vs.* the homozygous diplotype (GCTTC/GCTTC), and heterozygous diplotype (ATCCG/GCTTC) carriers *vs.* non-carriers.

Significantly, GCTTC haplotype non-carriers exhibited a greater protective effect against advanced tumor size (T3–T4) and metastasis compared to those with the homozygous diplotype (GCTTC/GCTTC) (OR = 0.367, 95% CI: 0.136–0.992, p = 0.038; OR = 0.098, 95% CI: 0.016–0.578, p = 0.010, respectively). This finding suggests a potential role of genetic variations represented by the GCTTC haplotype in promoting aggressive tumor behavior. For patients with advanced tumor stages (III–IV *vs.* I–II) and worse prognosis (T3–T4 + G3 *vs.* T1–T2 + G1–G2), individuals lacking the ATCCG haplotype showed a significantly higher likelihood of exhibiting advanced tumor stages and being in the worse prognosis group compared to those with the homozygous diplotype (ATCCG/ATCCG) (OR = 1.250, 95% CI: 0.454–3.444, p = 0.032; OR = 2.100, 95% CI: 0.638–6.916, p = 0.048, respectively) (Table 18).

Table 19 presents the results of multivariate logistic regression analyses focusing on diplotypes, with adjustments made for clinicopathological characteristics, particularly emphasizing tumor size and metastasis. Model No. 1: In the initial model, we adjusted for age (years) as an additional covariate. The association between GCTTC haplotype non-carriers and reduced odds of advanced tumor size remained significant (OR = 0.393, 95% CI: 0.188–0.822, p = 0.039). This underscores the robustness of our initial findings, indicating that age did not substantially alter the observed relationship between haplotype status and tumor size. Model No. 2: Further adjustments were made by including tumor grade (G3 *vs.* G1 + G2) in the analysis. Despite this additional adjustment, the association between GCTTC haplotype non-carriers and decreased odds of advanced tumor size remained statistically significant (OR = 0.392, 95% CI: 0.185–0.827, p = 0.041). This suggests that the observed association is independent of tumor grade, emphasizing the potential importance of genetic factors in influencing tumor progression. Model No. 3: Despite the inclusion of nodal status in the analysis, the association between GCTTC haplotype non-carriers and reduced odds of advanced tumor size remained statistically significant (OR = 0.391, 95% CI: 0.173–0.884, p = 0.041). Model No. 4: Finally, we included metastasis (M1 *vs.* M0) as an additional covariate in the analysis. The association between GCTTC haplotype non-carriers and advanced tumor size showed a trend towards significance (OR = 0.380, 95% CI: 0.166–0.869, p = 0.046). On the focus on metastasis, in Model No. 1, GCTTC haplotype non-carriers exhibit a substantial protective effect against metastasis (OR = 0.101, 95% CI: 0.017–0.598, p = 0.012). This suggests a potential role of genetic variations represented by the GCTTC haplotype in influencing metastatic propensity, even after adjusting for age. In Model No. 2, which includes additional adjustments for tumor grade (G3 *vs.* G1 + G2), the protective effect against metastasis remains significant (OR = 0.095, 95% CI: 0.016–0.577, p = 0.011), further

emphasizing the independent nature of this association. Model No. 3 incorporates adjustments for lymph node involvement (N1 *vs.* N0) along with age and tumor grade. Despite these additional adjustments, the protective effect against metastasis among GCTTC haplotype non-carriers persists (OR = 0.075, 95% CI 0.011–0.534, $p = 0.010$), highlighting the robustness of the observed association. Finally, in Model No. 4, which includes adjustments for tumor stage (T3–T4), in addition to age, tumor grade, and lymph node involvement, the protective effect against metastasis remains significant (OR = 0.150, 95% CI: 0.023–0.965, $p = 0.048$). This suggests that the influence of genetic variations represented by the GCTTC haplotype on metastatic propensity is independent of tumor size and other clinicopathological factors.

Overall, the consistent significance of the protective effect across all models underscores the potential importance of genetic variations represented by diplotypes in predicting tumor size and metastasis in cervical cancer patients, irrespective of traditional clinicopathological factors.

Table 18. *Univariate logistic regression analysis for the RRP1B gene: assessing odds ratios for the relationships between diplotypes and patients' age and tumor characteristics.*

Cinical characteristics	Diplotypes														
	Heterozygous diplotype (ATCCG/alternative hap) *vs.* homozygous diplotype (ATCCG/ATCCG)			**ATCCG haplotype non-carriers *vs.* homozygous diplotype (ATCCG/ATCCG)**			**Heterozygous diplotype (GCTTC/ alternative hap) *vs.* homozygous diplotype (GCTTC/GCTTC)**			**GCTTC haplotype non-carriers *vs.* homozygous diplotype (GCTTC/GCTTC)**			**Heterozygous diplotype (ATCCG/GCTTC) carriers *vs.* non-carriers**		
	OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
Positive T3–T4 *vs.* T1–T2	0.383	0.183–0.800	0.077	1.773	0.703–4.471	0.225	0.506	0.185–1.387	0.186	0.367	0.136–0.992	**0.038**	0.424	0.220–0.817	0.090
Positive N1 *vs.* N0	*	*	*	*	*	*	0.831	0.311–2.221	0.712	1.222	0.449–3.326	0.694	0.671	0.364–1.238	0.202
Positive M1 *vs.* M0	1.022	0.180–5.790	0.980	3.538	0.606–20.653	0.160	0.250	0.056–1.110	0.068	0.098	0.016–0.578	**0.010**	0.313	0.064–1.517	0.149
Positive G3 *vs.* G1 + G2	0.699	0.317–1.538	0.373	1.406	0.534–3.705	0.491	1.271	0.417–3.876	0.673	0.923	0.290–2.935	0.892	0.908	0.458–1.801	0.783
Age (groups): ≤ 50 *vs.* > 50	2.278	1.070–4.846	0.073	1.529	0.578–4.040	0.392	0.750	0.225–2.495	0.639	0.458	0.138–1.527	0.204	1.840	0.919–3.684	0.085
Positive stage III–IV *vs.* stage I–II	1.002	0.169–1.733	0.091	1.250	0.454–3.444	**0.032**	0.429	0.150–1.222	0.113	0.612	0.210–1.787	0.369	0.494	0.267–0.912	0.084
Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2	0.212	0.064–0.707	0.162	2.100	0.638–6.916	**0.048**	0.465	0.117–1.855	0.278	0.354	0.126–2.098	0.354	0.305	0.104–0.895	0.101

* OR could not be estimated because of zero value within a cell. Bolded *p*-values indicate statistical significance ($p < 0.05$).

Table 19. *Multivariate logistic regression analyses were conducted for RRP1B diplotypes, adjusting for clinicopathological characteristics, with a focus on tumor size and metastasis.*

SNPs	Dependent	Covariates	Multivariate											
			Model No. 1			Model No. 2			Model No. 3			Model No. 4		
			OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs2838342 rs7276633 rs2051407 rs9306160 rs762400	Positive T3–T4	GCTTC haplotype non-carriers *vs.* homozygous diplotype (GCTTC/GCTTC)	0.393	0.188–0.822	**0.039**	0.392	0.185–0.827	**0.041**	0.391	0.173–0.884	**0.041**	0.380	0.166–0.869	**0.046**
		Age (years)	1.004	0.981–1.032	0.614	1.006	0.983–1.034	0.567	1.031	1.002–1.063	0.131	1.032	1.002–1.064	0.132
		Positive G3 *vs.* G1 + G2				2.007	0.971–4.147	0.061	1.842	0.831–4.067	0.131	0.579	0.257–1.299	0.185
		Positive N1 *vs.* N0							0.000	0.000	0.996	0.000	0.000	0.996
		Positive M1 *vs.* M0										6.508	0.719–58.708	0.096
rs2838342 rs7276633 rs2051407 rs9306160 rs762400	Positive M	GCTTC haplotype non-carriers *vs.* homozygous diplotype (GCTTC/GCTTC)	0.101	0.017–0.598	**0.012**	0.095	0.016–0.577	**0.011**	0.075	0.011–0.534	**0.010**	0.150	0.023–0.965	**0.048**
		Age (years)	0.980	0.927–1.035	0.462	0.983	0.931–1.039	0.544	1.005	0.952–1.062	0.850	0.982	0.926–1.041	0.545
		Positive G3 *vs.* G1 + G2				2.051	0.515–8.170	0.309	1.657	0.377–7.294	0.504	1.324	0.312–5.579	0.702
		Positive N1 *vs.* N0							0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4										8.404	0.915–77.157	0.060

Bolded *p*-values indicate statistical significance ($p < 0.05$).

The *SRSF1* gene SNPs rs8819, rs11654058, rs2233908, and rs2585828 were not found to be significantly associated with tumor severity. Table 20 presents the results of the univariate logistic regression analysis assessing the relationships between *SRSF1* gene diplotypes and various clinical characteristics, including patients' age and tumor characteristics. This analysis compared non-carriers and heterozygous carriers of the CTGA haplotype against homozygous carriers (CTGA/CTGA). The analysis of *SRSF1* haplotypes did not find significant associations between SRSF1 diplotypes and the clinical characteristics. This suggests that the presence of the CTGA haplotype, whether in homozygous or heterozygous form, does not significantly impact tumor characteristics, metastasis, or prognosis in this cohort.

Table 20. *Univariate logistic regression analysis: assessing odds ratios for the relationships between SRSF1 gene diplotypes and patients' age, tumor characteristics.*

Cinical characteristics	***SRSF1* Rs8819 Rs11654058 Rs2233908 Rs2585828 Diplotypes**					
	CTGA haplotype non-carriers *vs.* homozygous diplotype (CTGA/CTGA)			**Heterozygous diplotype (CTGA/Alternative Hap) *vs.* homozygous diplotype (CTGA/CTGA)**		
	OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
Positive T3–T4 *vs.* T1–2	0.000	0.000	0.999	0.612	0.292–1.284	0.194
Positive N1 *vs.* N0	0.330	0.036–3.041	0.328	1.447	0.725–2.887	0.295
Positive M1 *vs.* M0	0.000	0.000	0.999	1.213	0.299–4.910	0.787
Positive G3 *vs.* G1+G2	0.742	0.080–6.892	0.793	1.385	0.652–2.943	0.397
Age (groups): ≤ 50 *vs.* > 50	0.000	0.000	0.999	0.739	0.353–1.545	0.421
Positive stage III–IV *vs.* stage I–II	0.189	0.021–1.743	0.142	0.996	0.497–1.996	0.992
Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2	0.000	0.000	0.999	0.905	0.317–2.582	0.853

Focusing on the impact of several frequent *HOTAIR* haplotypes on clinical outcomes, the univariate logistic regression analysis revealed that for the CGG haplotype, no significant associations were found when comparing non-carriers to heterozygous carriers. This included advanced tumor stage, nodal involvement, metastasis, high tumor grade, age groups, and prognosis. Similarly, the analysis showed no significant correlations for the CGA haplotype when comparing non-carriers to homozygous carriers, or between heterozygous and homozygous carriers, indicating that the CGA haplotype does not notably affect tumor characteristics or prognosis. For the TCA haplotype, comparisons between non-carriers and heterozygous carriers also showed no significant associations with advanced tumor stage, nodal involvement, metastasis, high tumor grade, age groups, or prognosis. Overall, the analysis found no significant correlations between *HOTAIR* gene diplotypes and clinical characteristics, suggesting that the presence of the CGG, CGA, or TCA haplotypes, whether in homozygous or heterozygous form, does not have a significant impact on tumor characteristics or prognosis in this population. All the results are detailed in Table 21.

Table 21. *Univariate logistic regression analysis: assessing odds ratios for the relationships between HOTAIR gene diplotypes and patients' age, tumor characteristics.*

Cinical characteristics	***HOTAIR*** **rs12826786 rs7958904 rs920778 diplotypes**											
	CGG haplotype non-carriers *vs.* (heterozygous diplotype (CGG/alternative hap)			**CGA haplotype non-carriers *vs.* homozygous diplotype (CGA/CGA)**			**Heterozygous diplotype (CGA/alternative hap) *vs.* homozygous diplotype (CGA/CGA)**			**TCA haplotype non-carriers *vs.* (heterozygous diplotype (TCA/alternative hap)**		
	OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
Positive T3–T4 *vs.* T1–2	0.893	0.480–1.661	0.720	0.847	0.444–1.615	0.614	0.000	0.000	1.000	1.011	0.540–1.891	0.973
Positive N1 *vs.* N0	0.824	0.452–1.505	0.529	1.164	0.619–2.187	0.638	0.000	0.000	1.000	0.920	0.501–1.687	0.787
Positive M1 *vs.* M0	1.576	0.429–5.795	0.494	0.533	0.148–1.921	0.336	0.000	0.000	1.000	1.821	0.455–7.292	0.397
Positive G3 *vs.* G1 + G2	0.566	0.285–1.126	0.105	1.582	0.758–3.304	0.333	0.000	0.000	1.000	0.864	0.438–1.705	0.674
Age (groups): ≤ 50 *vs.* > 50	1.625	0.831–3.178	0.156	0.729	0.359–1.482	0.382	0.000	0.000	1.000	0.713	0.367–1.388	0.320
Positive stage III–IV *vs.* stage I–II	0.794	0.435–1.451	0.454	1.137	0.606–2.134	0.689	0.000	0.000	1.000	0.849	0.462–1.561	0.599
Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2	0.559	0.219–1.429	0.225	1.545	0.551–4.336	0.408	0.000	0.000	1.000	0.824	0.327–2.073	0.680

3.5. Survival analysis

The influence of the SNPs on survival, including progression-free survival (PFS) and overall survival (OS), was evaluated using both genotype and allelic models. Cox univariate and multivariate analyses were employed to assess the impact of SNPs and haplotypes on these survival outcomes.

3.5.1. Survival analysis: focus on *TLR4* gene

No significant associations were found between the genotypes or alleles of SNPs rs1927906, rs11536865, rs10983755, rs4986790, rs4986791, and rs11536897 and survival outcomes. However, SNPs rs10759932 and rs11536898 were identified as having a significant impact on progression-free survival (PFS) and overall survival (OS). Kaplan-Meier analysis revealed that the rs10759932 CC genotype was associated with overall survival (OS) (Log Rank $p = 0.049$, Breslow $p = 0.018$, and Tarone–Ware $p = 0.028$). Cox regression analysis showed that the CC genotype was linked to shorter progression-free survival (PFS) and OS compared to the TT genotype, with odds ratios of 2.918 (95% CI: 0.894–9.530, $p = 0.049$) and 3.340 (95% CI: 1.006–11.095, $p = 0.048$), respectively. In the multivariate Cox regression model, which adjusted for tumor stage (T), nodal involvement (N), grade (G), and patient age, the CC genotype increased the risk of progression by nearly fourfold compared to the TT genotype (HR = 3.674, 95% CI: 1.115–12.108, $p = 0.032$) and the risk of earlier mortality (HR = 4.608, 95% CI: 1.344–15.801, $p = 0.015$). The T allele was significant in the allelic model for both PFS (Log Rank $p = 0.049$, Breslow $p = 0.042$, Tarone–Ware $p = 0.042$) and OS (Log Rank $p = 0.031$, Breslow $p = 0.018$, Tarone–Ware $p = 0.023$). Carriers of the T allele had a greater likelihood of longer PFS (HR = 0.331, 95% CI: 0.103–1.067, $p = 0.048$) and longer OS (HR = 0.284, 95% CI: 0.087–0.928, $p = 0.037$). After adjusting for tumor T, N, G, and patient age, T allele carriers had an increased chance of longer PFS (HR = 0.244, 95% CI: 0.075–0.795, $p = 0.019$) and a decreased risk of shorter OS (HR = 0.200, 95% CI: 0.059–0.674, $p = 0.009$), as shown in Table 22 and Supplementary Table 13.

Table 22. *Cox's univariate and multivariate models for progression-free survival (PFS) and overall survival (OS) assessed adjusted ratios for associations between rs10759932, age at diagnosis, and tumor characteristics.*

SNP	Variables	Progression-free survival						Overall survival					
		Univariate			Multivariate			Univariate			Multivariate		
		HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
rs10759932	TC *vs.* TT	0.884	0.472–1.653	0.699	0.658	0.338–1.280	0.217	0.818	0.382–1.752	0.606	0.747	0.351–1.590	0.449
	CC *vs.* TT	2.918	0.894–9.530	**0.049**	3.674	1.115–12.108	**0.032**	3.340	1.006–11.095	**0.048**	4.608	1.344–15.801	**0.015**
	Age at diagnosis				0.993	0.971–1.016	0.566				1.017	0.991–1.043	0.199
	T3–T4 *vs.* T1–T2				5.540	2.870–10.694	< 0.001				8.178	3.489–19.167	< 0.001
	N1 *vs.* N0				1.340	0.709–2.534	0.368				1.775	0.854–3.689	0.124
	G3 *vs.* G1–G2				0.913	0.490–1.704	0.776				0.773	0.384–1.556	0.471
rs10759932	T allele+ *vs.* T–	0.331	0.103–1.067	**0.048**	0.244	0.075–0.795	**0.019**	0.284	0.087–0.928	**0.037**	0.200	0.059–0.674	**0.009**
	Age at diagnosis				0.996	0.973–1.018	0.697				1.018	0.993–1.044	0.163
	T3–T4 *vs.* T1–T2				5.298	2.750–10.206	< 0.001				8.045	3.430–18.871	< 0.001
	N1 *vs.* N0				1.291	0.684–2.439	0.431				1.735	0.835–3.604	0.140
	G3 *vs.* G1–G2				0.962	0.520–1.779	0.902				0.797	0.399–1.593	0.521

Bolded *p*-values indicate statistical significance ($p < 0.05$).

The rs11536898 AA genotype was significantly associated with progression-free survival (PFS) and overall survival (OS) when compared to the CC genotype. Specifically, the AA genotype was linked to shorter PFS (Log Rank $p = 0.014$, Breslow $p = 0.001$, Tarone–Ware $p = 0.003$) and shorter OS (Log Rank $p = 0.003$, Breslow $p < 0.001$, Tarone–Ware $p < 0.001$). Compared to the CC genotype, the AA genotype decreased the likelihood of longer PFS (HR = 3.926, 95% CI: 1.201–12.837, $p = 0.024$) and increased the risk of shorter OS (HR = 5.057, 95% CI: 1.522–16.802, $p = 0.008$). In multivariate Cox regression analysis, the AA genotype continued to be associated with a higher risk of shorter OS (HR = 3.735, 95% CI: 1.051–13.278, $p = 0.042$) and showed a borderline effect on PFS (HR = 3.306, 95% CI: 0.967–11.299, $p = 0.057$) after adjusting for tumor stage (T), nodal involvement (N), grade (G), and patient age. Conversely, the rs11536898 C allele was linked to longer PFS (Log Rank $p = 0.015$, Breslow $p = 0.003$, Tarone–Ware $p = 0.005$) and longer OS (Log Rank $p = 0.004$, Breslow $p < 0.001$, Tarone–Ware $p = 0.001$). The CA genotype did not show a significant effect on PFS. The allelic model indicated that carriers of the C allele were less likely to experience shorter PFS compared to non-carriers. Specifically, the C allele was associated with longer PFS (HR = 0.261, 95% CI: 0.081–0.844, $p = 0.025$) and longer OS (HR = 0.212, 95% CI: 0.065–0.691, $p = 0.010$). After adjusting for tumor stage, nodal involvement, grade, and patient age, the association remained statistically significant for both PFS (HR = 0.291, 95% CI: 0.086–0.987, $p = 0.048$) and OS (OR = 0.274, 95% CI: 0.078–0.959, $p = 0.043$), as detailed in Table 23 and Supplementary Table 13. Kaplan–Meier analysis was performed to generate survival curves for genotypes and alleles for both PFS and OS (Figure 15).

The Cox univariate model for PFS and OS assessed the impact of the *TLR4* ACT haplotype on these outcomes. The analysis compared non-carriers of the ACT haplotype with homozygous carriers (ACT/ACT) and heterozygous carriers (ACT/alternative haplotype) with homozygous carriers. The results indicated no significant association between the ACT haplotype and either PFS or OS. All results are detailed in Supplementary Table 14.

Table 23. *Cox's univariate and multivariate models for progression-free survival (PFS) and overall survival (OS) assessed adjusted ratios for associations between rs11536898, age at diagnosis, and tumor characteristics.*

SNP	Variables	Progression-Free Survival						Overall Survival					
		Univariate			Multivariate			Univariate			Multivariate		
		HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
rs11536898	CA *vs.* CC	1.103	0.586–2.073	0.762	0.858	0.440–1.675	0.654	1.294	0.636–2.633	0.476	1.090	0.522–2.277	0.819
	AA *vs.* CC	3.926	1.201–12.837	**0.024**	3.306	0.967–11.299	**0.057**	5.057	1.522–16.802	**0.008**	3.735	1.051–13.278	**0.042**
	Age at diagnosis				0.993	0.971–1.017	0.578				1.018	0.992–1.045	0.171
	T3–T4 *vs.* T1–T2				5.158	2.675–9.947	< 0.001				7.658	3.280–17.876	<0.001
	N1 *vs.* N0				1.241	0.653–2.360	0.510				1.686	0.805–3.530	0.166
	G3 *vs.* G1–G2				1.009	0.538–1.894	0.977				0.819	0.405–1.654	0.577
rs11536898	C allele+ *vs.* C–	0.261	0.081–0.844	**0.025**	0.291	0.086–0.987	**0.048**	0.212	0.065–0.691	**0.010**	0.274	0.078–0.959	**0.043**
	Age at diagnosis				0.994	0.971–1.017	0.612				1.018	0.992–1.044	0.176
	T3–T4 *vs.* T1–T2				5.077	2.645–9.747	0.000				7.694	3.298–17.951	0.000
	N1 *vs.* N0				1.232	0.648–2.342	0.525				1.694	0.810–3.540	0.161
	G3 *vs.* G1–G2				1.018	0.543–1.907	0.955				0.817	0.405–1.651	0.574

Bolded *p*-values indicate statistical significance ($p < 0.05$).

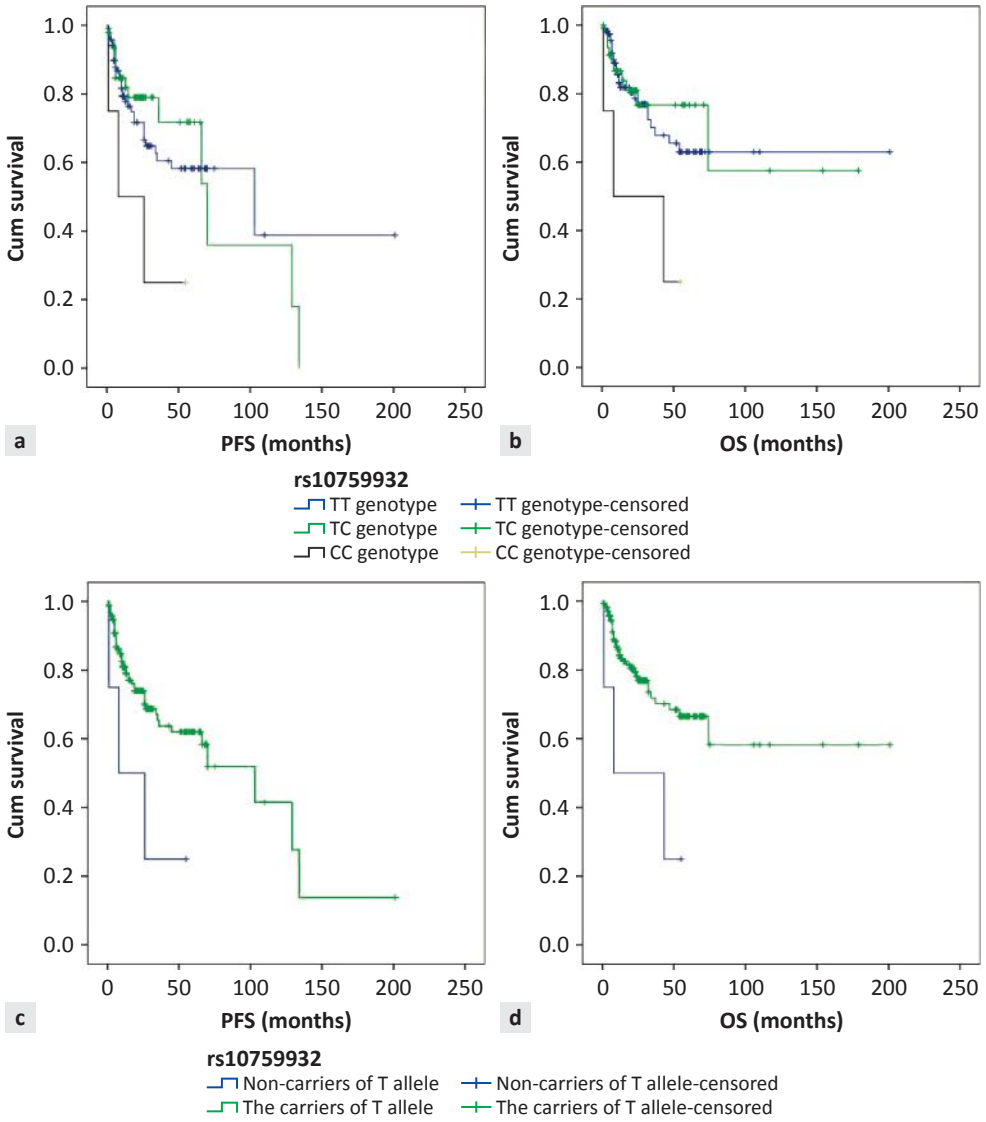

Figure 15 (a–d). *Kaplan–Meier survival curves for PFS and OS in patients with cervical cancer according rs10759932 and rs11536898 polymorphism (n = 172).*

Kaplan–Meier survival curves for rs10759932 and rs11536898 polymorphisms in the genotype and allelic models demonstrate PFS and OS differences. The X-axis displays the number of months from cervical cancer diagnosis, confirming the event date (PFS or OS), and the Y-axis indicates the survival probability. **(a, b)** The rs10759932 CC genotype increased the risk for shorter PFS and OS compared to patients with the TT genotype (95% CI: 0.894–9.530, $p = 0.049$; 95% CI: 1.006–11.095, $p = 0.048$, respectively); **(c, d)** Carrying the T allele increased the possibility of longer PFS (95% CI: 0.103–1.067, $p = 0.048$) and longer OS (95% CI: 0.087–0.928, $p = 0.037$).

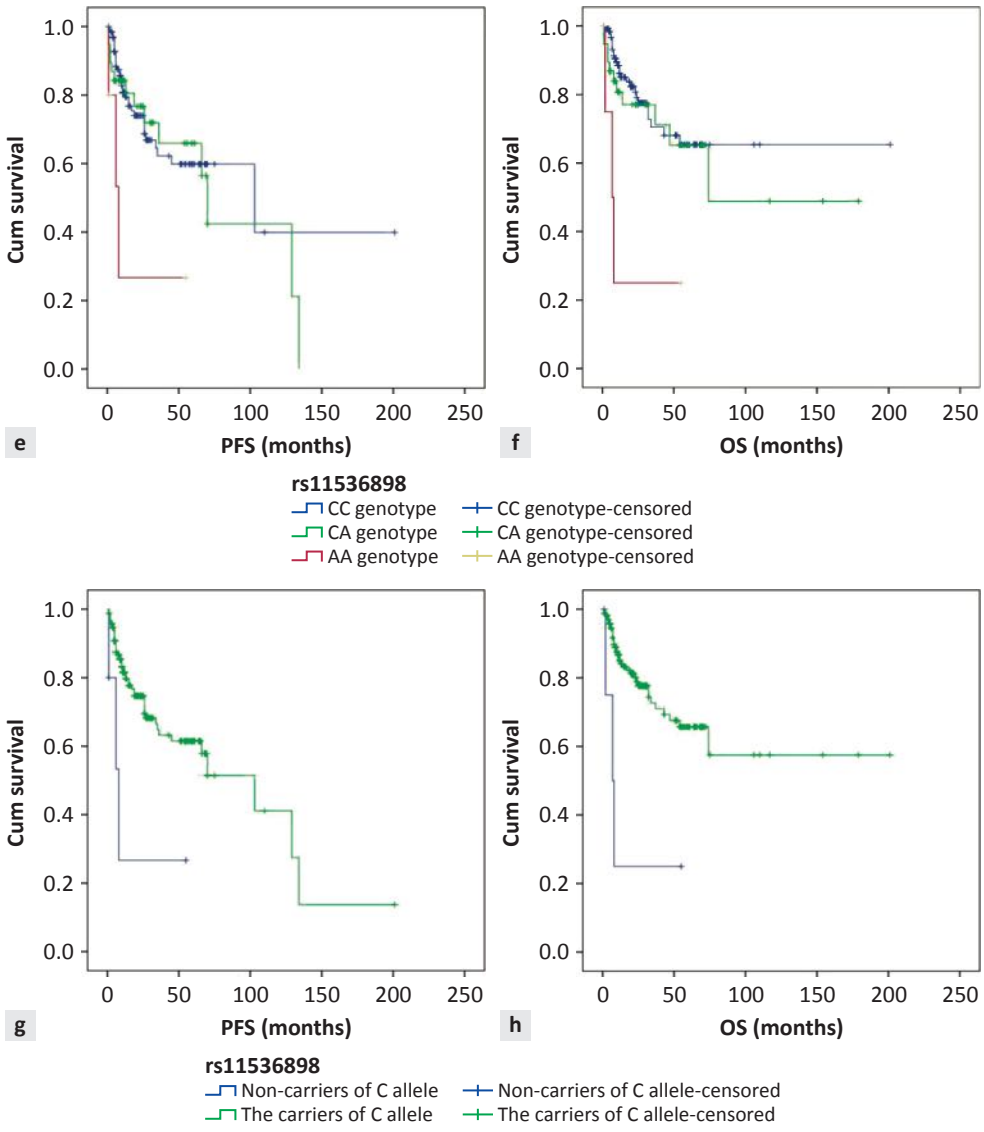

Figure 15 (e–h). *Kaplan–Meier survival curves for PFS and OS in patients with cervical cancer according rs10759932 and rs11536898 polymorphism (n = 172).*

Kaplan–Meier survival curves for rs10759932 and rs11536898 polymorphisms in the genotype and allelic models demonstrate PFS and OS differences. The X-axis displays the number of months from cervical cancer diagnosis, confirming the event date (PFS or OS), and the Y-axis indicates the survival probability. **(e, f)** The rs11536898 AA genotype, compared to patients with the CC genotype, shortened PFS (95% CI: 1.201–12.837, $p = 0.024$) and OS (95% CI: 1.522–16.802, $p = 0.008$); **(g, h)** The rs11536898 C allele predisposed for longer PFS (95% CI: 0.081–0.844, $p = 0.025$) and longer OS (95% CI: 0.065–0.691, $p = 0.010$).

3.5.2. Survival analysis: focus on *RRP1B* gene

No significant link between rs2838342, rs7276633, rs2051407, or rs762400 genotypes or alleles and PFS was detected. In the case of SNP rs9306160, the survival analysis did not yield differences for the genotypes and alleles. But the effect of four SNPs (rs2838342, rs7276633, rs2051407, rs762400) on OS has been identified as important.

The results indicate that for SNP rs2838342, there were no statistically significant associations between genotypes and survival outcomes. However, the presence of the A allele displayed a considerably lower hazard, signifying a potential protective role. This observation is particularly noteworthy, as the *p*-value of 0.031 indicates that the A allele may significantly contribute to improved OS outcomes (HR = 0.465, 95% CI: 0.232–0.931). Utilizing Cox's multivariate models, a effect of the A allele sustained even after adjusting for age at diagnosis (HR = 0.462, 95% CI: 0.231–0.926, $p = 0.030$, Model No. 1). When scrutinizing the influence of age at diagnosis and broader tumor characteristics (tumor T, N, G) in Model No. 2, we observed that the impact of the A allele on OS has now become statistically insignificant. We must note that the effect of tumor size was significant on the survival outcome, revealing an HR of 7.463 (T3–T4 *vs.* T1–T2, $p < 0.001$), reflecting its substantial impact on OS.

Similarly, for SNP rs7276633, the TC and CC genotypes did not show significant differences in survival when compared to TT, but the presence of the T allele was associated with better OS (HR = 0.465, CI: 0.232-0.931, p = 0.031). The T allele's protective effect persisted, with an adjusted HR of 0.462 (95% CI: 0.231–0.926, $p = 0.030$) after accounting for age-related factors (Model No. 1). These findings underscore the significance of allelic effects in influencing overall survival. Regrettably, Model No. 2 did not produce statistically significant results when adjusting for tumor T, N, G, and patients' age. The impact of tumor size on OS was significant, with an HR of 7.463 (95% CI: 3.195–17.432, $p < 0.001$).

Next, our attention turned to SNP rs2051407. Like previous SNPs, the different genotypes showed no substantial differences in survival outcomes. However, the C allele was a factor in modulating patient OS. The C allele carries had a decreased risk of dying faster (HR = 0.418, CI: 0.204–0.858, $p = 0.017$). In multivariate Cox's regression analysis, the C allele remains a factor for longer overall survival (HR = 0.404, 95% CI: 0.196–0.832, $p = 0.014$), when adjusting for the age of patients (Model No. 1). Unfortunately, Model No. 2 did not yield statistically significant results, with a significant effect of tumor size on overall survival persisting (HR = 7.484, 95% CI: 3.227–17.355, $p < 0.001$).

Finally, our analysis extended to SNP rs762400. In the univariate model, patients with the CC genotype, compared to the GG genotype, exhibited a significant impact on OS, with an HR of 2.550 (95% CI: 1.098–5.923, p = 0.030), indicating an elevated risk of adverse outcomes for individuals carrying this genotype. This result remained significant in multivariate Model No. 1, controlling for patient age (HR = 2.476, 95% CI: 1.064–5.758, p = 0.035). Exploring the interplay of tumor characteristics and age at diagnosis in Model No. 2, advanced tumor size (T3–T4 *vs.* T1–T2) once again emerged as a significant predictor, displaying a substantial HR of 7.546 (95% CI: 3.250–17.520, $p < 0.001$). In this model, the CC genotype still increases the risk for shorter OS, but the significance level (p) is > 0.05. Moreover, the scrutiny of SNP rs762400 showcased that the allelic model does not contradict the results of the genotypic model. The presence of the G allele emerged as a significant protective factor. The holders of G allele were less likely to have shorter OS when compared to the non-carriers (HR = 0.374, CI: 0.177–0.788, p = 0.010). The presence of the G allele (+) was associated with an HR of 0.370 (95% CI: 0.176–0.781, p = 0.009) in Model No. 1, after adjusting for age, indicating a substantially reduced risk of adverse OS outcomes linked to this genetic variant. In multivariate Model No. 2, advanced tumor size (T3-T4) exhibited a significant HR of 7.496 (95% CI: 3.235–17.373, $p < 0.001$). These results underline the considerable impact of tumor characteristics on OS outcomes, and once again, the significant influence of the G allele for OS was not observed. All the results are presented in Table 24 and Supplementary Table 15.

In our analysis using Cox's univariate model for progression-free survival (PFS) and overall survival (OS), we observed interesting trends in the association between *RRP1B* haplotypes and patients outcomes. Specifically, the ATCCG haplotype non-carriers *vs.* the homozygous diplotype (ATCCG/ATCCG) showed an elevated hazard ratio (HR) for both PFS and OS, indicating a potential link between this haplotype and poorer survival outcomes. However, statistical significance was not achieved in this comparison.

Conversely, the heterozygous diplotype of GCTTC/alternative haplotype compared to the homozygous diplotype (GCTTC/GCTTC) displayed a significantly decreased HR for OS (HR = 0.274, 95% CI: 0.120–0.626, p = 0.002), suggesting a possible protective effect associated with this haplotype. Similarly, GCTTC haplotype non-carriers compared to the homozygous diplotype (GCTTC/GCTTC) also exhibited a significantly decreased HR for OS (HR = 0.298, 95% CI: 0.128–0.695, p = 0.005), indicating a potentially favorable impact on survival outcomes.

In our comprehensive analysis using Cox's multivariate models for overall survival (OS), we meticulously examined the adjusted associations between diplotypes, age at diagnosis, and various tumor characteristics.

Focusing on diplotypes, particularly the comparison between the heterozygous diplotype (GCTTC/alternative hap) and the homozygous diplotype (GCTTC/GCTTC), our findings consistently demonstrated significantly decreased odds of overall survival (OS) across both Model No. 1 and Model No. 2 (HR = 0.259, 95% CI: 0.113–0.597, $p = 0.002$; OR = 0.372, 95% CI: 0.153–0.904, $p = 0.029$, respectively). This suggests a potential protective effect associated with certain diplotypes, indicating their relevance as prognostic indicators in cervical cancer. Similarly, when comparing GCTTC haplotype non-carriers to the homozygous diplotype (GCTTC/GCTTC), we observed notably reduced odds of OS in both Model No. 1 and Model No. 2 (HR = 0.303, 95% CI: 0.130–0.708, $p = 0.006$; HR = 0.363, 95% CI: 0.151–0.871, $p = 0.023$, respectively). This reinforces the importance of haplotype status in predicting survival outcomes, further highlighting the potential clinical significance of genetic variations represented by diplotypes.

In summary, our multivariate analysis within the Cox regression framework unraveled the intricate relationships between genetic variations, age at diagnosis, and tumor characteristics, providing a nuanced understanding of their combined impact on overall survival in this particular context. Tumor characteristics played a significant role, unveiling HRs, and reflecting their substantial impact on OS. All the results are presented in Tables 25 and 26.

Kaplan–Meier analysis was performed to generate survival curves for genotypes, alleles, and haplotypes showing significant associations with overall survival (OS) (Figures 16).

Table 24. *Cox's univariate and multivariate models for overall survival (OS) assessing adjusted ratios for associations between rs2838342, rs7276633, rs2051407, rs762400, age at diagnosis, and tumor characteristics.*

SNP	Covariates	Overall survival								
		Univariate			Multivariate					
					Model No. 1			Model No. 2		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
rs2838342	A allele+ *vs.* A–	0.465	0.232–0.931	**0.031**	0.462	0.231–0.926	**0.030**	0.802	0.382–1.686	0.561
	Age at diagnosis				1.011	0.987–1.037	0.366	1.020	0.995–1.046	0.110
	T3–T4 *vs.* T1–T2							7.463	3.195–17.432	< 0.001
	N1 *vs.* N0							1.874	0.907–3.872	0.090
	G3 *vs.* G1–G2							0.710	0.346–1.457	0.350
rs7276633	T allele+ *vs.* T–	0.465	0.232–0.931	**0.031**	0.462	0.231–0.926	**0.030**	0.802	0.382–1.686	0.561
	Age at diagnosis				1.011	0.987–1.037	0.366	1.029	0.995–1.046	0.110
	T3–T4 *vs.* T1–T2							7.463	3.195–17.432	< 0.001
	N1 *vs.* N0							1.874	0.907–3.872	0.090
	G3 *vs.* G1–G2							0.710	0.346–1.457	0.350
rs2051407	C allele+ *vs.* C–	0.418	0.204–0.858	**0.017**	0.404	0.196–0.832	**0.014**	0.604	0.285–1.281	0.189
	Age at diagnosis				1.013	0.988–1.039	0.297	1.022	0.997–1.048	0.082
	T3–T4 *vs.* T1–T2							7.484	3.227–17.355	< 0.001
	N1 *vs.* N0							1.824	0.892–3.732	0.100
	G3 *vs.* G1–G2							0.698	0.346–1.405	0.313

Table 24. *Continued.*

SNP	Covariates	Overall survival								
		Univariate			Multivariate					
					Model No. 1			Model No. 2		
		HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
rs762400	GC *vs.* GG	0.917	0.451–1.866	0.811	0.858	0.416–1.767	0.677	1.083	0.521–2.248	0.831
	CC *vs.* GG	2.550	1.098–5.923	**0.030**	2.476	1.064–5.758	**0.035**	1.865	0.785–4.431	0.158
	Age at diagnosis				1.013	0.987–1.040	0.325	1.021	0.996–1.047	0.100
	T3–T4 *vs.* T1–T2							7.546	3.250–17.520	< 0.001
	N1 *vs.* N0							1.814	0.882–3.731	0.105
	G3 *vs.* G1–G2							0.719	0.358–1.448	0.356
rs762400	G allele+ *vs.* G–	0.374	0.177–0.788	**0.010**	0.370	0.176–0.781	**0.009**	0.560	0.261–1.203	0.137
	Age at diagnosis				1.012	0.987–1.038	0.356	1.021	0.996–1.047	0.100
	T3–T4 *vs.* T1–T2							7.496	3.235–17.373	< 0.001
	N1 *vs.* N0							1.798	0.879–3.677	0.108
	G3 *vs.* G1–G2							0.728	0.365–1.452	0.367

Bolded *p*-values indicate statistical significance ($p < 0.05$).

Table 25. *Cox's univariate model for RRP1B diplotypes in PFS and OS.*

***RRP1B* diplotypes**	**Progression-free survival**			**Overall survival,**		
	HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
Heterozygous diplotype (ATCCG/alternative haplotype) *vs.* homozygous diplotype (ATCCG/ATCCG)	0.990	0.523–1.873	0.975	0.843	0.397–1.790	0.656
ATCCG haplotype non-carriers *vs.* homozygous diplotype (ATCCG/ATCCG)	2.244	0.991–5.080	0.052	2.121	0.910–4.943	0.081
Heterozygous diplotype (GCTTC/alternative haplotype) *vs.* homozygous diplotype (GCTTC/GCTTC)	0.485	0.208–1.132	0.094	0.274	0.120–0.626	**0.002**
GCTTC haplotype non-carriers *vs.* homozygous diplotype (GCTTC/GCTTC)	0.434	0.190–0.993	0.051	0.298	0.128–0.695	**0.005**
Heterozygous diplotype (ATCCG/GCTTC) carriers *vs.* non-carriers	0.872	0.479–1.588	0.655	0.694	0.362–1.331	0.271

Bolded *p*-values indicate statistical significance ($p < 0.05$).

Table 26. *Cox's multivariate models for overall survival: adjusted ratios for associations between RRP1B diplotypes, age at diagnosis, and tumor characteristics.*

SNPs	**Covariates**	**Overall Survival**					
		Model No. 1			**Model No. 2**		
		HR	**95%CI**	***p*-value**	**HR**	**95%CI**	***p*-value**
rs2838342 rs7276633 rs2051407 rs9306160 rs762400	Heterozygous diplotype (GCTTC/alternative hap) *vs.* homozygous diplotype (GCTTC/GCTTC)	0.259	0.113–0.597	**0.002**	0.372	0.153–0.904	**0.029**
	GCTTC haplotype non-carriers *vs.* homozygous diplotype (GCTTC/GCTTC)	0.303	0.130–0.708	**0.006**	0.363	0.151–0.871	**0.023**
	Age (years)	1.013	0.987–1.039	0.334	1.027	1.000–1.054	0.050
	G3 *vs.* G1–G2				0.760	0.374–1.547	0.449
	N1 *vs.* N0				1.913	0.933–3.922	0.076
	T3–T4 *vs.* T1–T2				7.412	3.196–17.188	< 0.001

Bolded *p*-values indicate statistical significance ($p < 0.05$).

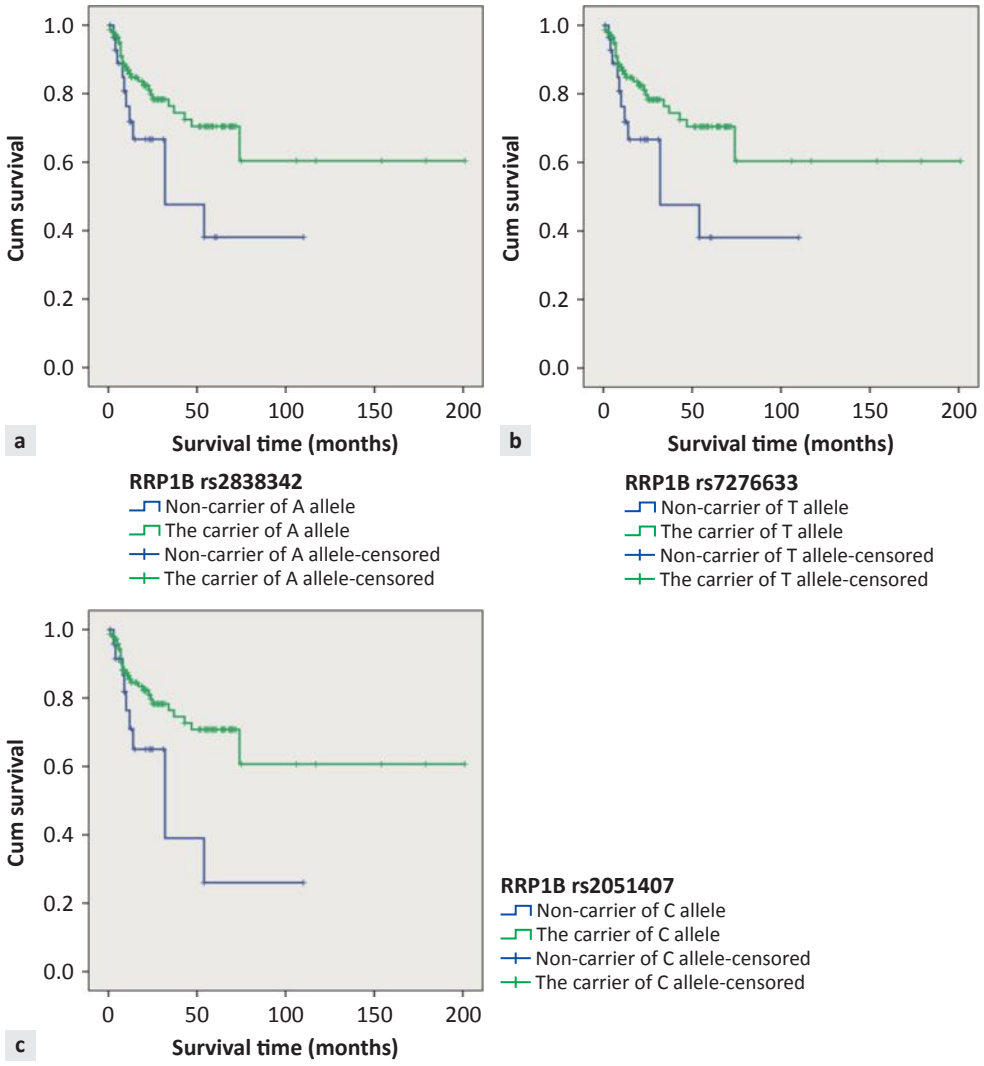

Figure 16 (a–c). *Kaplan–Meier survival curves in patients with cervical cancer according to RRP1B polymorphisms in the allelic and genotype models, and diplotypes, with a focus on the GCTTC haplotype, illustrating differences in OS.*

The y-axis displays the probability of survival, while the x-axis represents the duration in months from the diagnosis of cervical cancer, verifying the occurrence date of the event of interest (OS). Each vertical step in the curve signifies events (ie, deaths), and right-censored patients are denoted by a vertical mark in the curve at the censoring time. **(a)** Carrying the A allele of rs2838342 was associated with an increased likelihood of longer overall survival (HR = 0.465, 95% CI: 0.232–0.931, $p = 0.031$); **(b)** Carrying the T allele in rs7276633 increased the possibility for longer OS (HR = 0.465, CI: 0.232–0.931, $p = 0.031$); **(c)** Carriers of the rs2051407 C allele had an increased chance of longer OS in comparison with non-carriers (HR = 0.418, CI: 0.204-0.858, $p = 0.017$). Similarly, GCTTC haplotype non-carriers compared to the homozygous diplotype (GCTTC/GCTTC) also exhibited a significantly decreased hazard ratio for OS (HR = 0.298, 95% CI: 0.128–0.695, $p = 0.005$).

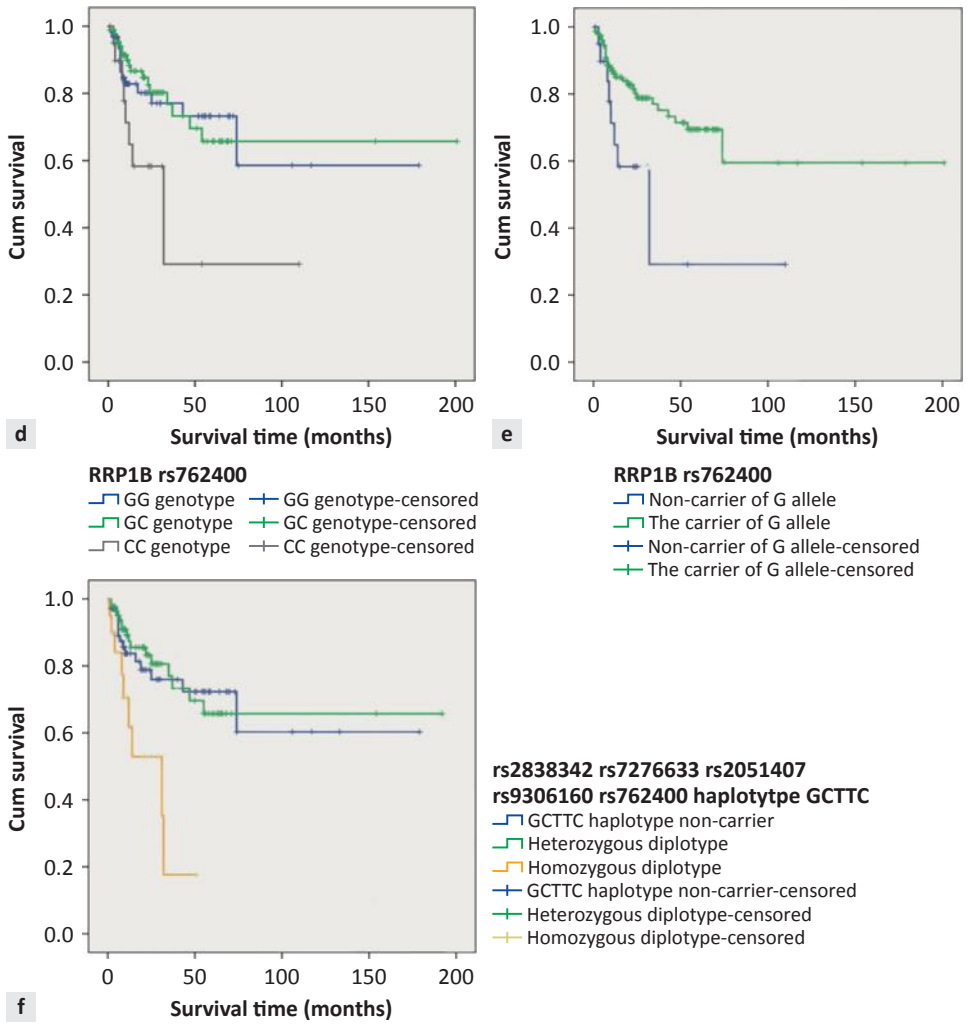

Figure 16 (d–f). *Kaplan–Meier survival curves in patients with cervical cancer according to RRP1B polymorphisms in the allelic and genotype models, and diplotypes, with a focus on the GCTTC haplotype, illustrating differences in OS.*

The y-axis displays the probability of survival, while the x-axis represents the duration in months from the diagnosis of cervical cancer, verifying the occurrence date of the event of interest (OS). Each vertical step in the curve signifies events (ie, deaths), and right-censored patients are denoted by a vertical mark in the curve at the censoring time. **(d, e)** rs762400 CC genotype increased the risk for shorter OS compared to patients with the GG genotype (HR = 2.550, 95% CI: 1.098–5.923, $p = 0.030$). Individuals carrying the G allele exhibited a heightened likelihood of longer OS compared to those without the G allele (HR = 0.374, CI: 0.177–0.788, $p = 0.010$); **(f)** The heterozygous diplotype (GCTTC/alternative haplotype) compared to the homozygous diplotype (GCTTC/GCTTC) displayed a significantly decreased hazard ratio (HR = 0.274, 95% CI: 0.120–0.626, $p = 0.002$), suggesting a possible protective effect associated with this haplotype. Similarly, GCTTC haplotype non-carriers compared to the homozygous diplotype (GCTTC/GCTTC) also exhibited a significantly decreased hazard ratio for OS (HR = 0.298, 95% CI: 0.128–0.695, $p = 0.005$).

3.5.3. Survival analysis: focus on *SIPA1* gene

The Cox's univariate analysis for the *SIPA1* SNPs rs746429, rs931127, and rs3741378 did not reveal any significant associations with progression-free survival (PFS) or overall survival (OS). The hazard ratios (HRs) and confidence intervals (CIs) indicate no significant impact of these SNPs, whether assessed by genotype (GA *vs.* GG, AA *vs.* GG for rs746429; AG *vs.* AA, GG *vs.* AA for rs931127; CT *vs.* CC, TT *vs.* CC for rs3741378) or allele (G *vs.* non-G, A *vs.* non-A for rs746429; A *vs.* non-A, G *vs.* non-G for rs931127; C *vs.* non-C, T *vs.* non-T for rs3741378). The *p*-values for all comparisons were above the threshold for statistical significance, suggesting that these particular *SIPA1* polymorphisms do not have a notable effect on PFS or OS in this cohort. All the results are presented in Supplementary Table 16.

3.5.4. Survival analysis: focus on *SRSF1* gene

Cox's univariate model for SRSF1 rs8819, rs11654058, rs2233908, and rs2585828 did not reveal any significant interactions with progression-free survival (PFS) or overall survival (OS), suggesting that these specific SNPs may not have a substantial impact on survival outcomes in this study.

The univariate analysis of SRSF1 rs34592492 genotypes on progression-free survival (PFS) showed no significant results. However, examining the influence of the G allele revealed that the G allele+ *vs.* G– comparison had a hazard ratio (HR) of 0.328 with a 95% confidence interval (CI) of 0.043 to 2.483 and a *p*-value of 0.022, indicating a significant protective effect on PFS. Moving to the multivariate analysis, two models are employed to adjust for age at diagnosis and tumor characteristics. In Model No. 1, which adjusts solely for age at diagnosis, the G allele+ *vs.* G- comparison yields an HR of 0.275, a 95% CI of 0.035 to 2.124, and a *p*-value of 0.216, indicating the previously observed significance was lost. Model No. 2 further adjusts for tumor characteristics. The G allele+ *vs.* G– comparison gives an HR of 0.351, a 95% CI of 0.043 to 2.892, and a *p*-value of 0.331, indicating no significant association. In summary, while the univariate analysis highlights potential associations, particularly the significant protective effect of the G allele of *SRSF1* rs34592492 and, these associations are not robust in the multivariate analysis when adjusting for age at diagnosis and tumor characteristics. Results are presented in Table 27.

In the univariate analysis of rs34592492 genotypes on OS, the CC *vs.* GG genotype comparison shows a substantial increase in risk with an HR of 19.947, a significant 95% CI of 2.489 to 159.836, and a *p*-value of 0.005, suggesting a strong association with reduced OS. The wide CI indicates a high degree of variability and uncertainty around the HR estimate. This could

be due to a small sample size, which limits the precision of the estimate, or substantial heterogeneity within the sample. Such a broad interval reflects that while the true HR could indicate an increased risk, it could also indicate a decreased risk or no effect at all. Examining the influence of the G allele, the G allele+ *vs.* G– comparison reveals an HR of 0.043, with a 95% CI of 0.005 to 0.348 and a *p*-value of 0.003, indicating a significant protective effect. Considering age at diagnosis, the HR is 1.012, with a 95% CI of 0.987 to 1.037, and a *p*-value of 0.364, showing no significant impact on OS.

Moving to the multivariate analysis, in Model 1, the GC *vs.* GG genotype comparison shows an HR of 0.787, with a 95% CI of 0.241 to 2.568 and a *p*-value of 0.692, indicating no significant association. The CC *vs.* GG genotype comparison maintains a high HR of 23.617, with a 95% CI of 0.241 to 196.252 and a *p*-value of 0.003, suggesting a continued significant association with reduced OS. The G allele+ *vs.* G- comparison yields an HR of 0.041, with a 95% CI of 0.005 to 0.343 and a *p*-value of 0.003, confirming its significant protective effect. Age at diagnosis in this model presents an HR of 1.012, with a 95% CI of 0.987 to 1.037 and a *p*-value of 0.355, indicating no significant effect. Model No. 2 further adjusts for tumor characteristics. The GC *vs.* GG genotype comparison has an HR of 0.748, with a 95% CI of 0.227 to 2.468 and a *p*-value of 0.633, showing no significant association. The CC *vs.* GG genotype comparison results in an HR of 12.582, with a 95% CI of 1.426 to 111.021 and a *p*-value of 0.023, indicating a significant association with reduced OS. The G allele+ *vs.* G– comparison gives an HR of 0.078, with a 95% CI of 0.009 to 0.687 and a *p*-value of 0.022, suggesting a continued significant protective effect. Age at diagnosis achieves near significance with an HR of 1.021, a 95% CI of 0.996 to 1.047, and a *p*-value of 0.096. Among tumor characteristics, the T3–T4 *vs.* T1–T2 comparison shows a significant HR of 7.738, with a 95% CI of 3.339 to 17.934 and a *p*-value of 0.000, indicating a strong association with reduced OS. The N1 *vs.* N0 comparison yields an HR of 1.894, with a 95% CI of 0.906 to 3.959 and a *p*-value of 0.090, indicating a trend towards significance. The G3 *vs.* G1–G2 comparison shows an HR of 0.697, with a 95% CI of 0.343 to 1.417 and a *p*-value of 0.319, indicating no significant association (Table 28).

In summary, the univariate analysis highlights the significant increase in risk with the CC genotype of *SRSF1* rs34592492 and a significant protective effect of the G allele. In the multivariate analysis, after adjusting for age at diagnosis and tumor characteristics, the CC genotype of *SRSF1* rs34592492 remains significantly associated with reduced OS, while the G allele maintains its significant protective effect. Age at diagnosis does not show a significant impact on OS in either model. Among tumor characteristics, the T3–T4 stage is significantly associated with reduced OS, while N stage shows a trend

towards significance, and tumor grade does not show a significant association. The wide confidence intervals, especially in the genotype comparisons, suggest variability and uncertainty, likely due to sample size or heterogeneity within the population. Kaplan-Meier survival curves were generated for OS and are shown in Figure 17.

Based on the analysis of Cox's univariate model for *SRSF1* haplotypes in progression-free survival (PFS) and overall survival (OS), no significant associations were found. Specifically, comparisons involving CTGA haplotype non-carriers and heterozygous diplotypes against the homozygous CTGA/CTGA diplotype did not yield statistically significant hazard ratios (HRs) or *p*-values. This indicates that variations in SRSF1 haplotypes do not significantly impact PFS or OS in this dataset (Supplementary Table 14).

Table 27. *Cox's univariate and multivariate models for progression-free survival (PFS) assessing adjusted ratios for associations between SRSF1 rs34592492, age at diagnosis, and tumor characteristics.*

SNP	Covariates	Progression-Free Survival								
		Univariate			Multivariate					
					Model No. 1			Model No. 2		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
rs34592492	G allele+ *vs.* G–	0.328	0.043–2.483	**0.022**	0.275	0.035–2.124	0.216	0.351	0.043–2.892	0.331
	Age at diagnosis				1.014	0.993–1.036	0.197	1.024	0.999–1.050	0.058
	T3–T4 *vs.* T1–T2							1.748	0.841–3.634	0.135
	N1 *vs.* N0							1.721	0.792–3.741	0.170
	G3 *vs.* G1–G2							0.977	0.501–1.906	0.946

Bolded *p*-values indicate statistical significance ($p < 0.05$).

Table 28. *Cox's univariate and multivariate models for overall survival (OS) assessing adjusted ratios for associations between SRSF1 rs34592492, age at diagnosis, and tumor characteristics.*

SNP	Covariates	Overall Survival								
		Univariate			Multivariate					
					Model No. 1			Model No. 2		
		HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
rs34592492	GC *vs.* GG	0.772	0.237–2.515	0.667	0.787	0.241–2.568	0.692	0.748	0.227–2.468	0.633
	CC *vs.* GG	19.947	2.489–159.836	**0.005**	23.617	0.241–196.252	**0.005**	12.582	1.426–111.021	**0.023**
	Age at diagnosis				1.012	0.987–1.037	0.364	1.022	0.997–1.047	0.088
	T3–T4 *vs.* T1–T2							7.738	3.339–17.934	0.000
	N1 *vs.* N0							1.894	0.906–3.959	0.090
	G3 *vs.* G1–G2							0.697	0.343–1.417	0.319
rs34592492	G allele+ *vs.* G–	0.043	0.005–0.348	**0.003**	0.041	0.005–0.343	**0.003**	0.078	0.009–0.687	**0.022**
	Age at diagnosis				1.012	0.987–1.037	0.355	1.021	0.996–1.047	0.096
	T3–T4 *vs.* T1–T2							7.820	3.371–18.143	0.000
	N1 *vs.* N0							1.839	0.885–3.824	0.103
	G3 *vs.* G1–G2							0.697	0.343–1.419	0.320

Bolded *p*-values indicate statistical significance ($p < 0.05$).

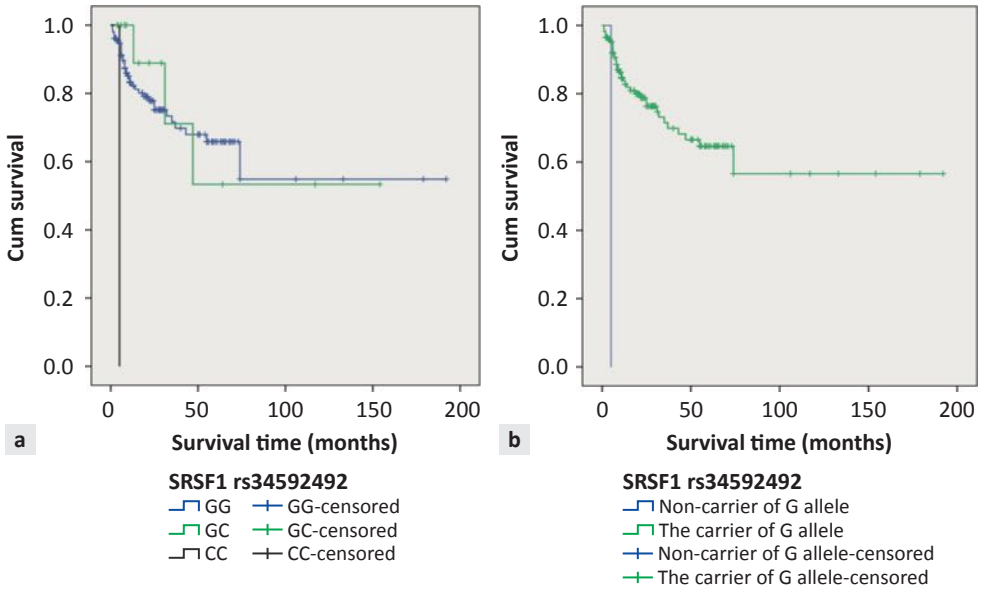

Figure 17. *Kaplan–Meier survival curves in patients with cervical cancer according to SRSF1 rs34592492 polymorphism in the allelic and genotype models, illustrating differences in OS.*

(a) The CC genotype increases the risk of shorter OS (HR = 19.947, 95% CI: 2.489–159.836, $p = 0.005$); **(b)** Carrying the G allele in rs34592492 increased the possibility for longer OS (HR = 0.043, CI: 0.005–0.348, $p = 0.003$).

3.5.5. Survival analysis: focus on *HOTAIR* gene

The analysis of Cox's univariate model for *HOTAIR* SNPs rs12826786, rs7958904, and rs920778 in progression-free survival (PFS) and overall survival (OS) revealed no significant associations between the genotypes or alleles and survival outcomes. For rs12826786, neither the CT nor TT genotypes compared to the CC genotype, nor the presence of the C or T alleles showed significant hazard ratios (HRs) or *p*-values in both PFS and OS. Similarly, for rs7958904, the CG and CC genotypes compared to the GG genotype, as well as the presence of the G or C alleles, did not demonstrate significant associations with survival outcomes. Additionally, for rs920778, neither the AG nor AA genotypes compared to the GG genotype, nor the presence of the G or A alleles, were significantly associated with PFS or OS. These findings suggest that the variations in *HOTAIR* SNPs rs12826786, rs7958904, and rs920778 do not have a significant impact on PFS or OS in this dataset. The results presented in Supplementary Table 18.

The analysis of Cox's univariate model for *HOTAIR* haplotypes in progression-free survival (PFS) and overall survival (OS), as presented in Supplementary Table 14, shows no significant associations. For the CGG haplotype, non-carriers *vs.* heterozygous diplotypes (CGG/alternative haplotype) yielded a hazard ratio (HR) of 1.171 (95% CI: 0.665–2.062, $p = 0.585$) for PFS and an HR of 1.231 (95% CI: 0.661–2.290, $p = 0.512$) for OS, indicating no significant impact on survival. Similarly, for the CGA haplotype, non-carriers *vs.* homozygous diplotypes (CGA/CGA) showed an HR of 0.691 (95% CI: 0.386–1.240, $p = 0.215$) for PFS and an HR of 0.593 (95% CI: 0.317–1.110, $p = 0.102$) for OS, both of which were not significant. The comparison between heterozygous diplotypes (CGA/alternative haplotype) and homozygous diplotypes (CGA/CGA) could not be estimated for PFS due to a zero value within a cell, and for OS, it showed an HR of 0.000 ($p = 0.976$), also indicating no significant association. For the TCA haplotype, non-carriers *vs.* heterozygous diplotypes (TCA/alternative haplotype) resulted in an HR of 1.077 (95% CI: 0.614–1.888, $p = 0.795$) for PFS and an HR of 1.119 (95% CI: 0.594–2.107, $p = 0.728$) for OS, neither of which were significant. Overall, these findings suggest that variations in *HOTAIR* haplotypes do not significantly influence PFS or OS in this dataset.

3.5.6. Survival analysis: focus on *MALAT1* gene

The analysis of Cox's univariate model for *MALAT1* SNPs rs619586, rs664589, and rs3200401 in progression-free survival (PFS) and overall survival (OS), as presented in Supplementary Table 19, indicates some significant associations. For rs619586, neither the AG genotype compared to AA nor the G allele compared to the A allele showed significant hazard ratios (HRs) or *p*-values for PFS or OS. For rs3200401, no significant associations were observed between the TC or TT genotypes compared to CC, nor between the T allele and C allele for either PFS or OS.

The analysis in Table 29 and Supplementary Table 19 investigates the associations between the *MALAT1* SNP rs664589, age at diagnosis, and tumor characteristics with overall survival (OS) using both univariate and four multivariate Cox models.

In the univariate model, the GG genotype of rs664589 showed a significant association with poorer OS (HR = 12.212, 95% CI: 1.594–93.558, $p = 0.016$). When adjusted for additional factors in multivariate models, the significance of this association decreased. In Model No. 1, adjusting for age, the association remained significant (HR = 11.615, 95% CI: 1.510–89.330, $p = 0.018$). However, in Model No. 2, which further adjusted for the N stage of the tumor, the association weakened and became non-significant

(HR = 5.729, 95% CI: 0.734–44.691, $p = 0.096$). Model No. 3, adding tumor grade, continued this trend (HR = 5.965, 95% CI: 0.754-47.210, $p = 0.091$), and by Model No. 4, which also included tumor stage, the association was further attenuated (HR = 3.449, 95% CI: 0.436–27.300, $p = 0.241$).

For the GC genotype of rs664589, there were no significant associations with OS in the univariate model (HR = 1.368, 95% CI: 0.328–5.701, $p = 0.667$) or any of the multivariate models, indicating that this genotype does not significantly impact OS.

Age at diagnosis showed no significant association with OS in the univariate model (HR = 1.009, 95% CI: 0.984–1.034, $p = 0.501$). In multivariate models, age approached significance in Models No. 2 and 3 (HR = 1.024, $p = 0.061$) but did not reach statistical significance overall. Tumor characteristics had notable impacts on OS. N1 stage (*vs.* N0) showed a strong, significant association with worse OS in Models No. 2 (HR = 3.954, 95% CI: 1.976–7.912, $p < 0.0001$) and Models No. 3 (HR = 3.883, 95% CI: 1.924–7.839, $p < 0.0001$), but this association was reduced in Model No. 4 (HR = 1.801, $p = 0.116$). Tumor grade (G3 *vs.* G1–G2) did not show a significant impact on OS. Tumor stage (T3–T4 *vs.* T1–T2) was highly significant in Model No. 4, indicating a markedly higher risk of poorer OS (HR = 7.811, 95% CI: 3.369–18.111, $p < 0.0001$).

Regarding the C allele of rs664589, a significant protective effect on OS was observed in the univariate model (HR = 0.083, 95% CI: 0.011–0.637, $p = 0.017$). This effect remained significant in the initial multivariate model (HR = 0.099, $p = 0.019$) but became non-significant in further adjusted models.

In conclusion, the analysis shows that while the *MALAT1* SNP rs664589 GG genotype is associated with poorer overall survival in the univariate model, this association weakens with adjustments for other factors in multivariate models. Age at diagnosis and tumor grade show more nuanced effects, with age approaching significance in some models. Tumor characteristics, particularly N1 stage and T3–T4 stage, demonstrate strong associations with overall survival, highlighting their importance in prognostic assessments. The rs664589 C allele initially appears to have a protective effect on overall survival, this effect diminishes and loses statistical significance when adjusting for age and tumor characteristics. This suggests that the observed protective effect may be influenced by these other factors.

Table 29. *Cox's univariate and multivariate models for overall survival (OS) assessing adjusted ratios for associations between MALAT1 rs664589, age at diagnosis, and tumor characteristics.*

SNP	Covariates	Overall survival														
		Univariate			Multivariate											
					Model No. 1			Model No. 2			Model No. 3			Model No. 4		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
rs664589	GC *vs.* CC	1.368	0.328–5.701	0.667	1.342	0.322–5.596	0.686	0.916	0.218–3.845	0.905	0.885	0.208–3.769	0.868	0.677	0.159–2.888	0.598
	GG *vs.* CC	12.212	1.594–93.558	**0.016**	11.615	1.510–89.330	**0.018**	5.729	0.734–44.691	0.096	5.965	0.7540–47.210	0.091	3.449	0.436–27.300	0.241
	Age (years)				1.009	0.984–1.034	0.501	1.024	0.999–1.050	0.061	1.024	0.999–1.050	0.061	1.019	0.994–1.044	0.145
	N1 *vs.* N0							3.954	1.976–7.912	0.000	3.883	1.924–7.839	0.000	1.801	0.865–3.751	0.116
	G3 *vs.* G1–G2										1.125	0.570–2.218	0.735	0.805	0.399–1.624	0.544
	T3–T4 *vs.* T1–T2													7.811	3.369–18.111	0.000
rs664589	C allele+ *vs.* C–	0.083	0.11–0.637	**0.017**	0.099	0.011–0.672	**0.019**	0.173	0.022–1.350	0.094	0.167	0.021–1.318	0.089	0.285	0.036–2.255	0.234
	Age (years)				1.009	0.984–1.034	0.492	1.024	0.999–1.050	0.061	1.024	0.999–1.050	0.062	1.018	0.993–1.044	0.150
	N1 *vs.* N0							3.934	1.975–7.838	0.000	3.860	1.918–7.771	0.000	1.779	0.854–3.706	0.124
	G3 *vs.* G1–G2										1.115	0.569–2.187	0.751	0.779	0.388–1.566	0.484
	T3–T4 *vs.* T1–T2													7.735	3.334–17.942	0.000

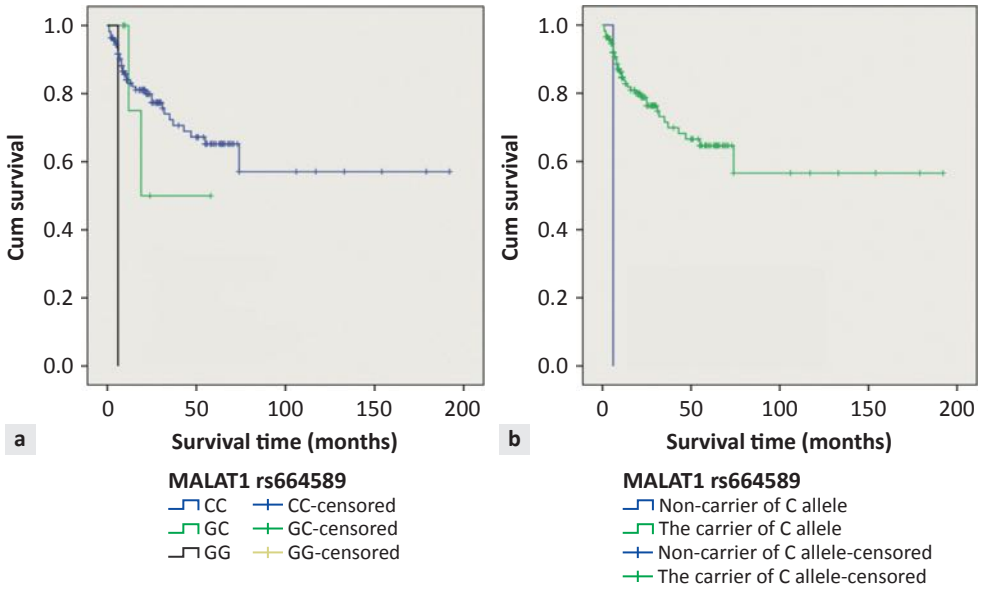

Figure 18. *Kaplan–Meier survival curves in patients with cervical cancer according to MALAT1 rs664589 polymorphism in the allelic and genotype models, illustrating differences in OS.*

(a) The GG genotype increases the risk of shorter OS (HR = 12.212, 95% CI: 1.594–93.558, $p = 0.016$); **(b)** Carrying the C allele in rs664589 increased the possibility for longer OS (HR = 0.083, CI: 0.11–0.637, $p = 0.017$).

4. DISCUSSION

4.1. Discussion of the *TLR4* gene

The active exploration of the correlation between *TLR4* SNPs and CC is highly compelling. This study represents the first to examine the specific SNPs in relation to both the clinicopathological characteristics and the progression of CC. Our findings establish a significant relationship between these SNPs in *TLR4* and the occurrence of CC, indicating their potential utility as biomarkers for prognosticating disease development. In the future, detecting SNPs in *TLR4* may enable patient stratification, prediction of clinical manifestations, assessment of risks for progression or relapse, and evaluation of treatment efficacy.

This study offers several strengths, including a comprehensive dataset that integrates genetic information, tumor phenotype data, and survival outcomes. However, there are also limitations to our research. A major challenge is the inability to compare our findings with those of other studies, as there are no existing investigations examining the associations between these polymorphisms and the clinicopathological characteristics of CC. Additionally, the limited sample size may have influenced our results. We plan to address this by expanding the study population in future research. Another notable limitation is the absence of a control group, which would have been valuable in assessing the risk of CC. While genotyping errors are anticipated to be minimal, any resulting biases are expected to be correspondingly small.

Two of the SNPs (rs10759932 and rs11536898) were significant in our analysis. Rs10759932 is located in the promoter region of the TLR4 gene and may regulate the *TLR4* expression level by influencing the binding affinity of transcription factors [691]. We found that the rare homozygous rs10759932 CC genotype is associated with shorter PFS and OS. The allelic model did not contradict the survival results. The T allele was linked to better survival, although the effect of the C allele on worse survival prognosis was not statistically significant.

However, previously published studies provide contradictory data regarding the correlation of rs10759932 with cancers. Some researchers' findings support our results, indicating that the CC genotype is an indicator of a worse outcome. T. Tongtawee et al. investigated 400 patients with gastric lesions, including chronic gastritis, gastric atrophy, intestinal metaplasia, and gastric cancer. They found that the rs10759932 CC homozygous genotype significantly increased the risk of premalignant and malignant gastric lesion development [692]. The Cleveland case-control study in Caucasians and African

Americans supported the influence of rs10759932 on prostate cancer risk. Men carrying the CC genotype for rs10759932 had a statistically significant increased risk of prostate cancer ($p = 0.006$) compared to men carrying the TT genotype [693].

On the other hand, other research provides opposite results. The study conducted in the Shandong Province of Northern China demonstrates that the rs10759932 polymorphism was associated with susceptibility to gastric cancer (GC) in both genotype and allelic frequency. However, genotype CC was identified as a protective factor for GC. The researchers believe that the genetic variant of *TLR4* rs10759932 might play an essential protective role in the development of GC [694]. Huang et al. found that the rs10759932 TC heterozygote and combined genotypes TC/CC were associated with a significantly reduced risk of gastric cancer in a high-risk population [691]. Similar results were obtained from a Japanese study where the rs10759932 TC/CC genotypes decreased the risk of gastric cancer, although this did not reach statistical significance ($p = 0.059$) [695].

Several studies have not shown any correlations between rs10759932 polymorphisms and cancer. These include studies on breast cancer in the Saudi population [686], the risk of non-cardia gastric cancer in Goyang [697], and the risk of colorectal cancer in Brazil [698]. A large nested case-control study of prostate cancer in the Physicians' Health Study (1982–2004), including 1267 controls and 1286 random prostate cancer cases, showed that genetic variation across this polymorphism is not strongly associated with prostate cancer risk or mortality [699].

The dissociation of research results may be due to sample size limitations, different ethnic groups, and the multifactorial backgrounds promoting cancer development, including genetic factors, race, environment, and lifestyle.

In our study, rs11536898 was also linked to clinical outcomes in CC patients. The rare AA genotype was associated with shorter progression-free survival (PFS) and overall survival (OS) compared to the CC genotype. Conversely, the C allele was inversely related to shorter PFS and OS. Furthermore, the AC genotype and A allele were associated with an increased risk of metastases. However, a notable association was found with the risk of prostate cancer. The Health Professionals Follow-up Study (HPFS) reported that men under 65 carrying two copies of the minor alleles of rs11536898 had a significantly lower risk of prostate cancer compared to non-carriers (CC and CA *vs.* AA: OR 0.59; 95% CI: 0.41–0.86) [700]. Nevertheless, many SNPs in this study were in high linkage disequilibrium with one another. The Physicians' Health Study found no significant association between this SNP and the overall prevalence of prostate cancer. Additionally, there were no

significant associations between the SNP and advanced, fatal, or severe cancer, nor was there evidence of associations between *TLR4* SNPs and prostate cancer-specific mortality or bone metastases [699]. Observational results from another population-based case-control study indicated that rs11536898 was associated with colon cancer, where the AA *vs.* CA/CC genotype showed a reduced colon cancer risk (OR 0.50, 95% CI: 0.29–0.87) [701]. However, other studies, including the Washington County Cancer Registry, the Maryland Cancer Registry, Sweden, and the Physicians' Health Study, did not find significant interactions between rs11536898 and cancer [699, 702, 703]. Although the data remain inconclusive, we speculate that the A allele may be associated with a worse prognosis.

Unfortunately, in our study, we did not find statistically significant associations between SNPs rs4986790 and rs4986791 and the pathomorphological features or outcomes of cervical cancer. These SNPs have been extensively studied worldwide and are potentially linked to other cancers, influencing both risk and prognosis.

Rs4986790 is a common polymorphism that results in an amino acid change from aspartate to glycine. In a study of 122 Tunisian women with cervical cancer compared to 260 healthy controls, the TLR4 polymorphism Asp299Gly (rs4986790) was associated with a higher risk of cervical cancer. The homozygous Asp/Asp genotype and the Asp allele were both linked to an increased risk of developing cervical cancer (OR 4.95, 95% CI: 1.97–13.22 and OR 5.17, 95% CI: 2.11–13.50, respectively) [704]. Another Tunisian case-control study with 130 cervical cancer patients and 260 controls found that the Asp/Asp genotype was significantly more common among cervical cancer cases at both early (I + II) and advanced stages (III + IV) compared to controls. The Asp allele was particularly associated with early-stage tumors [705].

Conversely, an Indian study involving 110 untreated cervical cancer patients and 141 healthy controls found that the minor allele G of rs4986790 was associated with an increased risk of cervical cancer, although no genotypic association was observed [178]. Pandey et al., in a study of North Indian women, found no association between rs4986790 and rs4986791 and cervical cancer risk at the genotype, allele, and haplotype levels. However, their study of 150 cervical cancer patients and 150 healthy controls did show that the Thr399Ile (rs4986791) polymorphism, particularly the Thr/Ile genotype, was significantly associated ($p = 0.044$) with stage II cervical cancer and conferred a 2.51-fold increased risk of developing cervical cancer at an early stage [706].

A study conducted in a Chinese Han population, including 1262 participants (420 cervical cancer patients and 842 controls), did not find a signi-

ficant association of rs4986791 with cervical cancer risk [707]. In evaluating other female-related cancers, a study at the Hunter Centre, Australia, comparing allele and genotype frequencies for rs4986790 between 191 endometrial cancer cases and 291 controls, found no associations with endometrial cancer risk [708]. Additionally, *TLR4* Asp299Gly and Thr399Ile alleles were not detected in a study of 105 ovarian cancer patients in northern China, indicating lower frequencies of these alleles in this population compared to other studies [709].

Among 70 women with ovarian cancer in Poland, the heterozygous variant and recessive G allele of rs4986790 were found more frequently than in 130 healthy controls, suggesting an increased risk of ovarian cancer for its carriers. However, no differences in the distribution of rs4986791 between cases and controls were observed [710]. In a study at the "Hippocratic" General Hospital of Athens, Greece, which included 261 breast cancer patients and 480 healthy individuals, Gly carriers of rs4986790 (Asp/Gly & Gly/Gly genotypes) and the Gly allele were more common among breast cancer cases ($p = 0.0031$ and $p = 0.0061$, respectively) [711]. In Saudi Arabia, rs4986790 showed a significant association with breast cancer malignancy in estrogen receptor (ER)-positive patients. Specifically, the AA genotype was significantly more frequent among patients compared to controls, while the AG genotype was less common [696].

TLR4 polymorphisms rs4986790 and rs4986791 may significantly increase the risk of gastric cancer. Two studies by Juliana Garcia de Oliveira highlight the substantial influence of these SNPs on gastric cancer risk within the Brazilian population [712, 713]. However, Garza-Gonzalez et al. found no correlation between *TLR4* polymorphisms and gastric cancer in the Mexican population [714]. Conversely, a study by Trejo-de la et al. observed that the D299G (rs4986790) polymorphism was significantly associated with duodenal ulcers and showed a trend towards association with gastric cancer in a Mexican cohort [715].

In Italy, Santini et al. found that the Thr399Ile polymorphism was linked to increased susceptibility to gastric cancer [716]. Data from a Caucasian population-based case-control study suggested that the *TLR4* +896A > G polymorphism might be a risk factor for non-cardia gastric carcinoma and its precursors [717]. However, a nested case-control study within the European Prospective Study Cancer Group reported low frequencies of risk alleles for rs4986790 and rs4986791, and the associations in the codominant model were not significant [718].

In the Ethnic Kashmiri population, no overall significance was found for gastric cancer risk, though odds ratio analysis indicated that carriers of the Asp299Gly G allele were significantly associated with tumors in the distal

part of the stomach. Conversely, carriers of the Thr399Ile T allele were associated with well-differentiated gastric adenocarcinoma [719]. A meta-analysis of gastric cancer risk suggests that *TLR4* polymorphisms (+896A/G and +1196C/T) might be associated with a significantly increased risk of gastric cancer among Caucasians [720]. However, no association between the Asp299Gly and Thr399Ile SNPs and gastric cancer susceptibility was found in Shandong Province, Northern China [694].

Regarding colorectal cancer (CRC), studies in Brazilian, Irish, Danish, and Iranian populations did not find significant effects of rs4986790 and rs4986791 on CRC risk [698, 721–723]. However, a meta-analysis indicated that *TLR4* genetic polymorphisms are associated with an increased risk of CRC among Asians, but not among Caucasians and Africans [724]. An Egyptian study found that carriers of the rs4986790 G allele were more frequent in the CRC group compared to controls, and the T allele of rs4986791 was linked to an increased CRC risk. Additionally, the rs4986791 CT/TT genotype was significantly associated with CRC. The G allele of rs4986790 was associated with CRC progression, including advanced cancer stages, high grade, positive lymph nodes (N2), and metastases [725]. A study of Russian individuals with various solid tumors suggested that the A/G genotype for the rs4986790 SNP correlated with an 80% increased risk of colorectal cancer, with rs4986790 polymorphisms being more pronounced in patients with rectal cancer specifically [726].

Large case-control studies and meta-analyses have found no significant association between rs4986790, rs4986791, and prostate cancer risk or its clinical features [698, 703, 727, 728].

Few studies have explored the relationships between these SNPs and other types of cancer. A Turkish case-control study on lung cancer (both NSCLC and SCLC) found no association between rs4986790 and lung cancer risk. However, the rs4986791 CT genotype was associated with a 3.857-fold increased risk of lung cancer compared to the CC genotype ($p = 0.041$) [729]. A study of patients with head and neck squamous cell carcinoma from Germany reported that the Asp299Gly genotype was associated with poorer disease-free survival (DFS) ($p = 0.04$) and worse overall survival (OS) ($p = 0.04$) compared to the Asp299Asp genotype. Patients with the rs4986790 wild-type genotype (*TLR4* Asp299Asp *vs.* *TLR4* Asp299Gly) had significantly longer DFS with adjuvant systemic treatment ($p = 0.004$). A similar pattern was observed for the rs4986791 SNP regarding DFS [730].

In an Indian case-control study, rs4986791 was significantly associated with an increased risk of gallbladder cancer [731]. Among melanoma patients and controls in Germany, carriers of the minor allele for the rs4986790 polymorphism had longer overall survival ($p = 0.01$) and improved survival

following metastases ($p = 0.02$) [732]. A study of Saudi Arabian patients with acute lymphoblastic leukemia (ALL) and healthy controls found that the rs4986790 and rs1927906 SNPs were associated with protective effects against ALL, with the AG genotype showing a significant protective effect ($p = 0.002$) [733].

Several meta-analyses have sought to generalize the data on *TLR4* polymorphisms. In a meta-analysis by Zhu L et al., which included 34 publications, *TLR4* rs4986790 and rs4986791 were associated with an increased overall cancer risk. The impact of rs4986790 was particularly notable in female-specific and digestive cancers, especially gastric cancer. The effect of rs4986791 was also significant for gastric cancer. However, no significant association was observed between rs4986790 and prostate cancer risk. The association between rs4986791 and cancer risk was significant in both South Asians and East Asians, but not in Caucasians [734].

Ding et al., in their meta-analysis of 55 publications, found that rs4986790 was not strongly associated with cancer risk. In contrast, the rs4986791 polymorphism was consistently linked to a reduced cancer risk in the general population. Their subgroup analysis revealed that Caucasian female-specific cancers were significantly associated with rs4986790 polymorphisms, while Asian digestive cancers were significantly influenced by the rs4986791 polymorphism [172].

While accumulated evidence suggests that *TLR4* polymorphisms may modulate the risk and development of various cancers, further replication of these findings is needed. In our study, the SNPs rs10983755, rs11536897, rs11536865, and rs1927906 did not correlate with clinical features or outcomes of cervical cancer (CC). Although rs10983755 is associated with gastric carcinogenesis and may provide some protection against *H. pylori* infection [697, 735], we found no significant association with *H. pylori* infection or with the overall survival of gastric cancer in another Chinese population study. Notably, patients with lymph node metastases undergoing postoperative chemotherapy and carrying the rs10983755 AA genotype had a hazard ratio (HR) of 0.328 compared to those with the GG + AG genotype [736].

The *TLR4* polymorphisms rs11536897 (3084), rs1927906 (3189), and rs11536865 (729G/C) are rare, and their functions remain unclear. In our study, all cases of rs11536865 were GG genotypes. There is evidence suggesting an interaction between *TLR4* polymorphisms and hepatitis C virus (HCV) status [737]. The 729GC polymorphism has been linked to an increased risk of bladder cancer, with the 729GC genotype significantly affecting lower TLR4 mRNA and protein levels, potentially leading to dysregulation of TLR4 expression [738]. In a case-control study of Korean men with prostate cancer, all 300 cases exhibited the GG genotype at rs11536897 [739]. No association

between rs11536897, rs1927906, and prostate cancer was found in a pooled Swedish case-control study and a meta-analysis by Weng et al. [703,727]. However, the rs1927906 heterozygous CT genotype was associated with a decreased cancer risk in Saudi Arabian patients with acute lymphoblastic leukemia (ALL) [733].

Our study indicates that *TLR4* SNPs rs10759932 and rs11536898 could potentially serve as markers for assessing survival prognosis in cervical cancer. Specifically, rs11536898 warrants further investigation due to its potential impact on cancer metastases. However, due to the limited sample size, additional research involving a larger cohort of cervical cancer patients is necessary to validate these findings.

Our results offer a foundation for future research on cervical cancer and other infection-related cancers, suggesting that these polymorphisms could be evaluated to understand their functional roles better. While there is growing evidence on the significance of genetic variation in the development of cervical cancer, the exploration of immune-related gene variants is still in its nascent stages. Larger studies encompassing diverse ethnic backgrounds are required to corroborate our findings. Identifying variants that influence the tumor immune response could provide targeted strategies for combating cervical cancer development and progression. In the future, detecting SNPs in *TLR4* may become a valuable tool for predicting the clinical manifestations, risk, and prognosis of cervical cancer.

4.2. Discussion of the *RRP1B, SIPA1*, and *SRSF1* genes

In our study, all examined SNPs in *RRP1B* gene exhibited significant associations with clinicopathological features of cervical cancer. Rs2838342, rs2051407, and rs762400 were linked to tumor size (T) and metastasis (M), while rs7276633 was associated with tumor size and rs9306160 was associated with metastasis. When analyzing the prognosis of the disease, considering tumor size and differentiation, significant results were observed in cases involving rs2838342, rs7276633, and rs2051407. Additionally, rs2838342 and rs7276633 were associated with the stage of the disease and patients' age groups. Based on these abundant and trending findings, it can be anticipated that *RRP1B* SNPs play a role in influencing the aggressiveness of cervical cancer and and the risk of metastasis.

Regrettably, our data could not be compared with that of other researchers, as we were unable to find publications specifically investigating and analyzing *RRP1B* polymorphisms in cervical cancer cases. Evaluating the results of rs2838342, rs7276633, rs2051407, and rs762400 polymorphisms poses

particular difficulties. In some cases, explaining the lack of correspondence between the genotypic model and the allelic model in the associations with the clinical characteristics of the tumor is challenging, especially due to the absence of published results from studies analyzing these SNPs. The analysis of these four polymorphisms clearly delineated the tendency of the more common allele to enhance overall survival. However, further replication of these findings is still needed.

A review of the global literature focused on the expression levels of RRP1B. Crawford et al. conducted research on breast cancer. Expression of RRP1B, and the activity of RRP1B expression, was investigated to be higher in low-metastatic mice inbred strains with mammary cancer compared to high-metastatic strains. Additionally, the variation in RRP1B expression within a highly metastatic mouse mammary tumor cell line was found to modify progression. Ectopic Expression of RRP1B reduced tumor growth and metastatic potential. Expression of this gene also predicted survival in human breast cancer. A significant difference in overall survival for the groups with good and poor prognosis, predicted by the RRP1B activation signature, was observed across various datasets [8, 207]. *RRP1B* has been represented as a likely biomarker for early gastric cancer. The expression level of RRP1B was significantly reduced in 76 early gastric cancer tissues compared with normal cases in the Chinese study [740]. The other study involved the analysis of 54 pairs of laryngeal tumor and adjacent normal tissues, it was revealed that RRP1B is significantly downexpressed in laryngeal squamous cell carcinoma [741]. There is a potential link between ALY (Aly/REF export factor), RRP1B, and metastasis in oral squamous cell carcinoma (OSCC). A knockdown of ALY reduces invasiveness and migration in OSCC cells, accompanied by an increase in RRP1B expression. Elevated RRP1B, alongside CD82, in ALY knockdown cells indicates that *RRP1B* may play a key role in regulating OSCC cellular invasiveness and migration [742].

Several studies have been conducted to evaluate the influence of RRP1B in non-oncological diseases. *RRP1B* is one of the genes regulating AREG (Amphiregulin) in endothelial cells, with HIF-1α playing a role in their upregulation in hypoxia. Silencing RRP1B reduces inflammation and apoptosis, highlighting its potential significance in pulmonary hypertension pathology [743]. It has been identified that *RRP1B* participates in the pathogenetic process of sepsis by regulating the activation and differentiation of lymphocytes. [744]. Based on a large-scale genome-wide association study, *RRP1B* is associated with a significant signal of blood pressure regulation [745]. The *RRP1B* gene was associated with blood pressure response to specific antihypertensive drugs, particularly atenolol [746]. The expression of *RRP1B* was analyzed in leucocytes of individuals with Down's syndrome (DS). The

results indicated that *RRP1B* showed significant upregulation in DS patients compared to the normal population [747].

It is interesting that data from the Cancer Genome Atlas (TCGA) project suggest the expression level of RRP1B is not a prognostic factor in cervical cancer survival analysis (https://tcga-data.nci.nih.gov/docs/publications/cesc_2016/, accessed on 15 December 2023).

There are few studies evaluating the associations of *RRP1B* rs9306160 polymorphisms with cancer risk or clinical data.

In our study, we found that the C allele of rs9306160 is more common and may have a significant protective effect against metastasis ($p = 0.008$). The variant T allele did not show statistically significant results, but the TT genotype increased the risk for metastasis (p-value close to the significance at 0.051). Unfortunately, we did not obtain significant associations between rs9306160 and clinical features such as lymph node metastasis, tumor differentiation, or survival rates. But if we consider that a frequent allele is a sign of a better prognosis, then when analyzing the results of other authors' studies, the data differ.

Crawford et al.'s study with breast cancer outcomes was conducted in two cohorts: one from Orange County and another from the Greater Baltimore Area. Consistent findings were observed between the cohorts, although some differences could be at tributed to cohort characteristics. They found a significant association between the variant A allele of rs9306160 and disease stage in a Caucasian cohort. The A allele was more prevalent in patients with localized disease compared to those with advanced regional or metastatic disease. The variant allele showed significant associations with various tumor characteristics, including estrogen receptor (ER) and progesterone receptor (PR) status, the presence of lymph node disease, and tumor grade. It was more fre quent in patients with ER-positive and PR-positive tumors, as well as in those with well-to-moderately differentiated tumors. Carriers of the variant allele had better breast cancer-specific survival compared to homozygous carriers of the common allele (G/G). This survival advantage was more pronounced in patients with ER-positive tumors [8].

Another study involved 1863 Dutch patients with operable primary breast cancer from Rotterdam, The Netherlands. The investigation identified a significant association of variants in rs9306160 with metastasis-free survival (MFS) ($p = 0.012$). Specifically, the study revealed a connection between the T allele of the *RRP1B* SNP (rs9306160) and a more favorable prognosis in MFS among breast cancer patients. Carrying the T allele (CT or TT genotypes) of rs9306160 was associated with a positive outcome in terms of MFS. Remarkably, this association maintained significance even in multivariate analysis, indicating that the T allele functions as an independent prognostic

factor. Notably, the association with patients' survival was confined to estrogen receptor-positive, lymph node-negative (ER+/LN−) patients ($p = 0.011$). Furthermore, combining the genotypes of two genes (*SIPA1* and *RRP1B*) demonstrated a significant ability to discriminate patients with poor metastasis-free survival (HR: 0.40, 95% CI: 0.24–0.68, $p = 0.001$). It is important to acknowledge the study's limitations, as the observed association was significant only for a specific subgroup (ER+/LN− patients) and not for other patient subgroups (ER+/LN+, ER−/LN+, ER−/LN−). The study was conducted within a Dutch patient population, and to establish broader applicability, the results may require validation in diverse populations [748].

On the other hand, the study of Nanchari et al., which included 493 breast cancer cases and 558 age-matched healthy female controls, could reflect a guideline for the results we obtained. The TT genotype and T allele frequencies of the *RRP1B* rs9306160 (1307T>C) polymorphism were significantly elevated in breast cancer cases compared to controls. The presence of the T allele conferred a 1.75-fold increased risk for breast cancer development. The TT genotype was associated with a higher risk under codominant and recessive models. Moreover, the TT genotype frequency was significantly elevated in obese patients, patients with advanced disease, and those with increased tumor size. The T allele was associated with positive lymph node status and Her2-negative receptor sta tus. In silico analysis of RNA secondary structures near the SNP site indicated that the T allele may result in a less stable mRNA structure compared to the C allele, potentially affecting functional interactions. The study suggests that the TT genotype may increase the risk for both breast cancer development and progression. It acknowledges deviations from the Hardy–Weinberg equilibrium and suggests the possibility of selective forces influencing genotype frequencies over generations. Additionally, the study highlights discrepancies in results compared to other cohorts, possibly due to ethnic variations. The C allele was more frequent in both controls and breast cancer cases, indicating that the C allele was more prevalent in both groups. However, there were differences in allele fre quencies between controls and breast cancer cases. It is important to note that the find ings are specific to the population studied (Southern Indian) and may not be directly applicable to other populations [749].

Earlier research from Lithuania characterized a group of young Lithuanian patients with breast cancer. Consistent with our findings, the prevalence of the C allele of rs9306160 (c.436T4C) was higher, constituting 59.5% in the allelic model. The study revealed a statistically significant association between rs9306160 and tumor grade (G). Specifically, the T allele was significantly linked to G3 tumor grade (high-grade tumors), indicating a higher probability of G3 grade in carriers of the T allele. This association remained

significant after adjustments, including age at diagnosis, tumor receptor status, tumor size, and lymph node involvement, suggesting an independent effect of the polymorphism on this breast cancer characteristic. The C allele was associated with ER-positive status, implying a higher likelihood of positive ER in individuals with the CC genotype or carriers of the C allele. Therefore, these findings support the notion of the T allele as a worse prognostic factor [750].

Moreover, a case-control study involving 100 Iraqi women (75 with confirmed breast cancer and 25 with normal breast tissue) could also corroborate the observed trend in our results. The results indicated a higher frequency of the CC genotype in the control group. The homozygous TT genotype was associated with histologic grade, and this association remained significant across all grades. Among cancer patients with a high-grade variant, T alleles were more prevalent compared to those in low-grade conditions. Furthermore, the (TT) genotype was more frequently observed in breast cancer cases with metastatic lymph node involvement compared to cases without lymph node involvement [751].

Our extended haplotype analysis of the investigated SNPs revealed that GCTTC haplotype non-carriers, predominantly consisting of ATCCG haplotypes, were less likely to exhibit advanced tumor size and metastasis. These findings were consistent with the results obtained from allelic models. The same trend was also noted in survival assessments. Consequently, we posit that these haplotypes could serve as independent markers.

In the present study, we examined the associations between five functional SNPs in the RRP1B gene and the clinicopathological profiles and survival rates in a cohort of Lithuanian women with cervical cancer. Our study is the first to analyze *RRP1B* SNPs for assessing the clinicopathological features and progression of CC. It establishes a link between SNPs in *RRP1B* and CC, suggesting these genetic variants as predictive biomarkers for prognosticating the development of the disease in the future. The study boasts several strengths, including a comprehensive dataset comprising genetic data, tumor phenotype information, and survival data. However, certain limitations warrant consideration. Notably, the absence of comparable studies on associations between these polymorphisms and clinicopathological characteristics of CC prevents a direct comparison of our results. Additionally, the limited sample size may have influenced the robustness of our findings. Furthermore, a notable weakness is the absence of a control group, hindering the assessment of CC risk.

Our investigation indicates a potential link between *RRP1B* polymorphisms and the pathomorphological features of cervical cancer, as well as disease outcomes. The association of these genetic variations with the aggres-

siveness of cervical cancer underlines the importance of considering germline factors in understanding cancer behavior. This observation opens avenues for further research to elucidate the mechanistic basis of *RRP1B*'s involvement in metastatic processes and its clinical implications. While *RRP1B* may not traditionally be classified as an oncogene, we believe that its inclusion in our investigation offers a unique opportunity to uncover novel facets of the disease's molecular underpinnings. Importantly, our decision to study *RRP1B* stems from a comprehensive approach aimed at elucidating the full spectrum of genetic factors contributing to cervical cancer development and progression. We recognize that the complexity of cancer biology extends beyond well-established oncogenes, and ex ploring genes like *RRP1B* allows us to broaden our understanding of the disease.

All investigated *RRP1B* polymorphisms (rs2838342, rs7276633, rs2051407, rs9306160, and rs762400) in our study have the potential to serve as markers for clinical characteristics and prognosis in cervical cancer. Among these, three (rs2051407, rs9306160, and rs762400) were found to be significant in relation to metastasis, while rs2838342 showed potential association with metastasis. Rs2838342, rs7276633, rs2051407, and rs762400 showed the assotiations with survival outcomes. Haplotypes analysis was in line with the allelic models. These results highlight the intricate interplay between genetic factors and clinical dynamics in the progression of tumors. Nevertheless, it is crucial to acknowledge that certain comparisons did not attain statistical significance, possibly owing to the relatively small sample size. Considering the clinical context is imperative, it is essential to interpret the results cautiously, especially for genotypes or alleles with borderline significance levels. Our results offer insights for subsequent studies on cervical cancer and other cancer types, examining these polymorphisms to ascertain their functionality. In the future, SNP detection in *RRP1B* may serve as a predictive tool for assessing the clinical manifestations and prognoses of cervical cancer.

In our study for the *SIPA1* SNPs rs931127 and rs3741378 SNPs, no significant associations with tumor phenotype were found. However, the rs746429 SNP was significantly linked to tumor differentiation (G), with the GA genotype and A allele associated with a decreased risk of poorly differentiated tumors (G3). Multivariate analyses confirmed that both the GA genotype and A allele offer protection against aggressive tumor characteristics. Additionally, the GA genotype and A allele were linked to a lower likelihood of being under 50 years old and a reduced risk of poor prognosis. Overall, rs746429, particularly the GA genotype and A allele, may help improve cervical cancer prognosis by mitigating poor tumor differentiation and adverse outcomes. Survival analysis showed no significant associations

between *SIPA1* SNPs rs746429, rs931127, and rs3741378 with progression-free survival (PFS) or overall survival (OS).

In comparison with other research on these three SNPs, only one study specifically addressed their role in cervical cancer. The study by Brooks et al. examined the association of SNPs in *SIPA1* with nodal metastases in early-stage cervical cancer. Their cohort included 101 patients with positive lymph nodes and 273 patients with negative lymph nodes as controls. Brooks et al. [752] similarly found no significant correlations between rs746429 and rs931127 with cervical cancer survival. They did not assess tumor differentiation, metastasis, or prognosis. However, the G allele at both rs931127 and rs746429 in *SIPA1* was associated with nodal disease overall. Specifically, in patients with smaller stage I B1 tumors, the G allele was linked to an increased risk of nodal metastases for both SNPs. Conversely, this association was not significant in stage I B2 tumors, which are larger lesions. The G allele in SIPA1 at rs746429 and rs931127 was significantly associated with nodal disease in patients without lymphovascular space invasion (LVSI), which was considered as an independent poor prognostic. The GG genotype was linked to a higher risk of nodal disease in both SNPs among patients without LVSI. Histologically, these SNPs were not related to histology types such as adenocarcinoma or squamous cell carcinoma. Additionally, the SNP genotype distributions in our study and those of Brooks slightly differ, and our study lacked a control group.

In studies of other cancers, research on the associations between seven *SIPA1* SNPs and recurrence and survival in 1,015 patients with primary breast cancer found that rs746429 was not associated with overall survival or recurrence [753].

Qu et al. investigated functional SNPs in the *SIPA1* gene for their impact on breast cancer risk and survival among Chinese women. The study included 1,134 breast cancer patients and 1,234 age-matched community controls from the Shanghai Breast Cancer Study, a large case-control study. The analysis revealed that *SIPA1* polymorphisms did not affect breast cancer risk. However, certain SNPs were linked to overall survival. Specifically, the GA/AA genotypes of rs746429 showed a marginally significant association with poorer overall survival compared to the GG genotype, with the effect being more pronounced in early-stage cancer patients than in late-stage patients. Conversely, the AA genotype of rs3741378 was associated with improved overall survival compared to the GG genotype. No significant association was found between *SIPA1* polymorphisms and disease-free survival. These results suggest that *SIPA1* genetic variations may influence breast cancer progression [754].

The *SIPA1* c.2760G>A (rs746429) polymorphism was significantly linked to lymph node status in the Lithuanian breast cancer cohort. Patients with the

GA genotype had a lower likelihood of positive lymph nodes compared to those with the GG genotype, with this association persisting after adjusting for age and tumor receptor status. In the case of the *SIPA1* c.545C>T (rs3741378) polymorphism, it was a significant prognostic factor for progression-free survival (PFS) and metastasis-free survival (MFS). Patients with the CT genotype had shorter PFS and MFS compared to those with the CC genotype, and T allele carriers had a higher risk of shorter MFS. Even after adjusting for age, treatment, and other factors, the CT genotype remained a strong negative predictor for both PFS and MFS [750].

Genetic variation in *SIPA1*, linked to aggressive breast tumors, was tested for associations with breast cancer risk and survival in two large case-control studies in Poland and Britain. Three *SIPA1* SNPs (rs931127, rs3741378, and rs746429) were genotyped in over 4,000 cases and controls. No significant associations were found between these variants and breast cancer risk or overall survival. The results do not support a link between *SIPA1* polymorphisms and breast cancer risk or prognosis [755].

Roberts et al. analyzed 154 SNPs in 12 metastasis-related genes, including rs746429, rs931127, and rs3741378, in a cohort of 2,671 women (European-American and African-American) to explore associations with breast cancer risk based on LN and ER status. Using the adaptive rank truncated product (ARTP) method, SIPA1 was significantly associated with ER− breast cancer. However, no single-SNP associations were significant after false discovery rate (FDR) adjustment [756].

In a case-control study conducted by Hsieh et al. on a Caucasian cohort in Queensland, Australia, 200 women diagnosed with breast cancer were compared to a control group of 200 women with no history of cancer. The results revealed a significant association with the *SIPA1* SNP rs3741378, where the CC genotype was more prevalent in the breast cancer group than in the cancer-free control group, suggesting that the variant C allele is linked to a higher incidence of breast cancer. While it showed association with hormonal receptor status in breast cancer group in a previous pilot study. While other *SIPA1* SNPs (rs931127 and rs746429) did not show a significant link to breast cancer incidence, they were associated with lymph node metastasis in earlier research, indicating *SIPA1*'s potential involvement in various stages of breast cancer progression [757].

In a study by Mackawy et al., 80 Egyptian women (50 breast cancer patients and 30 controls) were analyzed to examine the association of *SIPA1* SNPs with breast cancer risk and prognosis. The rs3741378 TT genotype was significantly associated with increased breast cancer risk, advanced tumor stages, higher grades, and lymph node metastasis. The T allele also correlated with negative ER status. In contrast, the rs746429 SNP showed no significant

association with breast cancer risk or progression. These findings suggest that the *SIPA1* rs3741378 C>T SNP may serve as a biomarker for breast cancer risk and progression [758].

Nguyen et al. investigated the association between the rs3741378 SNP in the *SIPA1* gene and breast cancer in a cohort of 50 breast cancer patients and 50 healthy controls from the Vietnamese population. The preliminary findings revealed a significant association between the C allele and an increased risk of breast cancer ($p = 0.006$, OR = 2.843). However, the analysis did not find a significant correlation between the mutant genotypes and the disease [759].

Yi et al. conducted a meta-analysis evaluating the association between three common *SIPA1* SNPs (rs746429, rs931127, and rs3741378) and breast cancer risk, pooling data from four studies with 4,907 cases and 5,294 controls. They found that the rs746429 GG and GA genotypes were linked to a reduced risk of breast cancer, while rs931127 and rs3741378 showed no significant association with the disease. Specifically, for the rs746429 SNP, significant reductions in breast cancer risk were observed for GG *vs.* AA (OR 0.88, $p = 0.04$), GA *vs.* AA (OR 0.88, $p = 0.03$), and GG+GA *vs.* AA (OR 0.88, $p = 0.04$). However, no significant difference was found between GG *vs.* GA+AA. For rs931127, no significant differences were observed: AA *vs.* GG, AG *vs.* GG, AA+AG *vs.* GG, and AA *vs.* AG+GG. Similarly, no significant association was found for rs3741378: CC *vs.*, CT *vs.* TT, CC+CT *vs.* TT, and CC *vs.* CT+TT [232].

In the study by Gdowicz-Kłosok et al., 351 Caucasian patients with inoperable non-small cell lung cancer (NSCLC) were analyzed, with most cases (91.5%) in advanced stages (IIIA–IV). The focus was on the *SIPA1* -313A>G (rs931127) polymorphism's impact on overall survival (OS) and progression-free survival (PFS). The minor allele frequency of the *SIPA1* -313G allele was 0.39, and genotype distribution followed Hardy-Weinberg equilibrium. The GG genotype was linked to significantly shorter PFS and OS, particularly in advanced-stage patients and those receiving radiotherapy alone. In all patients, GG homozygotes had a higher risk of disease progression under both codominant and recessive models. In advanced stages, the GG genotype further increased the risk of progression. For OS, the GG genotype was associated with worse outcomes in patients treated with radiotherapy alone (HR 2.41, $p = 0.020$ for OS; HR 2.34, $p = 0.020$ for PFS). Multivariate analysis confirmed the GG genotype as an independent predictor of poor PFS, especially when combined with factors like advanced stage, lack of chemotherapy, low radiation dose, and high smoking exposure. The study suggests that the SIPA1 -313A>G polymorphism, particularly the GG genotype, may serve as a biomarker for poor prognosis in unresectable NSCLC, indicating

earlier progression and reduced survival, especially in patients treated with radiotherapy alone [760].

Further research is needed to validate these findings on *SIPA1* gene polymorphisms and to understand the underlying mechanisms.

In our study, we investigated the *SRSF1* SNPs rs8819, rs34592492, rs11654058, and rs2233908 to explore potential associations with tumor phenotypes, prognosis, and patient age. However, no significant correlations were observed between the genotypes or alleles of these SNPs and the clinical outcomes examined.

When focusing on the *SRSF1* SNP rs34592492, we observed that the presence of the G allele (G+ *vs.* G–) appeared to confer a significant protective effect on progression-free survival (PFS). This suggests that the G allele may play a beneficial role in disease progression. However, the univariate analysis comparing the CC and GG genotypes for overall survival (OS) indicated a substantial increase in risk associated with the CC genotype, implying a potentially detrimental effect. The wide confidence interval (CI) around the hazard ratio (HR) estimate suggests a high degree of variability and uncertainty, likely due to our relatively small sample size. This limitation restricts the precision of our estimates and may reflect substantial heterogeneity within our sample population. We should note that the allele distribution in our sample differs significantly from the European population data ($p < 0.05$).

Further analysis using Cox's univariate model to assess the impact of *SRSF1* haplotypes on PFS and OS revealed no significant associations. Additionally, the evaluation of *SRSF1* diplotypes did not uncover any meaningful connections with clinical characteristics or patient survival outcomes.

Our ability to contextualize these findings within the broader scientific literature is constrained by the limited number of studies available on the specific *SRSF1* SNPs we investigated, namely rs34592492, rs11654058, rs2233908, and rs2585828. Of the few studies that have been conducted, only two explored the rs8819 variant. One study found no association between the SRSF1 rs8819 variant and pancreatic cancer in a case-control study involving 298 pancreatic cancer patients and 525 cancer-free controls from Central China [761]. Another study by Yang et al. identified a relationship between the T/C polymorphism at rs895819 and bipolar disorder, although this does not directly pertain to cancer phenotypes [762].

While evidence is accumulating regarding the significance of genetic variation in the etiology and development of cervical cancer, research exploring the role of metastasis-related gene variants in cervical cancer is still in its early stages. In conclusion, while our findings offer some insights into the potential influence of SRSF1 polymorphisms on cancer phenotypes, it is premature to draw definitive conclusions. The limited availability of related

studies, combined with the variability and uncertainty in our results, underscores the need for further research to clarify the role of *SIPA1* and *SRSF1* polymorphisms in cancer development and progression.

4.3. Discussion of the long non-coding RNAs HOTAIR and MALAT1

For the *HOTAIR* variants rs12826786, rs7958904, rs920778, and their haplotypes, no significant associations were found between these genotypes, alleles, or haplotypes and tumor characteristics (such as size, nodal involvement, metastasis, differentiation, stage, prognosis) or patient survival in our study

However, a review of the global literature reveals that some researchers have found significant associations, suggesting that rs12826786, rs7958904, and rs920778 are related to various cancers and other diseases.

In a study conducted in a northern Chinese population involving 515 gastric cardia adenocarcinoma (GCA) patients and 654 controls, higher HOTAIR expression was observed in tumor tissues compared to normal tissues. Among the three *HOTAIR* SNPs investigated, the T allele of rs12826786 was associated with an increased risk of GCA, and this risk was correlated with smoking habits and advanced TNM stage. Elevated HOTAIR expression was also linked to poorer survival outcomes in GCA patients [763].

A higher percentage of the T/T homozygous variant allele of *HOTAIR* rs12826786 C>T was found in Egyptian patients with breast cancer in the study by Aglan et al. This case-control study included 46 patients with pathologically proven invasive breast cancer and 49 age-matched healthy individuals as a control group [764].

In a hospital-based case-control study of breast cancer (BC) in a Turkish population, comprising 123 BC patients and 122 age-matched healthy controls, the TT genotype of the *HOTAIR* rs12826786 C>T polymorphism was associated with an increased risk of developing BC. This was observed in both and recessive models. Additionally, the TT genotype was significantly linked to worse clinicopathological features, including advanced TNM stage (III and IV), larger tumor size (T3 and T4), distant metastasis (M1), and poor histological grade (III) [765].

In a study involving 151 prostate cancer (PCa) cases and 180 cancer-free controls from a Caucasian ethnic group, the CC genotype of rs12826786 was significantly associated with shorter biochemical recurrence-free survival in patients with pT3-stage PCa [766].

In a meta-analysis by Li et al. on *HOTAIR* polymorphisms and cancer susceptibility, 12 case-control studies with 6,187 cases and 6,897 controls were included. For rs12826786 (C>T), pooled analyses of 1,048 cases and 1,432 controls indicated a significant increase in cancer susceptibility across recessive, dominant, allelic, and homozygous models (TT *vs.* CC: OR = 1.670; CT + TT *vs.* CC: OR = 1.233; TT *vs.* CT + CC: OR = 1.551; T *vs.* C: OR = 1.237), consistent with results from the hospital-based control subgroup [767].

A meta-analysis by Liu et al., which included 116 studies involving 122,832 subjects, found a significantly increased risk of cancer associated with the rs12826786 polymorphism [768].

In contrast, a study involving 106 Portuguese bladder cancer patients and 199 cancer-free controls, all of Caucasian ethnic background, found no association between rs12826786 genetic variants and the risk of developing bladder cancer. However, survival analysis revealed rs12826786 CC genotype was linked to better survival outcomes, particularly in male patients and in those with primary tumors classified as pathological stage pT2 [769].

The study by Xavier-Magalhães et al. investigated the effects of rs12826786 on glioma susceptibility and prognosis in a Portuguese population. No significant associations were found between these SNP and glioma risk. However, the rs12826786 CT genotype was linked to higher HOTAIR expression, and rs12826786 CT genotypes were associated with longer survival in patients with anaplastic oligodendroglioma [770].

Pooled analyses by Wang et al. on *HOTAIR* polymorphisms and breast cancer susceptibility included data on the rs12826786 polymorphism, with a total of 465 cases and 550 controls. The results indicated that rs12826786 was significantly associated with a decreased risk of breast cancer under the recessive, homozygous, and heterozygous models within the hospital-based control subgroup [771].

However, other studies did not find any significant association between rs12826786 and cancer. Similarly, our study did not find any significant results.

In a case-control study of the Iranian population involving 122 breast cancer patients and 200 controls, no significant differences were observed in the allele and genotype frequencies of rs12826786 between the case and control groups [772].

In the hospital-based case-control study involving 105 gastric cancer (GC) cases and 207 healthy controls from the Turkish population, no statistically significant differences were observed in the allele or genotype distributions of the *HOTAIR* rs12826786 C>T polymorphism between GC patients

and healthy controls. This suggests a lack of association between this polymorphism and gastric cancer risk in this population [773].

Dadas et al. reported that the *HOTAIR* polymorphism rs12826786 T>C was not significantly associated with an increased risk of lung cancer in any genetic inheritance models within the Turkish population. The study, which was a hospital-based case-control analysis, involved 180 participants, including 87 lung cancer cases (71 males and 16 females) and 93 healthy controls (67 males and 26 females) [774].

In a pooled case-control study by Xu et al., a total of 8 studies involving 1,532 cases and 2,113 controls were analyzed for the rs12826786 polymorphism. The combined analyses indicated a significantly increased cancer risk in four out of five genetic models. However, no significant association was found for specific cancer types or among different ethnic subgroups [775].

In the meta-analysis by Ke et al., examining the impact of SNP rs12826786 on lung cancer susceptibility, six studies involving 1,715 lung cancer patients and 2,745 healthy controls from China, Turkey, and Japan were included. No significant association was found between these SNPs and lung cancer susceptibility [776].

In a study by Kashani et al., the rs12826786 C>T was analyzed in 53 individuals with Hodgkin's lymphoma (HL) and 245 unrelated healthy controls. The results indicated that neither the overall chi-square comparison between cases and controls nor the logistic regression analysis revealed any significant association between *HOTAIR* polymorphisms and HL [777].

Additionally, we were unable to compare our data with existing literature, as there are no published studies on rs12826786 and cervical cancer.

In the case of *HOTAIR* rs7958904, a relationship was identified between the rs7958904 CC genotype and an elevated risk of cervical cancer compared to the GG/GC genotypes. Analysis of TCGA data revealed that tissues with the rs7958904 CC genotype exhibited higher levels of *HOTAIR* expression than those with the GG genotype. These findings suggest that the rs7958904 polymorphism in *HOTAIR* may affect susceptibility to cervical cancer [778].

In a case-control study of 850 individuals, including 450 colorectal cancer (CRC) patients and 400 healthy controls from CHA Bundang Medical Center (South Korea), the rs7958904 variant showed significant differences in genotype frequencies between CRC patients and controls. The rs7958904 CC genotype had a significantly higher mortality rate than the GG genotype, and the rs920778 CC genotype was associated with higher mortality than the TT genotype. Additionally, these *HOTAIR* variants were linked to altered mRNA expression levels ($p < 0.01$) [779].

A study conducted in a Chinese Han population involved a total of 1,939 participants, comprising 969 breast cancer cases and 970 healthy controls.

The study found that the C allele of rs7958904 showed significant differences between cases and controls in single locus analyses. Multivariate analyses further revealed that individuals with the rs7958904 CC genotype had a higher risk of developing breast cancer compared to those with the GG homozygous genotype [780].

In the Chinese population, the rs7958904 variant was not associated with susceptibility to head and neck squamous cell carcinoma [781].

As for rs7958904, several studies have indicated that the C allele is associated with a significantly decreased risk of osteosarcoma [782], colorectal cancer [783], and ovarian [784] compared to the G allele, which contrasts with the results of the study by Lin et al. This may be interpreted by the different susceptibilities to a disease among the different populations and the different kinds of cancer could have various etiologies, which involve diverse genetic or epigenetic modifications.

For the rs7958904 polymorphism, a meta-analysis by Ge et al. on cancer susceptibility in Caucasians and Asians examined various cancers, including gastric cancer (GC), esophageal squamous cell carcinoma (ESCC), osteosarcoma, breast cancer (BC), and colorectal cancer. The analysis included four case-control studies with 7,179 cases and 5,957 controls. These studies detected a significantly decreased susceptibility to overall cancer in all five genetic models, except for the heterozygous model [785].

In a meta-analysis by Li et al. on *HOTAIR* polymorphisms and cancer susceptibility, the rs7958904 (G>C) polymorphism, analyzed in six eligible studies within the Chinese population (totaling 5,123 cases and 5,701 controls), was associated with a significantly decreased overall cancer risk in most genetic models (C *vs.* G; CC *vs.* GG; GC *vs.* GG; GC/CC *vs.* GG; CC *vs.* GG/GC), except in the recessive and homozygous models [786].

A significant reduction in cancer risk was observed for the rs7958904 polymorphism, both overall and specifically within the colorectal cancer group, in a meta-analysis by Zhang et al. that covered 13 studies with 7,151 patients and 8,740 controls [787].

A case-control study of Korean women with primary ovarian insufficiency (POI) included 134 women with POI and 383 control women. The study found that the AA genotype of rs4759314 combined with the GC genotype of rs7958904 was associated with a decreased risk of POI ($p < 0.05$). In contrast, the GG genotype of rs1899663 combined with the GC genotype of rs7958904 was linked to an increased risk of POI ($p = 0.003$). Haplotype analysis revealed that certain haplotypes involving these polymorphisms were associated with either an increased or decreased risk of POI [788].

Analyzing the results of rs920778, a Chinese study involving 510 cervical cancer patients and 713 cancer-free controls found a significant association between the *HOTAIR* rs920778 polymorphism and cervical cancer risk, with the TT+CT genotypes showing a higher risk (adjusted OR = 1.51). The T allele was linked to increased risk in a dose-dependent manner, particularly in advanced-stage patients (adjusted OR = 2.17). Compared to the wild-type CC genotype, advanced-stage patients with CT+TT genotypes had a 2.17-fold increased risk. HOTAIR expression was higher in cervical cancer tissues than in normal tissues and was associated with the risk allele T. Cervical cancer tissues with CT and TT genotypes had significantly higher HOTAIR levels compared to those with the CC genotype. Analysis of 91 tissue pairs showed elevated HOTAIR levels in 72.5% of cancer cases *vs.* adjacent noncancerous tissues, and high HOTAIR expression correlated with advanced TNM stages (II+III+IV). The rs920778 polymorphism was strongly associated with cervical cancer, and high HOTAIR expression was associated with the risk allele T. These results confirm that the functional SNP rs920778 regulates HOTAIR expression and may ultimately influence the predisposition for cervical cancer [789].

In the Chinese population meta-analysis, involving 21 case-control studies, the rs920778 polymorphism in *HOTAIR* was significantly linked to cervical cancer susceptibility. Analysis of five studies with 1,884 cases and 2,087 controls revealed that rs920778 was associated with increased cancer risk in several genetic models: allele model (T *vs.* C: OR = 1.54, $p = 0.001$), homozygote model (TT *vs.* CC: OR = 1.74, $p = 0.03$), and recessive model (TT *vs.* TC + CC: OR = 1.84, $p = 0.01$). No publication bias was detected, and sensitivity analysis confirmed robustness of the results despite high heterogeneity in some models [790].

In a comparison with our study, we assume that the minour A allele is complementary to T, then in our study, the rs920778 A allele increased the risk for worse clinicopathological features in cervical cancer, although this result did not reach statistical significance.

Weng et al. analyzed the impact of *HOTAIR* SNP rs920778 on cancer recurrence and patient survival in Taiwanese women with cervical cancer. The study included 116 patients with invasive cervical cancer, 96 with preinvasive lesions, and 318 controls. No significant genotypic differences were found between patients and controls or among invasive cancer cases. However, genotype GG of rs920778 was associated with a significantly higher probability of cancer recurrence and worse overall survival. rs920778 may serve as an independent predictor for cancer recurrence and overall survival in cervical cancer patients [791].

Zhang et al. investigated the association between three haplotype-tagging SNPs (htSNPs) in the *HOTAIR* locus and the risk of esophageal squamous cell carcinoma (ESCC), focusing on the functional significance of the rs920778 SNP. The study involved three independent case-control sets, comprising 2098 ESCC patients and 2150 matched controls from Jinan, Shijiazhuang, and Huaian. The rs920778 TT carriers had a significantly increased risk of ESCC, with 1.37-fold, 1.78-fold, and 2.08-fold higher risk in the Jinan, Shijiazhuang, and Huaian populations, respectively, compared to CC carriers. Functional analysis revealed a novel intronic HOTAIR enhancer between +1719bp and +2353bp from the transcriptional start site. The rs920778 SNP showed allelic regulation of HOTAIR expression through this enhancer, with higher expression in T allele carriers [792].

In the Chinese population study, rs920778 was significantly associated with lung cancer susceptibility, particularly among male smokers with squamous cell carcinoma. Specifically, individuals with the C/T (C/T + TT) genotype for rs920778 showed a higher risk of developing lung cancer [793].

A case-control study in a Chinese population genotyped *HOTAIR* SNPs in hepatocellular carcinoma (HCC) with 482 cases and 520 controls. The *HOTAIR* rs920778 TT genotype, compared to the CC genotype, was significantly associated with increased HCC risk, particularly among drinkers and HBV-positive individuals. Additionally, the TT genotype was linked to higher HOTAIR expression and greater HCC cell proliferation [794].

In the northeastern Chinese population, rs920778 was significantly associated with breast cancer susceptibility and prognosis. The GG genotype of rs920778 showed a higher risk for breast cancer, with an OR of 2.426 compared to the AA genotype, while the AG genotype had an OR of 1.296. Both the GG and AG genotypes were linked to worse disease-free survival (DFS) ($p = 0.012$) [795].

In a study by Yan et al. involving 502 breast cancer cases and 504 cancer-free controls in a Chinese population, the rs920778 variant in *HOTAIR* was found to significantly increase the risk of breast cancer. Additionally, this variant may interact with reproductive factors, potentially influencing the progression of the disease [796].

However, Yan et al. found that the A allele is the most common genotype in the central Chinese population and is associated with an increased risk of breast cancer. This finding contrasts with studies from northeast China, southeast Iran, South India, and Turkey (including Lv et al. study, as well as those by Hassanzarei et al., Rajagopal et al., and Bayram et al., respectively). The distribution of rs920778 genotypes among breast cancer patients varies slightly across these studies.

A similar result regarding poor overall survival (OS) in cervical cancer was found in Weng et al.'s study [791].

In Bayram's study of 245 Turkish women, including 123 breast cancer patients and 122 healthy controls, the rs920778 SNP was found to be linked to several clinicopathological characteristics. The CC genotype significantly increased breast cancer risk in both codominant (TT *vs.* CC) and recessive (TT + TC *vs.* CC) models. Additionally, the CC genotype was associated with advanced TNM stage, larger tumor size, distant metastasis, perineural invasion, and poorer histological grade. In Bayram's study, the rs920778 SNP was linked to several clinicopathological characteristics in the Turkish population, including advanced TNM stage, increased tumor size, distant metastasis, perineural invasion, and lower histological grade [797].

Rajagopal's study in India, involving 502 newly diagnosed breast cancer cases and 509 healthy women, found that the rs920778 variant (TC + CC genotype) was associated with a 5.86-fold increased risk of breast cancer in pre-menopausal women (OR = 5.86; $p < 0.0001$) [798].

Hassanzarei et al. genotyped 220 breast cancer cases and 231 controls. The findings suggest that the rs920778 T>C variant significantly increased the risk of breast cancer, utilizing codominant (TC *vs.* TT; CC *vs.* TT), dominant (TC + CC *vs.* TT), recessive (CC *vs.* TT + TC), overdominant (TC *vs.* TT + CC), and allelic (C *vs.* T) inheritance models. The rs920778 variants were significantly associated only with ER status according to clinicopathological features [799].

In a cohort of sporadic breast cancer patients in Sri Lanka, the T allele of rs920778 was found to be prevalent [800].

The rs920778 polymorphism was significantly associated with reduced ovarian cancer risk and linked to advanced tumor stage, lymph node metastasis, and poor prognosis in a Chinese population. TT and TC carriers also exhibited shorter survival. These findings suggest that the rs920778 polymorphism may influence ovarian cancer susceptibility and prognosis [801].

A meta-analysis by Liu et al. found a significantly increased risk of cancer associated with the rs920778 polymorphism, which was specifically linked to an increased risk of gastrointestinal cancer [768].

In a study conducted on a Taiwanese population, 1,200 control participants and 907 patients were tested to examine the association between the rs920778 polymorphism and the risk of developing oral squamous cell carcinoma (OSCC). The results indicated that individuals carrying the polymorphic allele of rs920778 (TC and TC+CC) had a higher likelihood of developing OSCC compared to those homozygous for the wild-type allele. Additionally, the study observed that the rs920778 polymorphism was

associated with the development of larger tumors and an increased incidence of lymph node metastasis [802].

In a meta-analysis on cancer risk conducted by Zhang et al., the association between the rs920778 (C>T) polymorphism and cancer risk was analyzed across 6 studies involving 3,842 cases and 5,015 controls. The results revealed a significantly increased risk of cancer susceptibility in homozygote comparison, dominant model, and recessive model (TT *vs.* CC; CT/TT *vs.* CC; TT *vs.* CC/CT), but not in the allele contrast model or heterozygote comparison. Subgroup analysis showed that rs920778 was associated with a significantly increased risk of cancer in all genetic models within the Asian population. Additionally, in cancer type analysis, rs920778 was linked to a significantly increased risk of digestive cancers across all genetic models [786].

Ge et al. conducted a meta-analysis on cancer susceptibility across Caucasian and Asian populations, covering various cancers including gastric cancer (GC), esophageal squamous cell carcinoma (ESCC), osteosarcoma, breast cancer (BC), and colorectal cancer. This analysis, which included 8 case-control studies with 3,600 cases and 4,585 controls, found a significant association between the rs920778 polymorphism and increased cancer risk in both homozygous and recessive models. Furthermore, when stratified by cancer type, the analysis revealed heightened susceptibility to esophageal squamous cell carcinoma in all genetic models and to gastric cancer in the dominant model [785].

A pooled case-control study analyzing 10 studies with 7,258 cases and 9,007 controls examined the association between the rs920778 polymorphism and cancer risk. The combined analyses showed a significantly increased cancer risk for *HOTAIR* rs920778 across all five genetic models (allele, dominant, heterozygote, homozygote, and recessive). Stratified analyses revealed that this increased risk was significant among Asians and specific cancer types, particularly digestive and gynecologic cancers. However, no significant association was found between rs920778 and cancer risk in other ethnic groups [775].

In a meta-analysis by Zhang et al., significant associations were found between the *HOTAIR* rs920778 polymorphism and cancer risk across the total population and in subgroup analyses [787].

In a meta-analysis on cancer susceptibility, the pooled analysis revealed that the rs920778 (C>T) polymorphism significantly increased overall cancer risk across five genetic models (CT *vs.* CC; TT *vs.* CC; CT + TT *vs.* CC; TT *vs.* CT + CC; T *vs.* C). Similar associations were found for specific cancer types, including estrogen-dependent and digestive cancers, with a notably

increased risk observed in the Chinese population. Heterogeneity was reduced in both Caucasian and Chinese subgroups [767].

Nevertheless, some studies suggest that *HOTAIR* polymorphisms may reduce cancer risk. Pooled analyses by Wang et al. on *HOTAIR* polymorphisms and breast cancer susceptibility included data on the rs920778 variant, with the analysis comprising 845 cases and 856 controls. The results showed that rs920778 was associated with a significantly decreased breast cancer risk under the recessive, homozygous, and heterozygous models within the West Asian subgroup. Conversely, it was associated with an increased risk under the allele and dominant models within the East Asian subgroup. Additionally, rs920778 was linked to a decreased breast cancer risk under the recessive and heterozygous models in the hospital-based control subgroup [771].

Xavier-Magalhães et al. studied rs920778's impact on glioma in a Portuguese population and found no significant link to glioma risk. However, the rs920778 CT genotype was associated with longer survival in anaplastic oligodendroglioma patients [770].

Martins et al. found no link between rs920778 variants and bladder cancer risk in 106 Portuguese patients and 199 controls. However, the *HOTAIR* rs920778 TT genotype was associated with better survival, especially in men and those with stage pT2 tumors [769].

On the other hand, in a breast cancer case-control study involving 100 Egyptian patients and 100 healthy controls, the rs920778 C>T polymorphism was not found to be significantly associated with breast cancer [803].

Ke et al.'s meta-analysis of six studies with 1,715 lung cancer patients and 2,745 controls found no significant link between rs920778 and lung cancer susceptibility [776].

In a Japanese population study using the JG-SNP database, the *HOTAIR* rs920778 polymorphism was analyzed in 1,373 autopsy cases (827 cancer-positive and 546 cancer-negative). No significant association was found between the rs920778 polymorphism and overall cancer risk, nor with specific cancer types, except for a weak, likely incidental, association with lung cancer [804].

Oliveira et al. found no significant link between rs920778 and prostate cancer risk in a Caucasian cohort [766]. Kashani et al. found no significant association between rs920778 T>C and Hodgkin's lymphoma in 53 patients and 245 controls [777]. Overall, the literature overview suggests that rs920778 and rs12826786 are linked to increased cancer risk, while rs7958904 is possibly linked to decreased cancer risk.

Several studies have evaluated the influence of *HOTAIR* on non-oncological diseases. The rs920778 polymorphism is associated with a decreased risk of Hashimoto's thyroiditis [805]. Susceptibility to thyroid cancer was found to be associated with the rs920778 polymorphism in the northwest of

Iran [806]. The CT genotype of the rs920778 C/T polymorphism and the CT genotype of the rs12826786 C/T polymorphism were found to increase the risk of bipolar disorder [807]. The rs920778 variant was not associated with chronic kidney disease risk in the study by Majidpour et al. [808]. The rs12826786 polymorphism was associated with Autism Spectrum Disorder in the Iranian population under both the allelic (T *vs.* C) and recessive (TT *vs.* TC + CC) models [809]. The study by Sadeghi et al. demonstrated that the rs12826786 variants are associated with an increased susceptibility to developing rheumatoid arthritis (RA) in the Iranian population [810]. HOTAIR is implicated in the pathogenesis of diabetic retinopathy [811, 812].

In our study, we found no significant associations between the *MALAT1* gene SNPs rs619586, rs664589, and rs3200401 and various tumor characteristics, including tumor size, lymph node involvement, metastasis, differentiation, stage, prognosis, or patient age.

Several researchers did not find significant interactions between *MALAT1* rs619586 polymorphisms and cancers, consistent with our findings. However, due to the absence of a control group in our study, we could not compare our results with other studies.

In the Taiwanese study on uterine cervical cancer, which recruited 125 patients with invasive cancer, 98 women with precancerous lesions of the uterine cervix, and 325 female controls with no history of cervical cancer, the genotypic frequencies of rs619586 did not differ significantly between patients with cervical neoplasias and controls. Even when the patient group was divided into those with invasive cancer and those with precancerous lesions, no significant differences in genotypic distributions were observed among these subgroups and the controls. Additionally, when the study controlled for age, the analysis still showed no significant association between the genotypic frequencies and the risk of cervical neoplasias. The researchers also explored potential links between these genotypes and various clinicopathological features of cervical cancer, including tumor stage, cell grade, stromal invasion depth, and pelvic lymph node metastasis, but found no significant associations. Similarly, in both univariate and multivariate analyses, rs619586 was not associated with 5-year survival rates in cervical cancer patients; the only factor that significantly impacted survival was positive pelvic lymph node metastasis [813]. Notably, the distribution of genotypes for rs619586 in this study was similar to ours, with the AA genotype being more prevalent and the GG genotype absent.

Safan et al. studied the association between *MALAT1* rs619586 and hepatocellular carcinoma (HCC) with hepatitis C virus (HCV). Involving 40 HCC patients with HCV and 40 controls, genotyping via real-time PCR showed no significant differences in genotype or allele distributions between

the groups. The study concluded that rs619586 is not significantly associated with HCC risk in the context of HCV [814].

In a study of 624 hepatocellular carcinoma (HCC) cases and 618 controls from Southern China, no significant links were found between three *MALAT1* SNPs (rs619586 and rs3200401) and HCC risk. Analyses across various models and stratifications, including mediation and interaction effects, showed no significant associations. Additionally, these SNPs did not correlate with HCC progression factors such as TNM staging, metastasis, or cancer embolus. The results suggest that these *MALAT1* SNPs are not major risk factors for HCC susceptibility in this population [815].

Tong et al. investigated the impact of rs619586 and rs3200401 in the *MALAT1* gene on lung cancer risk. The study included 444 lung cancer patients and 460 healthy controls. No significant association was found between rs619586 and lung cancer risk, and gene-environment interactions with smoking were not notable [816].

The study by Lin et al. found that rs619586 were not linked to susceptibility to lung adenocarcinoma (LUAD). Statistical analysis revealed no significant association between these *MALAT1* SNPs and clinicopathological characteristics of lung adenocarcinoma, such as tumor stage, T status, lymph node status, distant metastasis, and cell differentiation [817].

Kelishadi et al. examined the expression levels and SNPs of genes in endometrial cancer (EC) patients with advanced stages (III or IV) or high-grade tumors (grade 3) who underwent six cycles of paclitaxel and carboplatin chemotherapy. The study also monitored cancer recurrence. Results indicated that MALAT1 expression was significantly lower in cancerous tissues compared to healthy tissues. Among patients who experienced recurrence, MALAT1 expression in cancerous tissue was lower than in those without recurrence, though this difference was not statistically significant. Furthermore, the rs619586 genotype was consistently AA in both patients and healthy controls [818].

Some studies have shown that *MALAT1* rs619586 are associated with cancer risk and are potential predictive biomarkers of cancer risk.

Gao et al.'s study found that the rs619586 polymorphism was significantly associated with colorectal cancer (CRC) risk. In their case-control study of 300 CRC patients and 300 healthy individuals, the results were as follows: AG *vs.* AA: OR = 0.64, $p = 0.03$; (AG + GG) *vs.* AA: OR = 0.62, $p = 0.02$; G *vs.* A: OR = 0.62, $p = 0.01$ [819].

In the study of differentiated thyroid carcinoma (DTC) survival and control, the rs619586 G allele was associated with reduced 5-year survival, higher DTC grade, and increased CTNNB1 expression, along with lower MALAT1 and miR-214 levels. The G allele or miR-214 mimic decreased

MALAT1 transcription, while miR-214 inhibition increased MALAT1 activity. Overall, the rs619586 G allele promoted cell proliferation, indicating it could be a prognostic marker for DTC [820].

A case-control study of 1,350 oral squamous cell carcinoma (OSCC) patients and 1,199 controls examined the association between *MALAT1* SNPs and OSCC risk and characteristics. The rs619586 AG/GG genotypes were associated with more advanced disease stages and larger tumors. MALAT1 expression was significantly higher in OSCC tissues compared to normal tissues, especially in those exposed to betel quid. These results suggest that *MALAT1* rs619586 play a role in OSCC development and interact with environmental carcinogens [821].

MALAT1 rs619586 was associated with platinum-based chemotherapy response in the dominant model [822].

Additionally, individuals carrying the G allele of the rs619586 polymorphism had a significantly higher risk of developing high-grade Gleason patterns in prostate cancer [823].

Cao et al. pooled data from 18 studies involving 11,843 cancer cases and 14,682 controls to analyze rs619586. They found that rs619586 was linked to cancer risk, with rs619586 also associated with hepatocellular carcinoma (HCC) risk. MALAT1 was significantly upregulated in colorectal cancer (CRC) and HCC compared to normal tissues [824].

Other studies have reported an association between the rs619586 polymorphism and a reduced risk of cancer.

Analyzing 917 lung cancer cases and 925 controls in a hospital-based case-control study, it was found that the AG and GG genotypes of *MALAT1* rs619586 were associated with a reduced risk of lung cancer (AG *vs.* AA: adjusted OR 0.65, $p = 0.001$; GG *vs.* AA: adjusted OR 0.22, $p = 0.003$). Both AG/GG variants and GG homozygotes showed significantly lower risk in dominant and recessive models. SNPs in hsa-miR-34b/c, pri-miR-124-1, and hsa-miR-423 were not linked to lung cancer risk. These results suggest that MALAT1 rs619586 A/G SNPs may reduce lung cancer risk, although additional studies are needed to validate these findings in diverse populations [825].

In Zhao et al.'s study, the association between four genetic variants in the lncRNA MALAT1 and colorectal cancer (CRC) susceptibility was evaluated in a Chinese population using a two-stage case-control design with 966 CRC cases and 988 controls. The minor G allele of rs619586 was significantly associated with reduced CRC risk. Individuals with AG or GG genotypes for rs619586 had a notably lower risk of CRC. These findings suggest that *MALAT1* variants may contribute to CRC development [826].

Wen et al. investigated the link between *MALAT1* SNPs and papillary thyroid cancer (PTC) risk in a case-control study with 1134 PTC patients and 1228 controls. They used luciferase assays, CCK-8, and flow cytometry to explore the functional impacts of these SNPs. The study found that the MALAT1 SNP rs619586 was significantly associated with a reduced risk of PTC. Functional tests showed that the G allele of rs619586 lowered MALAT1 expression, inhibited PTC cell proliferation, and increased apoptosis. These results suggest that rs619586 could be a valuable marker for PTC susceptibility and development [827].

Yuan et al.'s research found that younger patients (< 55 years) with the *MALAT1* rs619586 G allele had a reduced risk of hepatocellular carcinoma (HCC), both under the codominant model and dominant model. Additionally, MALAT1 expression increased progressively from normal to cancerous liver tissue and was associated with poorer survival rates in HCC patients, particularly those with hepatitis virus infection [828].

Ni et al. conducted a meta-analysis that included nine studies with 5,968 cases and 7,439 controls. The studies comprised eight focused on Asians and one on Caucasians, covering cancers such as HCC, breast cancer, CRC, ESCC, PTC, and melanoma. The analysis found a significant association between the *MALAT1* rs619586 polymorphism and cancer risk: (AG + GG) *vs.* AA: OR = 0.88; GG *vs.* (AG + AA): OR = 0.64; GG *vs.* AA: OR = 0.63; AG *vs.* AA: OR = 0.91; G *vs.* A: OR = 0.87. Subgroup analyses indicated significant associations in Asians and for cancers other than hepatocellular carcinoma, but not in Caucasians or for hepatocellular carcinoma [829].

Zheng et al. conducted a meta-analysis to explore the role of *MALAT1* in cancer susceptibility. The analysis included 12 studies with 7,007 cancer patients and 8,791 controls. rs619586 was linked to a reduced cancer risk across all models (G *vs.* A; GG *vs.* AA; GG + AG *vs.* AA; GG *vs.* AG + AA) [830].

In a case-control study by Qu et al. involving 245 esophageal squamous cell carcinoma (ESCC) patients and 490 controls, in ever drinkers, rs619586 GG was linked to a decreased ESCC risk (GG *vs.* AA: OR = 0.38). But the rs619586 finding was not significant after adjustment [831].

In a case-control study involving 1,300 HBV-positive HCC patients, the variant genotypes of rs619586 were associated with a decreased risk of HCC, though this finding was of borderline significance (AG/GG *vs.* AA: P = 0.057). However, no significant association was observed between these SNPs and the ability to eliminate HBV from the body [832].

In a study by Liu et al., 135 patients with brain metastatic lung cancer were recruited, and tissue samples were collected from each. The research found that MALAT1 expression was significantly reduced in the peripheral

blood of patients carrying the GGGT haplotype at rs11227209, rs619586, rs664589, and rs3200401 [833].

Results for *MALAT1* rs619586 also vary across different diseases.

Zheng et al. investigated the impact of the rs619586 polymorphism on invasive meningioma. Their results showed that with the rs619586 A>G polymorphism, levels of MALAT1 and COL5A1 decreased, while levels of miR-145 increased, indicating a negative relationship between MALAT1/COL5A1 and miR-145. They identified a MALAT1/miR-145/COL5A1 pathway, suggesting that the rs619586 polymorphism reduces MALAT1 and COL5A1 expression, potentially lowering meningioma invasiveness [834].

Cardiovascular disease and recurrent miscarriage share risk factors, and some cardiovascular-related genes are linked to miscarriage. Che et al. studied the rs619586 polymorphism in 284 patients and 392 controls, finding that the rs619586 G variant is protective against recurrent miscarriage. This study suggests that the rs619586 G variant may reduce recurrent miscarriage risk in the southern Chinese population [835].

The *MALAT1* rs619586 GG allele was significantly linked to a lower risk of congenital heart disease (CHD) in a Chinese pediatric population [836].

Smokers with the *MALAT1* rs619586 AA genotype and those with the GG + AG genotypes were at increased risk for coronary artery disease (CAD) [837].

In another study of a Chinese population, the rs619586 AG/GG genotypes and G allele were linked to a decreased risk of coronary artery disease (CAD). Multivariate logistic regression identified rs619586 and rs664589 as independent risk factors for CAD. Further combined analysis revealed that the presence of both rs619586 AG/GG and rs664589 CC genotypes was associated with a lower risk of CAD [838].

MALAT1 rs619586 AA and rs3200401 CT/TT genotypes were associated with an increased risk of cerebral ischemic stroke (CIS). The G variant of rs619586 was linked to higher serum MALAT1 expression levels. Multivariate logistic regression analysis identified serum MALAT1 and the *MALAT1* rs3200401 CT/TT genotypes as independent predictors of CIS [839].

A study of the Isfahan population in Iran found that the mutant allele (G) and mutant genotypes (AG/GG) were absent in type 2 diabetes mellitus patients [840].

MALAT1 rs619586 AG + GG genotypes were associated with a reduced risk of type 2 diabetes mellitus in Chinese Han population [841].

In an Iranian population study, the G allele of rs619586 was less frequent in psoriasis cases compared to controls. This SNP was associated with reduced psoriasis risk in both dominant (AG + GG *vs*. AA) and log-additive models [842].

In Yue et al.'s study, the rs619586, rs11227209, rs664589, and rs3200401 GGGT haplotype was associated with a decreased risk of Normal Tension Glaucoma (NTG) [843].

In our study, the GG genotype of *MALAT1* rs664589 was significantly associated with poorer overall survival (OS) in cervical cancer patients. However, we were unable to find similar studies for direct comparison. Nonetheless, the variant allele G of rs664589 appears to play a significant role in cancer risk, progression, and cardiovascular diseases.

Wu et al. found that colorectal cancer cells and tissues carrying the rs664589 CG/GG genotype exhibited notably higher MALAT1 expression compared to those with the rs664589 CC genotype. Multivariate Cox regression analysis identified *MALAT1* as a negative prognostic indicator for colorectal cancer. Overall, the presence of the rs664589 G allele is associated with altered MALAT1 binding to miR-194-5p in the nucleus, resulting in elevated MALAT1 levels and promoting colorectal cancer progression [844].

In a case-control study of Southern Chinese women involving 249 endometrial cancer patients and 446 cancer-free controls, the rs664589 C>G polymorphism in *MALAT1* was significantly associated with endometrial cancer risk. The association was evident in various models: heterogeneous, homogenous, and recessive. Stratified analysis revealed that this polymorphism increased endometrial cancer risk in patients with no prior surgery, higher numbers of deliveries, BMI between 25 and 29.9, and FIGO stages II–III [832].

In a pooled data analysis by Cao et al., which included 18 studies involving 11,843 cancer cases and 14,682 controls, rs664589 was found to be related to the risk of colorectal cancer in all three genetic models (CG *vs.* CC, GG *vs.* CC, and GG *vs.* CC+CG). Their findings also suggested that colorectal cancer (CRC) or hepatocellular carcinoma (HCC) patients with lower expression levels of MALAT1 might have better survival outcomes, including overall survival (OS), disease-specific survival (DSS), or progression-free interval (PFI) [824].

A borderline association was observed between rs664589 and cancer risk in the dominant model, with Zheng et al.'s meta-analysis further linking rs664589 to an increased risk of digestive cancers [830].

Carriers of the *MALAT1* rs664589 G allele were 1.39 times more likely to have Non-ST-Elevation Myocardial Infarction (NSTEMI) and 1.59 times more likely to have ST-Elevation Myocardial Infarction (STEMI) than C allele carriers in the Chinese Han population [845]. The G allele of the *MALAT1* rs664589 SNP is linked to a higher risk of hypertension [846].

We did not find any significant correlations between *MALAT1* rs3200401 and the clinical characteristics or survival outcomes of cervical cancer

patients. Similar results were found in a Taiwanese study on cervical cancer. The researchers explored potential links between these genotypes and various clinicopathological features of cervical cancer, including tumor stage, cell grade, stromal invasion depth, and pelvic lymph node metastasis, but found no significant associations. Both univariate and multivariate analyses revealed that the C/T+T/T and T/T genotypes of rs3200401 were not associated with 5-year survival rates in cervical cancer patients. The only factor that significantly impacted survival was positive pelvic lymph node metastasis. Furthermore, comparisons of rs3200401 genotypic frequencies between patients with cervical neoplasias and healthy controls showed no significant variation in the distributions of the C/C, C/T, and T/T genotypes. Even when the patient group was divided into those with invasive cancer and those with precancerous lesions, no significant differences in the genotypic distributions of C/C, C/T, and T/T were observed among these subgroups and the controls [813].

However, in a study by Yao et al. involving 1,248 samples (587 cervical cancer patients and 661 healthy controls), the link between specific SNPs and cervical cancer (CC) risk was investigated. The findings showed that the rs3200401 C allele was significantly associated with a lower risk of CC after Bonferroni correction (allele: $p = 0.001$, genotype: $p = 0.004$), suggesting it may serve as a protective factor. Subgroup analysis revealed that the rs3200401 C allele was notably linked to reduced risk of adenocarcinoma (AC) and squamous cell carcinoma (SCC) (AC: OR = 0.57; SCC: OR = 0.72), as well as early-stage cervical cancer (stage I: OR = 0.67). These results highlight the potential role of rs3200401 in MALAT1 in cervical cancer susceptibility [847].

A case-control study involving 1,350 oral squamous cell carcinoma (OSCC) patients and 1,199 controls investigated the relationship between *MALAT1* SNPs and OSCC risk and characteristics. The analysis revealed that the rs3200401 T allele was associated with a reduced overall risk of OSCC but an increased risk of developing moderately to poorly differentiated tumors. Among betel quid chewers, the T allele was linked to a higher risk of high-grade OSCC and a lower risk of lymph node metastasis [821].

In a case-control study by Qu et al. involving 245 esophageal squamous cell carcinoma (ESCC) patients and 490 controls, the analysis revealed that rs3200401 C>T was significantly associated with increased ESCC risk (CT *vs.* CC: OR = 1.59; TT *vs.* CC: OR = 2.27; CT + TT *vs.* CC: OR = 1.68). In never drinkers, rs3200401 TT and CT/TT genotypes increased ESCC risk (TT *vs.* CC: OR = 2.34; CT/TT *vs.* CC: OR = 1.52). The associations for rs3200401 remained significant after FDR adjustment. Thus, rs3200401 is a potential risk factor for ESCC, while rs619586 may be less relevant [831].

In a study involving 458 gastric cancer (GC) patients and 381 controls from Korea, the rs3200401 CT genotype in the codominant model and the CT + TT genotypes in the dominant model were associated with an increased risk of GC in males. This association was also observed in. The findings suggest that the *MALAT1* rs3200401 polymorphism is linked to increased susceptibility to GC and may influence its development [848].

Yuan et al.'s research on hepatocellular carcinoma (HCC) found that smokers with the rs3200401 CT + TT genotype had a higher prevalence of hepatitis B virus (HBV) infection and elevated aspartate aminotransferase levels [828].

Wang et al. carried out a case-control study followed by a meta-analysis to investigate the role of the *MALAT1* rs3200401 variant in the susceptibility to lung, colorectal, gastric, and liver cancers. The study involved 550 lung cancer patients, 787 colorectal cancer patients, 460 gastric cancer patients, 480 liver cancer patients, and 800 healthy controls from Hubei Cancer Hospital and Wuhan Xinzhou District People's Hospital. Their findings indicated that, within the Hubei Chinese population, the rs3200401 polymorphism was significantly associated with an increased risk of gastric cancer, while no significant association was found for lung, colorectal, or liver cancers. The subsequent meta-analysis supported the link between rs3200401 and a higher risk of gastric and colorectal cancers in the Chinese population, but not with liver cancer. These results suggest that the *MALAT1* rs3200401 variant may contribute to the susceptibility to colorectal and gastric cancers [849].

Li et al.'s meta-analysis included 10 case–control studies with a total of 6,630 cancer cases and 7,457 controls. The analysis found no significant association between the *MALAT1* rs3200401 C>T polymorphism and overall cancer risk across five genetic models. This lack of association persisted in subgroups based on control source, ethnicity, and study quality. However, in the cancer type-specific subgroup, the T allele was associated with an increased risk of colorectal cancer (CRC) compared to the C allele (C *vs.* T: OR = 1.16; 95% CI: 1.01–1.33). Overall, while there was no significant association with general cancer risk, the rs3200401 C>T polymorphism might be linked to an elevated risk of CRC, warranting further investigation [850].

A follow-up study by Wang et al. involving 538 non–small cell lung carcinoma (NSCLC) patients found that advanced lung adenocarcinoma patients with the rs3200401 CT and CT+TT genotypes had significantly longer median survival times (29.9 and 28.9 *vs.* 19.3 months) and lower death risks compared to those with the CC genotype. However, this survival benefit was not observed in early-stage NSCLC or advanced lung squamous cell

carcinoma patients. The rs3200401 T allele may serve as a prognostic biomarker for advanced lung adenocarcinoma [851].

A study by Volkogon et al. on bladder cancer found that patients with the rs3200401 TT genotype had lower hemoglobin levels and higher fasting glucose, creatinine, and tumor width compared to those with the CC genotype. Additionally, transitional cell carcinoma of the urinary bladder occurred later in individuals with the TT genotype, who also had a lower risk of developing the cancer compared to C allele carriers. The rs3200401 polymorphism in the *MALAT1* gene is associated with improved disease-free survival in Ukrainian patients with transitional cell carcinoma of the urinary bladder [852].

A study on *MALAT1* gene polymorphisms and prostate adenocarcinoma (PA) in a Ukrainian cohort involved 184 PA patients and 66 healthy controls. Results indicated that the rs3200401 T allele was associated with a significantly lower risk of PA compared to C allele carriers, and C allele homozygotes [853].

In the study by Tong et al., rs3200401 was significantly associated with a reduced risk of non-small cell lung cancer (NSCLC) and lung squamous cell carcinoma (LUSC), with the CT genotype showing a lower risk compared to the CC genotype [816].

Zheng et al. conducted a meta-analysis to investigate the role of *MALAT1* in cancer susceptibility and found no significant association between the rs3200401 polymorphism and overall cancer risk. However, the rs3200401 variant was associated with an increased risk of digestive cancers in both the allelic and dominant models [830].

In a study by Fawzy et al., 182 ovarian tissue samples (benign, borderline, and malignant) were analyzed for the *MALAT1* rs3200401 genotype using Real-time allelic discrimination PCR. The most common genotype was heterozygous (C/T), followed by homozygous (C/C). No significant differences in genotype distribution were observed among the tissue types, indicating that the *MALAT1* rs3200401 variant does not significantly impact ovarian cancer susceptibility [854].

The study by Lin et al. found that rs3200401 was not associated with susceptibility to lung adenocarcinoma (LUAD), nor was there any significant correlation between this *MALAT1* SNP and clinicopathological characteristics of LUAD, such as tumor stage, T status, lymph node status, distant metastasis, or cell differentiation. However, the dominant model of rs3200401 (CC *vs.* CT+TT) revealed a significant association between *MALAT1* genotypes and EGFR wild-type in relation to tumor stages [817].

In a study involving 624 hepatocellular carcinoma (HCC) cases and 618 controls from Southern China, no significant associations were observed between rs3200401 and HCC risk. The analyses, which included various models and explored mediation and interaction effects, did not reveal any significant links. Moreover, these SNPs showed no correlation with HCC progression factors such as TNM staging, metastasis, or cancer embolus [815].

The rs3200401 polymorphism has been associated with varying risks across different conditions, including increased risks of cerebral ischemic stroke [839], myocardial infarction [855], major adverse cardiac and cerebrovascular events [856], systemic arterial hypertension [857], systemic lupus erythematosus [858], type 2 diabetes mellitus [841], andchildhood obesity [859], while showing no significant association with rheumatoid arthritis [860], psoriasis [842], and indicating potential effects on inflammation severity and lipid levels.

These studies suggest that HOTAIR and MALAT1 polymorphisms may influence disease risk and progression, though they may not act as independent factors. The global literature contains a wealth of studies on various HOTAIR and MALAT1 polymorphisms, many of which demonstrate their impact on disease onset and progression. This body of evidence indicates that HOTAIR and MALAT1 could potentially serve as biomarkers for assessing cancer prognosis. However, further research is needed to validate these findings, particularly in the context of cervical cancer, and to better understand their role in disease mechanisms and clinical utility.

5. CONCLUSIONS

1. A relationship was found between *TLR4* polymorphisms and the clinicopathological features and survival of cervical cancer:
   1.1. The rs11536898 A allele was associated with a higher risk of cervical cancer metastasis.
   1.2. The rs11536898 AA genotype was associated with shorter overall survival and progression-free survival time, while the C allele of this polymorphism was associated with longer overall survival and longer progression-free survival time.
   1.3. The rs10759932 CC genotype significantly influenced shorter progression-free survival time and overall survival, whereas the T allele of this polymorphism was associated with longer progression-free survival time and overall survival.

2. Various associations of *RRP1B*, *SIPA1*, and *SRSF1* polymorphisms with clinicopathological features and survival indicators in cervical cancer were identified:
   2.1. The *RRP1B* rs2838342 A allele and rs7276633 T allele were associated with smaller tumor size.
   2.2. Individuals lacking the *RRP1B* GCTTC haplotype were significantly associated with smaller tumor size and had a lower risk of metastasis and worse overall survival.
   2.3. The *SIPA1* rs746429 GA genotype and A allele were significantly associated with a lower risk of poor tumor differentiation.
   2.4. The *SRSF1* rs34592492 G allele was associated with longer overall survival, while the CC genotype was associated with shorter overall survival.

3. Polymorphisms in the *HOTAIR* and *MALAT1* genes did not show significant associations with cervical cancer phenotype, progression-free survival, or overall survival.

SANTRAUKA

Gimdos kaklelio vėžys yra plačiai paplitusi sveikatos problema ir viena dažniausių onkologinių ligų. Pasak Pasaulio sveikatos organizacijos (PSO), tai yra ketvirtas dažniausiai diagnozuojamas vėžys moterims. Ši liga taip pat yra ketvirtoje vietoje pasaulyje pagal su vėžiu susijusius mirčių rodiklius tarp moterų [1]. Nepaisant profilaktikos ir gydymo pažangos, jis ir toliau išlieka viena pagrindinių su vėžiu susijusio sergamumo ir mirtingumo priežasčių tarp moterų, ypač šalyse, kurių gyventojai gauna mažas ir vidutines pajamas [2, 3].

Gimdos kaklelio vėžio patogenezė yra sudėtinga ir daugiaveiksnė, apimanti tiek aplinkos, tiek genetinius veiksnius. Nors pagrindinis etiologinis veiksnys yra nuolatinė didelės rizikos žmogaus papilomos viruso (ŽPV) infekcija, tačiau taip pat svarbų vaidmenį jautrumui ligoms, jų progresavimui ir gydymo atsakui atlieka genetiniai variantai [4].

Iš daugelio genetinių veiksnių, susijusių su gimdos kaklelio vėžiu, svarbiu įgimto imuniteto ir uždegimo veiksniu tapo Toll tipo receptoriai 4 (TLR4). Įrodyta, kad TLR4 aktyvacija moduliuoja šeimininko imuninį atsaką į ŽPV infekciją ir daro įtaką gimdos kaklelio vėžio vystymuisi ir progresavimui [5, 6].

Panašiai ir *RRP1B* genas, koduojantis ribosominės RNR apdorojimo 1B baltymą, buvo susijęs su įvairiais ląstelių procesais, įskaitant ląstelių proliferaciją, migraciją ir invaziją. Genetiniai *RRP1B* pakitimai buvo susieti su naviko agresyvumu ir metastazavimu [7, 8].

SIPA1, genas, svarbus signalų perdavimo keliams, taip pat buvo susijęs su gimdos kaklelio vėžio progresavimu. Tyrimai parodė, kad *SIPA1* skatina naviko ląstelių proliferaciją, migraciją ir invaziją, pabrėžiamas jo kaip terapinio taikinio potencialas gimdos kaklelio vėžio atveju [9].

Be to, sukirpimo (angl. *splicing*) faktorius SRSF1 sulaukė mokslininkų dėmesio dėl savo vaidmens apdorojant mRNR ir alternatyviame sukirpimo procese vystantis vėžiui. SRSF1 raiškos reguliavimo sutrikimai ir jo genetiniai variantai buvo susieti su netinkamo sukirpimo modeliais ir naviko progresavimu [10].

Ilgos nekoduojančios RNR (angl. *lncRNR*) HOTAIR ir MALAT1 taip pat buvo susietos su gimdos kaklelio vėžio patogeneze. Šios ilgos nekoduojančios RNR turi svarbią reikšmę epigenetiniam reguliavimui, metastazavimui ir atsparumui gydymui, todėl yra svarbios tolesniems tyrimams [11, 12].

Nepaisant pažangos aiškinantis gimdos kaklelio vėžio genetinį pagrindą, lieka didelių žinių spragų. Reikalingi tolesni tyrimai, siekiant išsiaiškinti tikslius genų, tokių kaip *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR* ir *MALAT1*, vaidmenis ligos patogenezėje ir klinikiniams rezultatams. Nors gimdos

kaklelio vėžio profilaktikos ir gydymo iššūkių apstu, naujausi molekulinės biologijos ir genetikos pasiekimai suteikia daug žadančių tobulėjimo galimybių. Svarbu giliau suprasti genetinius veiksnius, turinčius įtakos gimdos kaklelio vėžio jautrumui, progresavimui ir gydymo atsakui, kad būtų galima kurti tikslines intervencijas ir individualias gydymo strategijas.

Plečiantis mūsų žinioms apie šiuos genetinius ir molekulinius veiksnius, taikant individualesnį požiūrį į gimdos kaklelio vėžio atvejus, pasiekiami vis tikslesni ir individualūs gimdos kaklelio vėžio valdymo būdai, suteikiantys vilties ateityje taikyti geresnę profilaktiką, ankstyvąjį diagnozavimą ir gydymą.

Atsižvelgus į aptartą kontekstą, mūsų tyrimu siekta ištirti pagrindinių su gimdos kaklelio vėžio progresavimu susijusių genų germinacinių polimorfizmų įtaką. Atlikdami genetinių variantų įtakos naviko vystymuisi ir gydymo atsakui analizę, tyrimu siekiame nustatyti naujus prognostinės vertės biologinius žymenis ir prisidėti prie tiksliosios medicinos metodų gimdos kaklelio vėžiui suvaldyti. Pagrindinis šios disertacijos tikslas – ištirti ryšius tarp šių šešių genų genetinių variantų ir gimdos kaklelio vėžio jautrumo, progresavimo bei gydymo atsako. Norint atskleisti genetinius gimdos kaklelio vėžio veiksnius, siekiama nustatyti naujus rizikos stratifikacijos biologinius žymenis, kurie būtų naudingi ir individualizuotiems gydymo metodams, ir kartu prisidėti prie geresnių pacientų gydymo rezultatų.

Tyrimo tikslas

Šio tyrimo tikslas buvo įvertinti *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR* ir *MALAT1* germinacinių polimorfizmų reikšmę gimdos kaklelio vėžio prognozei.

Tyrimo uždaviniai

1. Nustatyti imuninio atsako moduliatoriaus *TLR4* geno polimorfizmus ir įvertinti jų sąsajas su gimdos kaklelio vėžio progresavimu bei prognoze.
2. Įvertinti metastazavimo procese dalyvaujančių *RRP1B*, *SIPA1* ir *SRSF1* genų variantų sąsajas su gimdos kaklelio vėžio eiga.
3. Analizuoti epigenetinių modifikatorių *HOTAIR* ir *MALAT1* genų variantus ir ištirti jų ryšį su gimdos kaklelio vėžio progresavimu.

Tyrimo naujumas ir praktinė reikšmė

Mūsų tyrimu siekiama ištirti gimdos kaklelio vėžį, vieną dažniausių moterų vėžio tipų. Tyrėme 27 genetinius polimorfizmus *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR* ir *MALAT1* genuose, kurie dalyvauja įvairiuose vėžio vystymosi, progresavimo ir gydymo atsako etapuose.

TLR4 vaidina nemažą vaidmenį, svarbų imuninei sistemai ir naviko reguliacijai. Dėl to tai yra labai svarbus dėmesio objektas. *RRP1B* ir *SIPA1* yra susiję su ląstelių proliferacija, migracija ir invazija, kurios turi potencialą naviko agresyvumui ir metastazėms. *SRSF1*, sukirpimo (angl. *splicing*) faktorius, yra svarbus alternatyviam sukirpimui (angl. *alternative splicing*), susijusiam su vėžiu, o HOTAIR ir MALAT1, ilgos nekoduojančios RNR, reguliuojančios epigenetiką ir metastazes, gali būti naudojamos kaip prognostiniai biologiniai žymenys.

Kaip rodo ankstesni tyrimai, paveldėti polimorfizmai yra susiję su specifinėmis naviko savybėmis ir vėžio baigtimis. Pripažindami potencialią germinacinių polimorfizmų įtaką ligos patomorfologinėms savybėms ir ligos progresavimui, ištyrėme dvidešimt septynių funkcinių genetinių variantų asociacijas *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR* ir *MALAT1* genuose su klinikiniais patologiniais profiliais ir išgyvenamumo rodikliais moterų, sergančių gimdos kaklelio vėžiu, kohortoje. Mūsų tyrimas yra pirmasis, kuriame analizuojami šių genų variantai, siekiant įvertinti jų įtaką klinikinėms vėžio savybėms bei gimdos kaklelio vėžio progresavimui. Nustatant ryšį tarp genetinių variantų ir gimdos kaklelio vėžio, galima rekomenduoti naudoti šiuos genetinius variantus kaip prognostinius biologinius žymenis įvertinant numanomą ligos vystymąsi.

Tyrimas išsiskiria keliais privalumais, o vienas iš jų – išsamus duomenų rinkinys, kuriame pateikiama genetinė informacija, naviko fenotipų duomenys ir išgyvenamumo duomenys. Pažymėtina, kad, mūsų žiniomis, iki šiol panašių tyrimų, kuriuose būtų nagrinėjamos šių polimorfizmų ir gimdos kaklelio vėžio klinikinių savybių sąsajos nebuvo atlikta, todėl negalima tiesiogiai palyginti mūsų gautų rezultatų. Todėl mūsų tyrimu siekta išsiaiškinti šių polimorfizmų poveikį ligos klinikinėms apraiškoms ir rezultatams.

Apskritai, tiriant šių šešių genų genetinius variantus ir jų sąsajas su gimdos kaklelio vėžio progresavimu, ne tik užkamšomos esamos spragos, bet ir suteikiama vilčių nustatyti naujus prognostinius žymenis ir gydymo taikinius, todėl taip bus prisidedama prie geresnių gydymo rezultatų suvaldant gimdos kaklelio vėžį.

TYRIMO METODAI

Tyrimo dizainas

Retrospektyvusis kohortinis tyrimas, kuriame tirtos suaugusios gimdos kaklelio vėžiu sergančios pacientės, buvo patvirtintas Kauno regiono biomedicininių tyrimų etikos komiteto (Nr. BE-2-10 ir P1-BE-2-10/2014). Visos pacientės buvo tiriamos Lietuvos sveikatos mokslų universiteto ligoninės

Kauno klinikose, Kaune, Lietuvoje, nuo 2014 m. spalio iki 2020 m. rugpjūčio mėn. Iš viso į tyrimą buvo įtrauktos 172 pacientės, sergančios I–IV stadijos gimdos kaklelio vėžiu. Jų diagnozės buvo patvirtintos klinikiniais (ginekologiniais ir radiologiniais tyrimais) bei histologiniais (gimdos kaklelio biopsijomis) vertinimais. Kraujo mėginiai genetiniams tyrimams buvo paimti iš periferinių venų. Vėžio gydymas buvo taikomas pagal institucines gaires ir laikantis tarptautinių standartų.

Pacienčių įtraukimo ir neįtraukimo kriterijai:

Įtraukimo kriterijai:

- Visiškas klinikinių charakteristikų duomenų prieinamumas.
- Pacientės raštiškas sutikimas dalyvauti.

Neįtraukimo kriterijai:

- Kitos piktybinės ligos buvimas.
- Reikšmingos gretutinių ligų būklės.
- Medicininiai įrašai, kurie buvo naudojami klinikinėms ir patologinėms savybėms bei ligos eigą apibūdinantiems duomenims gauti, buvo neišsamūs.

Polimorfizmų atranka ir genotipavimas

Genotipų duomenys buvo gauti iš Tarptautinio *HapMap* projekto (http://www.HapMap.org) ir 1000 genomų projekto (http://www.1000genomes.org). Genetiniai variantai (angl. *SNP*) buvo atrinkti pagal ankstesnį identifikavimą įvairiose populiacijose ir sąsajas su ligų baigtimis, aprašytomis literatūroje. Mes sutelkėme dėmesį į polimorfizmus, kurie mažiau tirti gimdos kaklelio vėžio kontekste ir kurių retojo alelio dažnis Europos populiacijoje yra bent 5 proc., – taip buvo siekta užtikrinti statistinį reikšmingumą. Prioritetas buvo teikiamas polimorfizmams, kurie gali turėti funkcionalų pagrindinių biologinių procesų reikšmingumą.

DNR buvo išgauta iš periferinio kraujo mėginių leukocitų. Išgavimo procedūrai buvo naudojamas genominės DNR išskyrimo rinkinys iš *Thermo Fisher Scientific Baltics*, Vilnius, Lietuva. Pasirinktų polimorfizmų genotipavimas *TLR4*, *RRP1B*, *SIPA1*, *SRSF1*, *HOTAIR* ir *MALAT1* genuose buvo atliktas Onkologijos institute, Lietuvos sveikatos mokslų universitete, naudojant *TaqMan*® polimorfizmų genotipavimo analizės rinkinius. Molekulinės genetinės analizės buvo atliktos Tikralaikės polimerazės grandininės reakcijos metodika.

Klinikinių ypatybių vertinimas

Visų karcinomos atvejų stadijos buvo nustatytos pagal FIGO gaires, o naviko diferenciacijos laipsnis buvo vertinamas pagal architektūrinius ir

citologinius kriterijus. Analizė apėmė klinikinius patologinius požymius, įskaitant pacienčių amžių diagnozės metu, naviko dydį (T), limfmazgių pažeidimą (N), metastazių plitimą (M), stadiją, diferenciacijos laipsnį (G), gydymo atsaką, ligos progresavimą ir mirtingumą.

Buvo tiriami ryšiai tarp polimorfizmų ir naviko ypatybių, tokių kaip amžius (≤ 50 *vs.* > 50 m.), naviko dydis (T1–T2 *vs.* T3–4), limfmazgių būklė (N0 *vs.* N1), tolimosios metastazės (M0 *vs.* M1), naviko diferenciacijos laipsnis (G1 + G2 *vs.* G3), stadija (I–II *vs.* III–IV) ir prognozė (T3–T4 + G3 *vs.* T1–T2 + G1–G2). Taip pat buvo nagrinėjami klinikiniai rezultatai, įskaitant išgyvenamumą be ligos progresavimo ir bendrąjį išgyvenimą.

Statistinė analizė ir programinės įrangos naudojimas

Identifikuoti genetiniai variantai buvo analizuojami naudojant *SPSS 25.0* versiją. Genotipų, alelių ir naviko charakteristikų ryšiams tirti buvo taikyti Pearsono chi kvadrato ir Fisherio *Exact* testai. Naudojant vienmačius ir daugiamačius logistinės regresijos modelius, buvo apskaičiuoti šansų santykiai (ŠS) su 95 proc. pasikliautinaisiais intervalais (PI) ir *p* vertėmis, atsižvelgiant į amžių ir klinikinius patologinius požymius. Išgyvenamumų skirtumams analizuoti buvo apskaičiuoti rizikos santykiai, naudojant Cox modelius, o išgyvenimo kreivės buvo sudarytos naudojant Kaplano–Meierio metodą ir vertintos log-rank testu. *p* vertė < 0,05 buvo laikoma statistiškai reikšminga. Haplotipų reikšmė (angl. *linkage disequilibrium (LD)*) tarp polimorfizmų buvo vertinama naudojant *Haploview v4.1*, o haplotipai buvo nustatyti naudojant *Phase v2.1* programinę įrangą. Buvo analizuojami haplotipų ryšiai su klinikinėmis ligos apraiškomis ir išgyvenimo rezultatais, siekiant nustatyti potencialius genetinius veiksnius, galinčius turėti įtakos ligos eigai.

REZULTATAI

Klinikiniai ypatumai

Tyrime dalyvavo 172 pacientės. Daugumą jų sudarė Lietuvos pilietės (90,1 proc.), o kitos atvyko iš kitų Europos šalių. Dalyvių amžius svyravo nuo 22 iki 83 metų, vidutinis amžius buvo 55,4 metų su 13,5 metų standartiniu nuokrypiu. Naviko dydžio analizė parodė, kad 48,8 proc. atvejų buvo T2 kategorijos. Limfmazgių pažeidimas buvo nustatytas 44,8 proc. pacienčių, o metastazės paraaortiniuose limfmazgiuose dokumentuotos 5,2 proc. Tolimosios metastazės buvo nustatytos 5,8 proc. atvejų. Dažniausios stadijos buvo IIB ir IIIC1 – atitinkamai 32,0 ir 31,0 proc. atvejų. 44,2 proc. tyrimo dalyvių nustatytos mažesnės stadijos (I–II), o 55,8 proc. – didesnės stadijos (III–IV). Naviko diferenciacija: 7,6 proc. gerai diferencijuoti (G1), 65,7 proc. –

vidutiniškai (G2) ir 26,7 proc. – blogai. Didžiajai daliai pacienčių (69,2 proc.) buvo taikytas standartinis chemospindulinis gydymas. 70,3 proc. atvejų gydymo atsakas buvo labai geras. Laikas iki ligos progresavimo buvo vidutiniškai 13 mėnesių, t. y. nuo 1 iki 201 mėnesio, o 30,2 proc. dalyvių liga progresavo. Iš jų 51 pacientei nustatyta sritinių limfmazgių metastazių, 18 – paraaortinių limfmazgių metastazių, o 16 – tolimųjų metastazių. Tyrimo metu užregistruota 40 mirčių (23,3 proc. dalyvių), vidutinė bendrojo išgyvenamumo trukmė buvo 16,5 mėnesio. 45,9 proc. pacienčių turėjo lėtinių ligų, tačiau visos mirtys buvo nuo vėžio progresavimo.

Genetinių variantų dažniai ir pasiskirstymas

Mūsų tyrime 172 pacientės buvo genotipuotos pagal šiuos genetinius variantus: *TLR4* (rs10983755, rs10759932, rs11536865, rs4986790, rs4986791, rs11536897, rs1927906, rs11536898), *RRP1B* (rs2838342, rs7276633, rs2051407, rs9306160, rs762400), *SIPA1* (rs746429, rs931127, rs3741378), *SRSF1* (rs8819, rs34592492, rs11654058, rs2233908, rs2585828), *HOTAIR* (rs12826786, rs7958904, rs920778) ir *MALAT1* (rs619586, rs664589, rs3200401). *RRP1B* rs9306160 buvo identifikuotas 169 atvejais, trys atvejai buvo atmesti dėl neamplifikacijos. Dauguma polimorfizmų atitiko Hardy-Weinbergo pusiausvyrą, išskyrus *TLR4* rs619586, kuris parodė reikšmingą nuokrypį, kadangi nenustatyta C alelio. Palyginus mūsų kohortos alelių dažnius su Europos duomenimis iš 1000 genomo projekto, buvo pastebėta nedidelių, statistiškai nereikšmingų skirtumų dvidešimt šešių polimorfizmų atvejais ($p > 0,05$), tačiau *SRSF1* polimorfizmo rs746429 atveju skirtumai buvo statistiškai reikšmingi ($p < 0,05$).

Sąsajų pusiausvyra ir haplotipų paskirstymas

Mes tirtuose genuose analizavome sąsajas (angl. *linkage disequilibrium*) tarp polimorfizmų, naudodami D' ir r^2 rodiklius.

***TLR4* genas:** vertinant sąsajas tarp 8 *TLR4* geno polimorfizmų, D' vertės svyruoja nuo 0 iki 1, nustatant polimorfizmų poras su reikšminga sąsajų reikšme. Sąsajų blokas, sudarytas iš rs4986790, rs4986791 ir rs1927906, rodo aukštas D' (0,95–1,0) ir r^2 vertes (0,6–0,95), turinčias minimalią rekombinaciją. Dažniausias haplotipas „ACT“ buvo rastas 304 chromosomose (88,37 proc.). „ACC“ haplotipas nustatytas 14 chromosomų (4,07 proc.), „GTC“ – 25 chromosomose (7,27 proc.), o „ATC“ buvo labai retas, pastebėtas tik vienoje chromosomoje (0,29 proc.). Tolesnėje analizėje dėmesys buvo sutelktas į „ACT“ haplotipą dėl jo gausumo.

***RRP1B* genas:** vidutinė D' vertė buvo 0,949 ± 0,037, rodanti stiprų ryšį. Vidutinė r^2 vertė buvo 0,802 ± 0,065, taip pat atspindinti vidutinį – aukštą

ryšį. Šie rezultatai rodo, kad yra reikšmingų sąsajų tarp *RRP1B* tirtų penkių polimorfizmų. Atsižvelgiant į šį stiprų ryšį, visi penki variantai (rs2838342, rs7276633, rs2051407, rs9306160 ir rs762400) buvo įtraukti į haplotipų analizę. Buvo nustatyta trylika haplotipų. Dažniausias haplotipas „ATCCG“ sudarė 55 proc. visų haplotipų, o „GCTTC“ – 36 proc. Kiti haplotipai, tokie kaip „ATCTG“ ir „ATCTC“, buvo retesni. Tolesnėje analizėje sutelkėme dėmesį į du dažniausius haplotipus: „ATCCG“ ir „GCTTC“.

***SIPA1* genas:** haplotipų blokų nebuvo nustatyta.

***SRSF1* genas:** D' vertės svyravo nuo 0,922 iki 1,0, o r^2 vertės – nuo 0,005 iki 1,0. Į analizę buvo įtraukti keturi polimorfizmai (rs8819, rs11654058, rs2233908 ir rs2585828). Nustatyti penki *SRSF1* haplotipai. Dažniausias haplotipas „CTGA“ buvo rastas 290 chromosomose (84,30 proc.), todėl jis yra vyraujantis. „TCAG“ buvo retesnis, rastas 34 chromosomose (9,89 proc.). Reti haplotipai, tokie kaip „CTAG“ ir „TTGA“, buvo pastebėti tik vienoje chromosomoje (0,29 proc.), o „TTAG“ – 18 chromosomų (5,23 proc.). Dėl šios priežasties tolesnei analizei pasirinktas dažniausias haplotipas „CTGA“.

***HOTAIR* genas:** analizuojant tris *HOTAIR* geno polimorfizmus (rs12826786, rs7958904 ir rs920778), buvo nustatyta stipri sąsaja. D' vertės svyravo nuo 0,972 iki 1,0, o r^2 vertės – nuo 0,812 iki 0,988. Visi trys polimorfizmai sudarė vieną bloką. Dažniausias haplotipas „CGA“ buvo rastas 123 chromosomose (35,76 proc.). Antras pagal dažnumą haplotipas „CGG“ buvo aptiktas 88 chromosomose (25,58 proc.). Kiti haplotipai, tokie kaip „TCA“, „TCG“, „CCA“, „TGA“ ir „CCG“, buvo ne tokie dažni, nuo 0,29 proc. iki 21,51 proc. Analizė buvo sutelkta į vyraujančius „CGA“ ir „CGG“ haplotipus.

***MALAT1* genas:** buvo nustatytas silpnas ryšys tarp trijų *MALAT1* polimorfizmų, su D' vertėmis nuo 0,653 iki 1,0 ir r^2 vertėmis – nuo 0,004 iki 0,383. Haplotipo blokų nebuvo nustatyta, todėl jie nebuvo naudojami tolesnei analizei.

Asociacijų analizė

***TLR4* genas:**

Mūsų analizė, nagrinėjant rs10759932, rs1927906, rs11536865, rs10983755, rs4986790, rs4986791 ir rs11536897 sąsajas su navikų ypatybėmis, parodė, kad nėra statistiškai reikšmingų koreliacijų su naviko dydžiu, limfmazgių būkle, metastazėmis, naviko diferenciacija, stadija, prognoze arba pacienčių amžiumi diagnozės metu ($p > 0,05$).

Naudojant logistinės regresijos modelį, nenustatyta statistiškai reikšmingų koreliacijų tarp rs11536898 ir naviko dydžio, limfmazgių būklės, naviko ląstelių diferenciacijos, stadijos, prognozės ar pacientės amžiaus. Tačiau

nustatėme reikšmingą ryšį tarp rs11536898 ir metastazių (M). Turint A alelį, metastazių rizika buvo gerokai didesnė (ŠS = 5,068, 95 proc. PI: 1,357–18,918, p = 0,008). Genotipinis modelis iš dalies patvirtino šį radinį: pacientėms, turinčioms CA genotipą, buvo 4,735 karto didesnė tolimųjų metastazių rizika, nei pacientėms, turinčioms CC genotipą (95 proc. PI: 1,204–18,626, p = 0,026). Norime atkreipti dėmesį, kad mūsų tyrime buvo tik penkios pacientės, turinčios AA genotipą, o tai galėjo turėti įtaką p vertei atliekant šį lyginimą.

Daugiamate logistinės regresijos analize patvirtintas statistiškai reikšmingas ryšys tarp rs11536898 ir metastazių. Daugiamatėje analizėje (Modelis Nr. 1) CA genotipas gerokai padidino metastazių riziką (ŠS = 4,609, 95 proc. PI: 1,166–18,212, p = 0,029), o A alelis padidino metastazių riziką (ŠS = 5,044, 95 proc. PI: 1,346–18,899, p = 0,016), atsižvelgiant į pacientės amžių diagnozės metu. Šis ryšys išliko statistiškai reikšmingas, kai buvo atsižvelgiama tiek į pacientės amžių diagnozės metu, tiek į naviko diferenciaciją (G) (Modelis Nr. 2): CA genotipas labai padidino metastazių riziką (ŠS = 4,419, 95 proc. PI: 1,111–17,576, p = 0,035), taip pat A alelis didino metastazių riziką (ŠS = 4,884, 95 proc. PI: 1,297–18,392, p = 0,019).

***RRP1B* genas:**

Visi tiriami polimorfizmai parodė reikšmingas sąsajas su gimdos kaklelio vėžio klinikiniais požymiais, tačiau nebuvo susiję su limfmazgių pažeidimu ar naviko diferenciacija. Kita vertus, visi polimorfizmai buvo susiję su naviko dydžiu arba metastazėmis, o kai kurie taip pat turėjo įtakos vėžio stadijai ir prognozei.

Rs2838342 analizė atskleidė reikšmingus rezultatus. Vienmatė logistinės regresijos analizė parodė, kad asmenys, turintys A alelį, turėjo daug mažesnę didesnio naviko dydžio (T3–T4) tikimybę (ŠS = 0,281, 95 proc. PI: 0,122–0,643, p = 0,002), palyginti su tais, kurie neturėjo A alelio. Daugiamatės analizės visais keturiais modeliais nuosekliai išryškino, kad A alelis buvo susijęs su mažesne didesnio naviko dydžio tikimybe, ŠS svyravo nuo 0,244 iki 0,299. Regioninio limfmazgių pažeidimo (N1) ir tolimųjų metastazių (M1) buvimas taip pat turėjo įtakos šiems ryšiams. Vienmatė logistinės regresijos analizė parodė, kad A alelis gerokai sumažino tolimųjų metastazių tikimybę (ŠS = 0,274, 95 proc. PI: 0,072–1,040, p = 0,044). Priešingai, turint bent vieną G alelį, tolimųjų metastazių tikimybė šiek tiek padidėjo (OR = 1,199), tačiau ši sąsaja nebuvo reikšminga (p = 0,798). Daugiamatės analizės duomenimis, matomas galimas A alelio apsauginis poveikis tolimųjų metastazių atsiradimui, nors statistinė reikšmė buvo ribota (p > 0,05). Šie rezultatai rodo, kad A alelis rs2838342 gali padėti apsisaugoti nuo tolimųjų metastazių. Blogesnės prognozės grupėje (T3–T4 + G3) A alelis reikšmingai sumažino blogos prognozės tikimybę (ŠS = 0,182, 95 proc. PI: 0,061–0,538,

p = 0,002). Nors GG genotipas turėjo aukštesnę ŠS, esant blogai prognozei, šis ryšys nepasiekė įprastų reikšmingumo lygių (p = 0,071). A alelis taip pat rodė mažesnę blogesnės prognozės vėžio tikimybę (ŠS = 0,341, 95 proc. PI: 0,137–0,849, p = 0,017) ir galimą apsauginį poveikį. Pacientėms, turinčioms AG genotipą, buvo mažesnė gimdos kaklelio vėžio išsivystymo iki 50 metų rizika (ŠS = 0,471, 95 proc. PI: 0,226–0,983, p = 0,045). Šie rezultatai rodo, kad A alelis rs2838342 gali turėti apsauginį poveikį ir apsaugoti nuo gimdos kaklelio vėžio progresavimo ir ligos sunkumo galbūt būdamas svabus kaip prognostinis žymuo.

Rs7276633 T alelio turėtojoms buvo reikšmingai mažesnė didesnio naviko dydžio tikimybė (T3–T4) (ŠS = 0,281, 95 proc. PI: 0,122–0,643, p = 0,003). Šis ryšys išliko tiriant visais keturiais daugiamatės analizės modeliais, kurių ŠS svyravo nuo 0,264 iki 0,299. Priešingai, C alelis neparodė reikšmingos sąsajos su naviko dydžiu (p = 0,145). T alelis taip pat mažino didesnės naviko stadijos (III–IV) (ŠS = 0,341, 95 proc. PI: 0,137–0,849, p = 0,021) ir blogesnės prognozės (T3–T4 + G3) (ŠS = 0,182, 95 proc. PI: 0,061–0,538, p = 0,002) riziką. C alelio turėtojoms buvo didesnė rizika susirgti šia liga iki 50 metų (ŠS = 2,138, 95 proc. PI: 1,080–4,230, p = 0,029). Šie rezultatai rodo, kad T alelis rs7276633 gali turėti apsauginį poveikį ir sąlygoti mažesnį išplitusio naviko dydį bei geresnę ligos prognozę.

Tarp rs2051407 genotipų ir klinikinių ligos požymių reikšmingų ryšių nenustatyta. Tačiau C alelio turėtojoms buvo mažesnė didesnio naviko (T3–T4) rizika, palyginti su tomis, kurios šio alelio neturėjo (ŠS = 0,393, 95 proc. PI: 0,166–0,929, p = 0,033). Ši sąsaja išliko reikšminga trijuose daugiamatės analizės modeliuose (ŠS svyruoja nuo 0,354 iki 0,414), tačiau ne Modelyje Nr. 4 (ŠS = 0,409, 95 proc. PI: 0,149–1,123, p = 0,083). Vienmatė analizė parodė, kad C alelis reikšmingai sumažino metastazių riziką (ŠS = 0,223, 95 proc. PI: 0,058–0,858, p = 0,019), o šį ryšį patvirtino ir daugiamatės analizės Modeliai Nr. 1, 2 ir 3. Vis dėlto Modelis Nr. 4 šios sąsajos nepatvirtino. C alelis taip pat mažino blogesnės prognozės (T3–T4 + G3) riziką (ŠS = 0,267, 95 proc. PI: 0,087–0,823, p = 0,021), o tai leidžia manyti, kad šis polimorfizmas gali atlikti apsauginį vaidmenį.

Rs9306160 polimorfizmo C alelis rodo reikšmingą apsauginį poveikį nuo metastazių (ŠS = 0,179, 95 proc. PI: 0,044–0,721, p = 0,008). Reikšmingo ryšio su CT genotipu nenustatyta, tačiau TT genotipas parodė tendenciją didinti metastazių riziką (ŠS = 5,889, 95 proc. PI: 0,993–34,906, p = 0,051). Daugiamatė analizė patvirtino C alelio apsauginį poveikį Modeliuose Nr. 1, 2 ir 3, kur ŠS svyravo nuo 0,151 iki 0,187. Tačiau Modelyje Nr. 4 šis poveikis nebuvo statistiškai reikšmingas, nors ir išliko apsauginio poveikio tendencija.

Rs762400 parodė reikšmingų sąsajų: G alelis buvo susijęs su mažesne išplitusio naviko dydžio (T3–T4) rizika, palyginti su G alelio nebuvimu

(ŠS = 0,383, 95 proc. PI: 0,151–0,967, $p = 0,037$). Ši sąsaja išliko reikšminga Modeliuose Nr. 1, 2 ir 3, tačiau Modelyje Nr. 4 statistiškai reikšmingo lygio nepasiekė ($p = 0,106$), o tai rodo kitų kintamųjų įtaką. Be to, G alelis buvo susijęs su reikšmingai mažesne metastazių rizika (ŠS = 0,176, 95 proc. PI: 0,045–0,686, $p = 0,006$) vienmatėje analizėje. Daugiamatės analizės patvirtino apsauginį poveikį Modeliuose Nr. 1, 2 ir 3, nors Modelyje Nr. 4 ši tendencija išliko, bet nebuvo statistiškai reikšminga. Amžius ir kiti klinikiniai veiksniai reikšmingų sąsajų su metastazėmis neparodė.

***SIPA1* genas:**

Polimorfizmų rs931127 ir rs3741378 atvejais nenustatyta reikšmingo genotipų arba alelių ryšio su naviko fenotipu.

Vienmatė logistinės regresijos analizė parodė, kad rs746429 GA genotipą turintys asmenys turi reikšmingai mažesnę blogai diferencijuotų navikų (G3) riziką, palyginti su GG genotipo neturinčiais asmenimis (ŠS: 0,329, $p = 0,007$). A alelis taip pat siejamas su mažesne blogos naviko diferenciacijos rizika (ŠS: 0,424, $p = 0,019$). Siekiant patvirtinti GA genotipo ir A alelio apsauginį poveikį, minėtos sąsajos išlieka reikšmingos daugiamačiuose modeliuose, kuriuose buvo atsižvelgta į amžių, limfmazgių būklę, metastazes ir naviko dydį. Taip pat rs746429 GA genotipas siejamas su mažesne tikimybe susirgti gimdos kaklelio vėžiu jaunesnėms nei 50 metų moterims (ŠS: 0,435, $p = 0,036$) ir mažesne blogesnės prognozės rizika (ŠS: 0,255, $p = 0,012$). A alelis taip pat rodo reikšmingą šių rizikų sumažėjimą, o tai leidžia manyti, kad jis taip pat gali turėti apsauginį poveikį.

Apibendrinant galima teigti, kad rs746429, ypač GA genotipas ir A alelis, yra reikšmingai susiję su mažesne agresyvių naviko savybių ir nepalankių klinikinių rezultatų rizika ir turi galimą apsauginį poveikį, lemiantį geresnę naviko diferenciaciją ir prognozę.

***SRSF1* genas:**

Tiriant rs8819, rs34592492, rs11654058 ir rs2233908 polimorfizmus, reikšmingų sąsajų su naviko fenotipais, prognoze ar pacienčių amžiumi nenustatyta.

Vis dėlto *SRSF1* rs34592492 G alelis rodo reikšmingą sąsają su metastazėmis (M). Tiriant G alelį turinčius asmenis, daugiausia buvo tų, kurie turi metastazes, o remiantis atliktais chi kvadrato testais ($p < 0,001$) matomas statistiškai reikšmingas ryšys. Nepaisant tikrai tikslių reikšmingumo testų rezultatų (p = 0,058), vis dėlto dėl mažo šio alelio turėtojų skaičiaus siūloma rezultatus vertinti atsargiai. Įvertinus riziką matyti, kad G alelį turintiems asmenims buvo 19 kartų didesnė metastazių rizika, nors platus pasikliautinasis intervalas rodo neapibrėžtumą dėl mažo imties dydžio. Todėl nors sąsaja yra, įrodymai nėra pakankamai tvirti, kad būtų galima neabejotinai teigti, jog G alelis turi tiesioginę įtaką metastazėms atsirasti.

***HOTAIR* genas:**

Tiriant rs12826786, rs7958904 ir rs920778 polimorfizmus, reikšmingo ryšio tarp genotipų ar alelių ir naviko fenotipo (naviko dydžio, plitimo limfmazgiuose, metastazių, naviko diferenciacijos, stadijos, ligos prognozės ir paciento amžiaus) nenustatyta.

***MALAT1* genas:**

Tiriant rs619586, rs664589 ir rs3200401 polimorfizmus, reikšmingų sąsajų tarp genotipų ar alelių ir įvairių naviko fenotipų, įskaitant naviko dydį, limfmazgių pažeidimus, metastazes, naviko diferenciaciją, stadiją, ligos prognozę ir paciento amžių, nenustatyta.

Haplotipų asociacijų analizė

Analizuojant dažniausią *TLR4* geno polimorfizmų rs4986790, rs4986791 ir rs1927906 haplotipą (ACT), reikšmingų sąsajų tarp *TLR4* diplotipų ir klinikinių charakteristikų nenustatyta. ACT haplotipo buvimas, nesvarbu, ar homozigotinis, ar heterozigotinis, neturi reikšmingos įtakos naviko agresyvumui, metastazėms ar prognozei.

Manydami, kad *RRP1B* polimorfizmai gali neveikti nepriklausomai, analizavome haplotipus, siekdami nustatyti bendrą kelių polimorfizmų poveikį. Tyrėme įvairių diplotipų ir klinikinių savybių sąsajas, lygindami heterozigotines ir haplotipų (ATCCG, GCTTC) neturinčias turėtojas su jų homozigotiniais atitikmenimis. Svarbu pažymėti, kad GCTTC haplotipo neturintys asmenys turėjo reikšmingai mažesnę išplitusio naviko dydžio (T3–T4) ir metastazių riziką, palyginti su homozigotiniais turėtojais (atitinkamai ŠS = 0,367, 95 proc. PI: 0,136–0,992, $p = 0,038$ ir ŠS = 0,098, 95 proc. PI: 0,016–0,578, $p = 0,010$). Pacientėms, neturinčioms ATCCG haplotipo, buvo didesnė išplitusio naviko stadijos ir blogesnės prognozės tikimybė, palyginti su homozigotinėmis ATCCG turėtojomis (ŠS = 2,100, 95 proc. PI: 0,638–6,916, $p = 0,048$). Daugiamatės analizės patvirtino šias sąsajas. GCTTC haplotipo neturėjimas nuosekliai rodė jo apsauginį poveikį, lemiantį mažesnį išplitusio naviko dydį (ŠS = 0,393, 95 proc. PI: 0,188–0,822, $p = 0,039$) ir apsaugą nuo metastazių atsiradimo (ŠS = 0,101, 95 proc. PI: 0,017–0,598, $p = 0,012$), net po amžiaus, naviko laipsnio, limfmazgių būklės ir kitų veiksnių įtakos vertinimo.

Apibendrinant galima teigti, jog šie statistiškai reikšmingi rezultatai patvirtina *RRP1B* haplotipų, ypač GCTTC, reikšmingą genetinių pokyčių vaidmenį ir jų įtaką naviko dydžiui ir metastazėms, sergant gimdos kaklelio vėžiu, nepriklausomai nuo kitų klinikinių veiksnių.

Logistinės regresijos analizė, lyginant *SRSF1* geno polimorfizmų rs8819, rs11654058, rs2233908 ir rs2585828 diplotipus su klinikinėmis savybėmis,

įskaitant amžių ir naviko ypatybes, šioje kohortoje neparodė reikšmingo CTGA haplotipo (nesvarbu, ar homozigotinis, ar heterozigotinis) poveikio naviko savybėms, metastazėms arba prognozei.

Vienmatės logistinės regresijos analizė, vertinant *HOTAIR* haplotipus (CGG, CGA, TCA), neparodė reikšmingų sąsajų su klinikiniais rezultatais, įskaitant naviko stadiją, limfmazgių įtraukimą, metastazes, naviko laipsnį, amžių ar prognozę. Šių haplotipų buvimas, nesvarbu, ar homozigotinis, ar heterozigotinis, neturi reikšmingos įtakos naviko savybėms ar prognozei šioje populiacijoje.

Išgyvenamumo analizė

***TLR4* genas:**

Daugumos *TLR4* polimorfizmų genotipų ar alelių ir išgyvenamumo rezultatų sąsajos nebuvo statistiškai reikšmingos. Tačiau rs10759932 ir rs11536898 turėjo reikšmingos įtakos laikui be ligos progresavimo (PFS) ir bendram išgyvenamumui (OS).

Ištyrus rs10759932, nustatyta, kad CC genotipas buvo susijęs su trumpesniu PFS ir OS, palyginti su TT genotipu. Kaplano–Meierio analizė parodė reikšmingas sąsajas su OS (*Log Rank p* = 0,049, Breslow *p* = 0,018, Tarone–Ware *p* = 0,028). Cox regresijos analizė parodė, kad CC genotipas beveik tris kartus padidino trumpesnio PFS riziką (RS = 2,918, 95 proc. PI: 0,894–9,530, *p* = 0,049) ir daugiau nei tris kartus padidino trumpesnio OS riziką (RS = 3,340, 95 proc. PI: 1,006–11,095, *p* = 0,048). Po koregavimo pagal naviko stadiją, limfmazgių įtraukimą, diferenciacijos laipsnį ir paciento amžių CC genotipas vis dar reikšmingai didino trumpesnio laiko iki ligos progresavimo riziką (RS = 3,674, 95 proc. PI: 1,115–12,108, *p* = 0,032) ir ankstyvesnės mirties riziką (RS = 4,608, 95 proc. PI: 1,344–15,801, *p* = 0,015). Priešingai, T alelio turėtojoms buvo reikšmingai didesnė tikimybė, kad bus ilgesnis PFS (RS = 0,244, 95 proc. PI: 0,075–0,795, *p* = 0,019) ir ilgesnis OS (RS = 0,200, 95 proc. PI: 0,059–0,674, *p* = 0,009).

Ištyrus rs11536898, nustatyta, kad AA genotipas buvo susijęs su trumpesniu PFS ir OS, palyginti su CC genotipu. Kaplano–Meierio analizė parodė reikšmingas sąsajas tiek su PFS (*Log Rank p* = 0,014, Breslow *p* = 0,001, Tarone–Ware *p* = 0,003), tiek su OS (*Log Rank p* = 0,003, Breslow *p* < 0,001, Tarone–Ware *p* < 0,001). AA genotipas reikšmingai sumažino ilgesnio PFS tikimybę (RS = 3,926, 95 proc. PI: 1,201–12,837, *p* = 0,024) ir padidino trumpesnio OS riziką (RS = 5,057, 95 proc. PI: 1,522–16,802, *p* = 0,008). Daugiamatėje analizėje AA genotipas išliko reikšmingai susijęs su didesne trumpesnio OS rizika (RS = 3,735, 95 proc. PI: 1,051–13,278, *p* = 0,042) ir parodė ribinį poveikį PFS (RS = 3,306, 95 proc. PI: 0,967–11,299, *p* = 0,057).

C alelis rs11536898 buvo susijęs su ilgesniu PFS (RS = 0,291, 95 proc. PI: 0,086–0,987, $p = 0,048$) ir ilgesniu OS (RS = 0,274, 95 proc. PI: 0,078–0,959, $p = 0,043$) po klinikinių veiksnių koregavimo.

Priešingai, *TLR4* ACT haplotipas šioje kohortoje nerodė reikšmingos sąsajos su PFS arba OS.

***RRP1B* genas:**

Tarp rs2838342, rs7276633, rs2051407 ar rs762400 genotipų ar alelių ir PFS reikšmingų sąsajų nenustatyta. Tačiau rs2838342 A alelis buvo susijęs su geresniu OS (RS = 0,465, 95 proc. PI: 0,232–0,931, $p = 0,031$), ir ši sąsaja išliko reikšminga po amžiaus koregavimo (RS = 0,462, $p = 0,030$). Rs7276633 T alelis taip pat buvo susijęs su geresniu OS (RS = 0,465, $p = 0,031$), šis poveikis išliko reikšmingas po koregavimo pagal amžių (RS = 0,462, $p = 0,030$). Rs2051407 C alelis taip pat turėjo apsauginį poveikį OS (RS = 0,418, $p = 0,017$) ir šis poveikis išliko reikšmingas daugiamatėje analizėje (RS = 0,404, $p = 0,014$).

Rs762400 CC genotipas parodė didesnę trumpesnio OS (RS = 2,550, $p = 0,030$) riziką, ir šis poveikis išliko reikšmingas po koregavimo pagal amžių (RS = 2,476, $p = 0,035$). Tačiau šis poveikis buvo ne toks reikšmingas, kai buvo atsižvelgta į platesnes naviko charakteristikas, kur išplitusio naviko dydis turėjo didelį poveikį OS (RS = 7,546, $p < 0,001$).

RRP1B haplotipų analizė parodė, kad GCTTC haplotipo neturinčios tiriamosios turėjo geresnį OS, palyginti su homozigotinėmis GCTTC haplotipo turėtojomis (RS = 0,298, $p = 0,005$), o tai rodo jo apsauginį vaidmenį. Šis poveikis buvo nuoseklus visuose daugiamačiuose modeliuose, pabrėžiant haplotipo buvimo reikšmę išgyvenamumo rezultatams.

Apibendrinant galima teigti, kad daugiamatė analizė leidžia akcentuoti reikšmingą tam tikrų genetinių variantų ir naviko charakteristikų vaidmenį, darančius įtaką OS, o kai kurie aleliai ir haplotipai rodo esant galimą apsauginį poveikį.

***SIPA1* genas:**

Cox vienmatė *SIPA1* polimorfizmų rs746429, rs931127 ir rs3741378 analizė neparodė reikšmingų sąsajų su laiku be ligos progresavimo (PFS) ar bendruoju išgyvenamumu (OS). Rizikos santykiai (HRs), pasikliautinumo intervalai (CIs) ir *p* vertės rodo, kad šioje kohortoje šie polimorfizmai neturi reikšmingo poveikio PFS ar OS.

***SRSF1* genas:**

Cox vienmatė analizė dėl *SRSF1* polimorfizmų rs8819, rs11654058, rs2233908 ir rs2585828 neparodė reikšmingos įtakos laikui be ligos progresavimo (PFS) ar bendrajam išgyvenamumui (OS). Rs34592492 atveju G alelis iš pradžių buvo susijęs su apsauginiu poveikiu PFS (RS = 0,328, $p = 0,022$), tačiau šis reikšmingumas neišryškėjo daugiamatėje analizėje, atsižvelgiant į

amžių ir naviko ypatybes. Kita vertus, rs34592492 CC genotipas buvo reikšmingai susijęs su sumažėjusiu OS tiek vienmatėje (RS = 19,947, p = 0,005), tiek daugiamatėje analizėje (RS = 12,582, p = 0,023), o G alelis išlaikė savo apsauginį poveikį (RS = 0,078, p = 0,022).

Naviko stadija T3–T4 taip pat parodė stiprią sąsają su sumažėjusiu OS (RS = 7,738, $p < 0,001$). Amžius diagnozės metu ir naviko diferenciacijos laipsnis neturėjo reikšmingos įtakos OS. *SRSF1* haplotipai, įskaitant CTGA, neparodė reikšmingų sąsajų su PFS ar OS.

***HOTAIR* genas:**

Cox vienmatė *HOTAIR* polimorfizmų rs12826786, rs7958904 ir rs920778 analizė neparodė reikšmingų sąsajų su laiku be ligos progresavimo (PFS) ar bendruoju išgyvenamumu (OS). Genotipų (pvz., CT *vs.* CC, CG *vs.* GG, AG *vs.* GG) ir alelių skirtumai neturėjo reikšmingos įtakos išgyvenamumo rezultatams. *HOTAIR* haplotipai (CGG, CGA, TCA) taip pat neturėjo reikšmingos įtakos PFS ar OS. Visos p vertės buvo statistiškai nereikšmingos. Šie rezultatai rodo, kad *HOTAIR* polimorfizmų ir haplotipų variantai šioje duomenų imtyje neturi reikšmingos įtakos išgyvenamumo rezultatams.

***MALAT1* genas:**

Cox vienmatė *MALAT1* polimorfizmų rs619586 ir rs3200401 analizė neparodė reikšmingų sąsajų su PFS ar OS. Tačiau rs664589 GG genotipas buvo reikšmingai susijęs su blogesniu OS (RS = 12.212, p = 0.016), bet ši reikšmė sumažėjo daugiamačiuose modeliuose, kai buvo atsižvelgta į pacienčių amžių ir naviko charakteristikas. Rs664589 C alelis pradžioje rodė apsauginį poveikį OS (RS = 0.083, p = 0.017), bet šis poveikis taip pat sumažėjo daugiamačiuose modeliuose.

Amžius diagnozės metu neturėjo reikšmingos įtakos OS, o naviko charakteristikos, ypač N1 stadija, ir T3–T4 buvo stipriai susijusios su blogesniu OS. Remiantis analize, nors rs664589 gali turėti įtakos OS, jos poveikiui tikriausiai turėjo kiti veiksniai, pvz., naviko charakteristikos.

IŠVADOS

1. Remiantis tyrimais, nustatytas ryšys tarp *TLR4* polimorfizmų ir gimdos kaklelio vėžio klinikinių patologinių ypatybių bei išgyvenamumo:
   1.1. Rs11536898 A alelis buvo susijęs su didesne vėžio metastazavimo rizika.
   1.2. Rs11536898 AA genotipas buvo susijęs su trumpesniu bendruoju išgyvenamumu ir laiku be ligos progresavimo, o šio polimorfizmo C alelis – su ilgesniu bendruoju išgyvenamumu ir ilgesniu laiku be ligos progresavimo.

1.3. Rs10759932 CC genotipas turėjo reikšmingos įtakos trumpesniam laikui be ligos progresavimo ir bendrajam išgyvenamumui, o šio polimorfizmo T alelis buvo susijęs su ilgesniu laiku be ligos progresavimo ir bendruoju išgyvenamumu.

2. Nustatytos įvairios *RRP1B*, *SIPA1* ir *SRSF1* polimorfizmų sąsajos su gimdos kaklelio vėžio klinikiniais patologiniais požymiais ir išgyvenamumo rodikliais:
   2.1. *RRP1B* rs2838342 A alelis ir rs7276633 T alelis buvo susijęs su mažesniu naviko dydžiu.
   2.2. Tiriamosios, neturinčios *RRP1B* GCTTC haplotipo, turėjo reikšmingą sąsają su mažesniu naviko dydžiu ir mažesnę metastazavimo bei blogesnio bendrojo išgyvenamumo riziką.
   2.4. *SIPA1* rs746429 GA genotipas ir A alelis buvo reikšmingai susiję su mažesne blogos naviko diferenciacijos rizika.
   2.5. *SRSF1* rs34592492 G alelis buvo susijęs su ilgesniu bendruoju išgyvenamumu, o CC genotipas – su trumpesniu bendruoju išgyvenamumu.

3. *HOTAIR* ir *MALAT1* genų polimorfizmai nebuvo reikšmingi gimdos kaklelio vėžio fenotipui ir laikui be ligos progresavimo ar bendrajam išgyvenamumui.

REFERENCES

1. World Health Organization (WHO). Cervical Cancer. **2024**. Available online: https://www.who.int/news-room/fact-sheets/detail/cervical-cancer (accessed on 7 August 2024).
2. Crosbie, E. J.; Einstein, M. H.; Franceschi, S.; Kitchener, H. C. Human papillomavirus and cervical cancer. *The Lancet*. **2013**, 382(9895), 889-899.
3. Stelzle, D.; Tanaka, L. F.; Lee, K. K.; Khalil, A. I.; Baussano, I.; Shah, A. S. V.; McAllister, D. A.; Gottlieb, S. L.; Klug, S. J.; Winkler, A. S.; Bray, F.; Baggaley, R.; Clifford, G. M.; Broutet, N.; Dalal, S. Estimates of the global burden of cervical cancer associated with HIV. *The Lancet Global Health*. **2021**, *9*(2), e161–e169. https://doi.org/10.1016/s2214-109x(20)30459-9.
4. Lee, S.; Vigliotti, J.; Vigliotti, V.; Jones, W. From human papillomavirus (HPV) detection to cervical cancer prevention in clinical practice. *Cancers*. **2014**, *6*(4), 2072–2099. https://doi.org/10.3390/cancers6042072.
5. Agarwal, M.; Kumar, M.; Pathak, R.; Bala, K.; Kumar, A. Chapter Six – Exploring TLR signaling pathways as promising targets in cervical cancer: The road less traveled. *International Review of Cell and Molecular Biology*. **2024**, 385, 227-261. https://doi.org/10.1016/bs.ircmb.2023.11.005.
6. Zhang, C.; Yang, Z.; Luo, P.; Ye, M.; Gong, P.; Gong, Q.; Mei, B. Association of TLR4 and TLR9 gene polymorphisms with the risk and progression of cervical lesions in HPV-infected women. *Biomarkers in Medicine*. **2023**, *17*(3), 133–142. https://doi.org/10.2217/bmm-2022-0702.
7. Lee, M.; Dworkin, A. M.; Lichtenberg, J.; Patel, S. J.; Trivedi, N. S.; Gildea, D.; Bodine, D. M.; Crawford, N. P. Metastasis-Associated Protein Ribosomal RNA Processing 1 Homolog B (RRP1B) Modulates Metastasis through Regulation of Histone Methylation. *Molecular Cancer Research*. **2014**, *12*(12), 1818–1828. https://doi.org/10.1158/1541-7786.mcr-14-0167.
8. Crawford, N. P. S.; Qian, X.; Ziogas, A.; Papageorge, A. G.; Boersma, B. J.; Walker, R. C.; Lukes, L.; Rowe, W. L.; Zhang, J.; Ambs, S.; Lowy, D. R.; Anton-Culver, H.; Hunter, K. W. RRP1B, a new candidate susceptibility gene for breast cancer progression and metastasis. *PLoS Genetics*. **2007**, *3*(11), e214. https://doi.org/10.1371/journal.pgen.0030214.
9. Liu, C.; Jiang, W.; Hargest, R.; Martin, T. The role of SIPA1 in the development of cancer and metastases (Review). *Molecular and Clinical Oncology*. **2020**, 13, 32. https://doi.org/10.3892/mco.2020.2102.
10. Lo Giudice, A.; Asmundo, M. G.; Broggi, G.; Cimino, S.; Morgia, G.; Di Trapani, E.; Luzzago, S.; Musi, G.; Ferro, M.; De Cobelli, O.; Russo, G. I. The Clinical role of SRSF1 expression in Cancer: A review of the current literature. *Applied Sciences*. **2022**, *12*(5), 2268. https://doi.org/10.3390/app12052268.
11. Zhou, Y.; Cui, Y.; Wang, T.; Luo, Y. Long non-coding RNA HOTAIR in cervical cancer: Molecular marker, mechanistic insight, and therapeutic target. *Advances in Clinical Chemistry*. **2020**, 117–140. https://doi.org/10.1016/bs.acc.2019.12.004.
12. Sun, R.; Qin, C.; Jiang, B.; Fang, S.; Pan, X.; Peng, L.; Liu, Z.; Li, W.; Li, Y.; Li, G. Down-regulation of MALAT1 inhibits cervical cancer cell invasion and metastasis by inhibition of epithelial–mesenchymal transition. *Molecular BioSystems*. **2016**, *12*(3), 952–962. https://doi.org/10.1039/c5mb00685f.

13. Bruni, L., Albero, G.; Serrano, B.; Mena, M.; Collado, J.J.; Gómez, D.; Muñoz, J.; Bosch, F. X.; de Sanjosé, S. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in the World. Summary Report 10 March **2023**. [(accessed on 17 August 2024).
14. Arbyn, M.; Weiderpass, E.; Bruni, L.; de Sanjosé, S.; Saraiya, M.; Ferlay, J.; Bray, F. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *The Lancet Global Health*, **2020**, *8*(2), e191-e203. https://doi.org/10.1016/S2214-109X(19)30482-6.
15. Denny, L.; Anorlu, R.; Boa, R. Cervical cancer in Africa. *Cancer Epidemiology Biomarkers & Prevention*. *2012*, 21(9), 1434-1438. https://doi.org/doi.org/10.1158/1055-9965.EPI-12-0334.
16. Dzinamarira, T.; Moyo, E.; Dzobo, M.; Mbunge, E.; Murewanhema, G. Cervical cancer in sub-Saharan Africa: an urgent call for improving accessibility and use of preventive services. *International Journal of Gynecological Cancer*. **2022**, *33*(4), 592–597. https://doi.org/10.1136/ijgc-2022-003957.
17. Mulongo, M.; Chibwesha, C. J. Prevention of cervical cancer in Low-Resource African settings. *Obstetrics and Gynecology Clinics of North America*. **2022**, *49*(4), 771–781. https://doi.org/10.1016/j.ogc.2022.08.008.
18. Mantula, F.; Toef.y, Y.; Sewram, V. Barriers to cervical cancer screening in Africa: a systematic review. *BMC Public Health*. **2024**, *24*(1). https://doi.org/10.1186/s12889-024-17842-1.
19. Vaccarella, S.; Lortet-Tieulent, J.; Plummer, M.; Franceschi, S.; Bray, F. Worldwide trends in cervical cancer incidence: Impact of screening against changes in disease risk factors. *European Journal of Cancer*. **2013**, *49*(15), 3262-3273. https://doi.org/10.1016/j.ejca.2013.04.024.
20. Ritchie, D.; Arbyn, M.; Basu, P.; Corbex, M.; Fidarova, E.; Ivanuš, U.; Papi, G.; Torode, J.; Zacharopoulou, C. Europe's path to eliminating cervical cancer as a public health problem. *The Lancet Regional Health – Europe*. **2022**, *12*, 100276. https://doi.org/10.1016/j.lanepe.2021.100276.
21. European Centre for Disease Prevention and Control (ECDC). Cervical cancer screening in the EU/EEA: Quality assurance and organization. **2021**. Retrieved from https://www.ecdc.europa.eu/en/publications-data/cervical-cancer-screening-eueea-quality-assurance-and-organization. (accessed on 15 August 2024).
22. Crosbie, E. J.; Einstein, M. H.; Franceschi, S.; Kitchener, H. C. Human papillomavirus and cervical cancer. *The Lancet Journal*. **2013**, *382*(9895), P889-899. https://doi.org/10.1016/s0140-6736(13)60022-7.
23. Schiffman, M.; Herrero, R.; DeSalle, R.; Hildesheim, A.; Wacholder, S.; Rodríguez, A. C.; Bratti, M. C.; Sherman, M. E.; Morales, J.; Guillén, D. M. G.; Alfaro, M.; Hutchinson, M. L.; Wright, T.; Solomon, D.; Chen, Z.; Schussler, J.; Castle, P. E.; Burk, R. D. The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology*. **2005**, *337*(1), 76–84. https://doi.org/10.1016/j.virol.2005.04.002.
24. Castle, P. E.; Rodríguez, A. C.; Burk, R. D.; Herrero, R.; Wacholder, S.; Alfaro, M.; Morales, J.; Guillén, D. M. G.; Sherman, M. E.; Solomon, D.; Schiffman, M. Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. *The BMJ*. **2009**, *339*(jul28 2), b2569. https://doi.org/10.1136/bmj.b2569.

25. De Sanjosé, S.; Díaz, M.; Castellsagué, X.; Clifford, G. M.; Bruni, L.; Muñóz, N.; Bosch, F. X. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *The Lancet Infectious Diseases*. **2007**, *7*(7), 453–459. https://doi.org/10.1016/s1473-3099(07)70158-5.
26. Li, N.; Franceschi, S.; Howell-Jones, R.; Snijders, P. J.; Clifford, G. M. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. *International Journal of Cancer*. **2010**, *128*(4), 927–935. https://doi.org/10.1002/ijc.25396.
27. Guan, P.; Howell-Jones, R.; Li, N.; Bruni, L.; De Sanjosé, S.; Franceschi, S.; Clifford, G. M. Human papillomavirus types in 115,789 HPV-positive women: A meta-analysis from cervical infection to cancer. *International Journal of Cancer*. **2012**, *131*(10), 2349–2359. https://doi.org/10.1002/ijc.27485.
28. Brown, D. R.; Kjær, S. K.; Sigurðsson, K.; Iversen, O.; Hernández-Ávila, M.; Wheeler, C. M.; Pérez, G.; Koutsky, L. A.; Tay, E. H.; García, P. J.; Ault, K. A.; Garland, S. M.; Leodolter, S.; Olsson, S.; Tang, G.; Ferris, D. G.; Paavonen, J.; Steben, M.; Bosch, F. X.; Barr, E. The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 Virus-Like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-Naive women aged 16–26 years. *The Journal of Infectious Diseases.* **2009**, *199*(7), 926–935. https://doi.org/10.1086/597307.
29. Paavonen, J.; Naud, P.; Salmerón, J.; Wheeler, C. M.; Chow, S. N.; Apter, D.; Kitchener, H. C.; Castellsagué, X.; Teixeira, J. C.; Skinner, S. R.; Hedrick, J.; Jaisamrarn, U.; Limson, G.; Garland, S. M.; Szarewski, A.; Romanowski, B.; Aoki, F. Y.; Schwarz, T. F.; Poppe, W.; Dubin, G. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. *The Lancet*. **2009**, *374*(9686), 301–314. https://doi.org/10.1016/s0140-6736(09)61248-4.
30. Einstein, M. H.; Baron, M.; Levin, M. J.; Chatterjee, A.; Fox, B.; Scholar, S.; Rosen, J. B.; Chakhtoura, N.; Lebacq, M.; Van Der Most, R.; Moris, P.; Giannini, S. L.; Schuind, A.; Datta, S.; Descamps, D. Comparison of the immunogenicity of the human papillomavirus (HPV)-16/18 vaccine and the HPV-6/11/16/18 vaccine for oncogenic non-vaccine types HPV-31 and HPV-45 in healthy women aged 18–45 years. *Human Vaccines.* **2011**, *7*(12), 1359–1373. https://doi.org/10.4161/hv.7.12.18282.
31. Roteli-Martins, C. M.; Naud, P.; De Borba, P. C.; Teixeira, J. C.; De Carvalho, N. S.; Zahaf, T.; Sánchez, N.; Geeraerts, B.; Descamps, D. Sustained immunogenicity and efficacy of the HPV-16/18 AS04-adjuvanted vaccine. *Human Vaccines & Immunotherapeutics*. **2012**, *8*(3), 390–397. https://doi.org/10.4161/hv.18865.
32. Wheeler, C. M.; Castellsagué, X.; Garland, S. M.; Szarewski, A.; Paavonen, J.; Naud, P.; Salmerón, J.; Chow, S. N.; Apter, D.; Kitchener, H. C.; Teixeira, J. C.; Skinner, S. R.; Jaisamrarn, U.; Limson, G.; Romanowski, B.; Aoki, F. Y.; Schwarz, T. F.; Poppe, W.; Bosch, F. X.; Lehtinen, M. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *The Lancet Oncology.* **2012**, *13*(1), 100–110. https://doi.org/10.1016/s1470-2045(11)70287-x.

33. Naud, P.; Roteli-Martins, C. M.; De Carvalho, N. S.; Teixeira, J. C.; De Borba, P. C.; Sánchez, N.; Zahaf, T.; Catteau, G.; Geeraerts, B.; Descamps, D. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine. *Human Vaccines & Immunotherapeutics*. **2014**, *10*(8), 2147–2162. https://doi.org/10.4161/hv.29532.
34. Zhu, F.; Hu, S.; Hong, Y.; Hu, Y.; Zhang, X.; Zhang, Y.; Pan, Q.; Zhang, W.; Zhao, F.; Zhang, C.; Yang, X.; Yu, J.; Zhu, J.; Zhu, Y.; Chen, F.; Zhang, Q.; Wang, H.; Wang, C.; Bi, J.; Struyf, F. Efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine in Chinese women aged 18–25 years: event-triggered analysis of a randomized controlled trial. *Cancer Medicine*. **2016**, *6*(1), 12–25. https://doi.org/10.1002/cam4.869.
35. Lei, J.; Ploner, A.; Elfström, K. M.; Wang, J.; Roth, A.; Fang, F.; Sundström, K.; Dillner, J.; Sparén, P. HPV vaccination and the risk of invasive cervical cancer. *The New England Journal of Medicine*. **2020**, *383*(14), 1340–1348. https://doi.org/10.1056/nejmoa1917338.
36. Kamolratanakul, S.; Pitisuttithum, P. Human Papillomavirus Vaccine Efficacy and Effectiveness against Cancer. *Vaccines.* **2021**, *9*(12), 1413. https://doi.org/10.3390/vaccines9121413.
37. Oh, N. L.; Biddell, C. B.; Rhodes, B. E.; Brewer, N. T. Provider communication and HPV vaccine uptake: A meta-analysis and systematic review. *Preventive Medicine.* **2021**, *148*, 106554. https://doi.org/10.1016/j.ypmed.2021.106554.
38. Pappa, K.; Kontostathi, G.; Lygirou, V.; Zoidakis, J.; Anagnou, N. Novel structural approaches concerning HPV proteins: Insight into targeted therapies for cervical cancer (Review). *Oncol. Rep*. **2018**, 39, 1547–1554. https://doi.org/10.3892/or.2018.6257.
39. De Sanjosé, S.; Brotons, M.; Pavón, M.Á. The natural history of human papillomavirus infection. *Best Pract. Res. Clin. Obstet. Gynaecol*. **2018**, 47, 2–13. https://doi.org/10.1016/j.bpobgyn.2017.08.015.
40. Drolet, M.; Bénard, É.; Pérez, N.; Brisson, M.; Ali, H.; Boily, M.; Baldo, V.; Brassard, P.; Brotherton, J.; Callander, D.; et al. Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: Updated systematic review and meta-analysis. *Lancet.* **2019**, 394, 497–509. https://doi.org/10.1016/s0140-6736(19)30298-3.
41. Small, W.; Bacon, M.; Bajaj, A.; Chuang, L.; Fisher, B.J.; Harkenrider, M.M.; Jhingran, A.; Kitchener, H.C.; Mileshkin, L.; Viswanathan, A.N.; et al. Cervical cancer: A global health crisis. *Cancer*. **2017**, 123, 2404–2412. https://doi.org/10.1002/cncr.30667.
42. European Centre for Disease Prevention and Control (ECDC). Cervical Cancer Screening in the EU/EEA: Quality Assurance and Organization. **2021**. Available online: https://www.ecdc.europa.eu/en/publications-data/cervical-cancer-screening-eueea-quality-assurance-and-organization (accessed on 8 January 2024).
43. Bruni, L.; Saura-Lázaro, A.; Montoliu, A.; Brotons, M.; Alemany, L.; Diallo, M. S.; Afsar, O. Z.; LaMontagne, D. S.; Mosina, L.; Contreras, M.; Velandia-González, M.; Pastore, R.; Gacic-Dobo, M.; Bloem, P. HPV vaccination introduction worldwide and WHO and UNICEF estimates of national HPV immunization coverage 2010–2019. *Preventive Medicine.* **2021**, *144*, 106399. https://doi.org/10.1016/j.ypmed.2020.106399.

44. Aziz, S. Q.; Aziz, M. Cervical cancer metastasis. *In Elsevier eBooks*. 2017, pp. 77–94. https://doi.org/10.1016/b978-0-12-804003-4.00005-0.
45. Zhang, S.; Xu, H.; Zhang, L.; Qiao, Y. Cervical cancer: Epidemiology, risk factors and screening. *Chinese Journal of Cancer Research.* **2020**, *32*(6), 720–728. https://doi.org/10.21147/j.issn.1000-9604.2020.06.05.
46. Basaran, D.; Leitao, M. M. The landmark series: Minimally Invasive Surgery for Cervical cancer. *Annals of Surgical Oncology.* **2020**, *28*(1), 204–211. https://doi.org/10.1245/s10434-020-09265-0.
47. Patel, D.; Tayade, S.; Thakur, A. S.; Singh, S. Revolutionizing Early-Stage Cervical Cancer Treatment: A Comprehensive review of Radical trachelectomy as a minimally Invasive approach. *Cureus.* **2024**, *16*(2): e53958. https://doi.org/10.7759/cureus.53958.
48. Faye, M. D.; Alfieri, J. Advances in radiation oncology for the treatment of cervical cancer. *Current Oncology.* **2022**, *29*(2), 928–944. https://doi.org/10.3390/curroncol29020079.
49. Anghel, B.; Serboiu, C.; Marinescu, A.; Taciuc, I.; Bobirca, F.; Stanescu, A. D. Recent advances and adaptive strategies in image guidance for cervical cancer radiotherapy. *Medicina.* **2023**, *59*(10), 1735. https://doi.org/10.3390/medicina59101735.
50. Mbodi, L.; Maringa, V. D.; Moroeng, M. W.; Bassa, S.; Kaufmann, A. M.; Dlamini, Z. An overview of cervical cancer, chemotherapy as treatment and chemotherapy resistance. In *Elsevier eBooks.* 2024, pp. 1–16. https://doi.org/10.1016/b978-0-443-28985-9.00017-3.
51. Watkins, D. E.; Craig, D. J.; Vellani, S. D.; Hegazi, A.; Fredrickson, K. J.; Walter, A.; Stanbery, L.; Nemunaitis, J. Advances in targeted therapy for the treatment of cervical cancer. *Journal of Clinical Medicine.* **2023**, *12*(18), 5992. https://doi.org/10.3390/jcm12185992.
52. Xing, Y.; Yasinjan, F.; Du, Y.; Geng, H.; Zhang, Y.; He, M.; Guo, R.; Yang, L.; Cui, J.; Mu, D.; Liu, Z.; Wang, H. Immunotherapy in cervical cancer: From the view of scientometric analysis and clinical trials. *Frontiers in Immunology.* **2023**, *14*. https://doi.org/10.3389/fimmu.2023.1094437.
53. Damane, B. P.; Mulaudzi, T. V.; Kgokolo, M. C.; Luvhengo, T.; Skepu, A.; Rotimi, S. O.; Dlamini, Z. Future directions in cervical cancer treatment. In *Elsevier eBooks.* **2024**, pp. 155–177. https://doi.org/10.1016/b978-0-443-28985-9.00004-5.
54. Braun, C.; Campana, B.; Brambs, C. Recent advances in cervical cancer treatment. *Healthbook TIMES Oncology Hematology.* **2024**, *19*. https://doi.org/10.36000/hbt.oh.2024.19.135.
55. Cafforio, P.; Palmirotta, R.; Lovero, D.; Cicinelli, E.; Cormio, G.; Silvestris, E.; Porta, C.; D'Oronzo, S. Liquid biopsy in cervical Cancer: Hopes and pitfalls. *Cancers.* **2021**, *13*(16), 3968. https://doi.org/10.3390/cancers13163968.
56. Choi, P.; Liu, T. L.; Wong, C. W.; Liu, S. K.; Lum, Y.; Ming, W. The dysregulation of MicroRNAs in the development of cervical Pre-Cancer – An update. *International Journal of Molecular Sciences.* **2022**, *23*(13), 7126. https://doi.org/10.3390/ijms23137126.
57. Vidlarova, M.; Rehulkova, A.; Stejskal, P.; Prokopova, A.; Slavik, H.; Hajduch, M.; Srovnal, J. Recent advances in methods for circulating tumor cell detection. *International Journal of Molecular Sciences.* **2023**, *24*(4), 3902. https://doi.org/10.3390/ijms24043902.

58. Mittelstadt, S.; Kelemen, O.; Admard, J.; Gschwind, A.; Koch, A.; Wörz, S.; Oberlechner, E.; Engler, T.; Bonzheim, I.; Staebler, A.; Weidner, N.; Stubenrauch, F.; Iftner, T.; Riess, O.; Schroeder, C.; Kommoss, S.; Ossowski, S. Detection of circulating cell-free HPV DNA of 13 HPV types for patients with cervical cancer as potential biomarker to monitor therapy response and to detect relapse. *British Journal of Cancer.* **2023**, *128*(11), 2097–2103. https://doi.org/10.1038/s41416-023-02233-x.
59. Wong, C.; Chen, Y. Clinical significance of exosomes as potential biomarkers in cancer. *World Journal of Clinical Cases.* **2019**, *7*(2), 171–190. https://doi.org/10.12998/wjcc.v7.i2.171.
60. Van Raemdonck, G. A. A.; Tjalma, W. A. A.; Coen, E. P.; Depuydt, C. E.; Van Ostade, X. W. M. Identification of protein biomarkers for cervical cancer using human cervicovaginal fluid. *PLoS ONE.* **2014**, *9*(9), e106488. https://doi.org/10.1371/journal.pone.0106488.
61. Kishore, V. Expression of P16INK4A protein in cervical intraepithelial neoplasia and invasive carcinoma of uterine cervix. *Journal of Clinical and Diagnostic Research.* 2017. https://doi.org/10.7860/jcdr/2017/29394.10644.
62. Downham, L.; Jaafar, I.; Rol, M. L.; Nyaga, V. N.; Valls, J.; Baena, A.; Zhang, L.; Gunter, M. J.; Arbyn, M.; Almonte, M. Accuracy of HPV E6/E7 oncoprotein tests to detect high-grade cervical lesions: a systematic literature review and meta-analysis. *British Journal of Cancer.* **2023**, *130*(4), 517–525. https://doi.org/10.1038/s41416-023-02490-w.
63. Nandakishore, N. A.; Alva, S.; Shetty, P.; Mysorker, V. A study of cytokeratin-7 expression and clinicopathological correlation in dysplasia and squamous cell carcinoma of the cervix. *Asian Pacific Journal of Cancer Prevention.* **2022**, *23*(3), 885–891. https://doi.org/10.31557/apjcp.2022.23.3.885.
64. He, X.; Wang, Y.; Ping, J.; Xu, W.; Fang, W.; Liu, J. The serum CK17 and CK19 expressions in cervical cancer patients and their prognostic value. *American journal of translational research.* **2021**, *13*(6), 6439–6445.
65. Zhang, J.; Liu, J.; Zhu, C.; He, J.; Chen, J.; Liang, Y.; Yang, F.; Wu, X.; Ma, X. Prognostic role of vascular endothelial growth factor in cervical cancer: a meta-analysis. *Oncotarget.* **2017**, *8*(15), 24797–24803. https://doi.org/10.18632/oncotarget.15044.
66. Piškur, I.; Topolovec, Z.; Bakula, M.; Zagorac, I.; Vranješ, I. M.; Vidosavljević, D. Expression of Vascular endothelial Growth Factor-A (VEGF-A) in adenocarcinoma and squamous cell cervical cancer and its impact on disease progression: Single institution experience. *Medicina.* **2023**, *59*(7), 1189. https://doi.org/10.3390/medicina59071189.
67. Zhang, J.; Dong, D.; Wei, Q.; Ren, L. CXCL10 serves as a potential serum biomarker complementing SCC-Ag for diagnosing cervical squamous cell carcinoma. *BMC Cancer.* **2022**, *22*(1). https://doi.org/10.1186/s12885-022-10142-0.
68. Ran, C.; Sun, J.; Qu, Y.; Long, N. Clinical value of MRI, serum SCCA, and CA125 levels in the diagnosis of lymph node metastasis and para-uterine infiltration in cervical cancer. *World Journal of Surgical Oncology.* **2021**, *19*(1). https://doi.org/10.1186/s12957-021-02448-3.
69. Tilborghs, S.; Corthouts, J.; Verhoeven, Y.; Arias, D.; Rolfo, C.; Trinh, X. B.; Van Dam, P. A. The role of Nuclear Factor-kappa B signaling in human cervical cancer. *Critical Reviews in Oncology/Hematology.* **2017**, *120*, 141–150. https://doi.org/10.1016/j.critrevonc.2017.11.001.

70. Liu, J.; Su, S.; Liu, Y. The value of Ki67 for the diagnosis of LSIL and the problems of p16 in the diagnosis of HSIL. *Scientific Reports.* **2022**, *12*(1). https://doi.org/10.1038/s41598-022-11584-z.
71. Chen, W.; Huang, S.; Shi, K.; Yi, L.; Liu, Y.; Liu, W. Prognostic Role of matrix metalloproteinases in cervical Cancer: A Meta-Analysis. *Cancer Control.* **2021**, *28*, 107327482110337. https://doi.org/10.1177/10732748211033743.
72. Tawe, L.; Grover, S.; Zetola, N.; Robertson, E. S.; Gaseitsiwe, S.; Moyo, S.; Kasvosve, I.; Paganotti, G. M.; Narasimhamurthy, M. Promoter hypermethylation analysis of host genes in cervical cancer patients with and without human immunodeficiency Virus in Botswana. *Frontiers in Oncology.* **2021**, *11*. https://doi.org/10.3389/fonc.2021.560296.
73. Zhao, T.; Liu, B.; Zhang, M.; Li, S.; Zhao, C.; Cheng, L. Assessment of alterations in histone modification function and guidance for death risk prediction in cervical cancer patients. *Frontiers in Genetics.* **2022**, *13*. https://doi.org/10.3389/fgene.2022.1013571.
74. Okawa, R.; Banno, K.; Iida, M.; Yanokura, M.; Takeda, T.; Iijima, M.; Kunitomi-Irie, H.; Nakamura, K.; Adachi, M.; Umene, K.; Nogami, Y.; Masuda, K.; Kobayashi, Y.; Tominaga, E.; Aoki, D. Aberrant chromatin remodeling in gynecological cancer (Review). *Oncology Letters.* 2**017**, *14*, 5107-5113. https://doi.org/10.3892/ol.2017.6891.
75. Fischer, M.; Uxa, S.; Stanko, C.; Magin, T. M.; Engeland, K. Human papilloma virus E7 oncoprotein abrogates the p53-p21-DREAM pathway. *Scientific Reports.* **2017**, *7*(1). https://doi.org/10.1038/s41598-017-02831-9.
76. Yeo-Teh, N.; Ito, Y.; Jha, S. High-Risk human papillomaviral oncogenes E6 and E7 target key cellular pathways to achieve oncogenesis. *International Journal of Molecular Sciences.* **2018**, *19*(6), 1706. https://doi.org/10.3390/ijms19061706.
77. Pergialiotis, V.; Nikolaou, C.; Haidopoulos, D.; Frountzas, M.; Thomakos, N.; Bellos, I.; Papapanagiotou, A.; Rodolakis, A. PIK3CA Mutations and Their Impact on Survival Outcomes of Patients with Cervical Cancer: A Systematic Review. *Acta Cytologica.* **2020**, *64*(6), 547–555. https://doi.org/10.1159/000509095.
78. Bahar, M. E.; Kim, H. J.; Kim, D. R. Targeting the RAS/RAF/MAPK pathway for cancer therapy: from mechanism to clinical studies. *Signal Transduction and Targeted Therapy.* **2023**, *8*(1). https://doi.org/10.1038/s41392-023-01705-z.
79. Wang, B.; Li, X.; Liu, L.; Wang, M. β-Catenin: oncogenic role and therapeutic target in cervical cancer. *Biological Research.* **2020**, *53*(1). https://doi.org/10.1186/s40659-020-00301-7.
80. Samarzija, I.; Beard, P. Hedgehog pathway regulators influence cervical cancer cell proliferation, survival and migration. *Biochemical and Biophysical Research Communications.* **2012**, *425*(1), 64–69. https://doi.org/10.1016/j.bbrc.2012.07.051.
81. Jing, J.; Wu, Z.; Wang, J.; Luo, G.; Lin, H.; Fan, Y.; Zhou, C. Hedgehog signaling in tissue homeostasis, cancers, and targeted therapies. *Signal Transduction and Targeted Therapy.* **2023**, *8*(1). https://doi.org/10.1038/s41392-023-01559-5.
82. Sun, Y.; Zhang, R.; Zhou, S.; Ji, Y. Overexpression of Notch1 is associated with the progression of cervical cancer. *Oncology Letters.* **2015**, *9*(6), 2750–2756. https://doi.org/10.3892/ol.2015.3143.
83. Aubrey, B. J.; Kelly, G. L.; Janic, A.; Herold, M. J.; Strasser, A. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? *Cell Death and Differentiation.* **2017**, *25*(1), 104–113. https://doi.org/10.1038/cdd.2017.169.
84. Sun, G.; Zhang, Q.; Liu, Y.; Xie, P. Role of Phosphatidylinositol 3-Kinase and its catalytic unit PIK3CA in Cervical Cancer: A Mini-Review. *Applied Bionics and Biomechanics.* **2022**, 1–6. https://doi.org/10.1155/2022/6904769.

85. Janku, F.; Tsimberidou, A. M.; Garrido-Laguna, I.; Wang, X.; Luthra, R.; Hong, D. S.; Naing, A.; Falchook, G. S.; Moroney, J. W.; Piha-Paul, S. A.; Wheler, J. J.; Moulder, S. L.; Fu, S.; Kurzrock, R. PIK3CA Mutations in Patients with Advanced Cancers Treated with PI3K/AKT/mTOR Axis Inhibitors. *Molecular Cancer Therapeutics.* **2011**, *10*(3), 558–565. https://doi.org/10.1158/1535-7163.mct-10-0994.
86. Jiang, W.; Xiang, L.; Pei, X.; He, T.; Shen, X.; Wu, X.; Yang, H. Mutational analysis ofKRASand its clinical implications in cervical cancer patients. *Journal of Gynecologic Oncology.* **2018**, *29*(1). https://doi.org/10.3802/jgo.2018.29.e4.
87. Mustachio, L. M.; Chelariu-Raicu, A.; Szekvolgyi, L.; Roszik, J. Targeting KRAS in Cancer: Promising therapeutic Strategies. *Cancers.* **2021**, *13*(6), 1204. https://doi.org/10.3390/cancers13061204.
88. Liu, F.; Chang, L.; Hu, J. Activating transcription factor 6 regulated cell growth, migration and inhibiteds cell apoptosis and autophagy via MAPK pathway in cervical cancer. *Journal of Reproductive Immunology*. **2020**, *139*, 103120. https://doi.org/10.1016/j.jri.2020.103120.
89. Aisagbonhi, O.; Zare, S. Y.; Hasteh, F.; Binder, P.; Roma, A. A.; Fadare, O. PTEN Loss and ARID1A Mutation in an HPV-positive Metastatic Adenocarcinoma Diagnosed Almost 18 yr After an Intact Cone Excision for Endocervical Adenocarcinoma In Situ. *International Journal of Gynecological Pathology.* **2021**, *41*(3), 307–312. https://doi.org/10.1097/pgp.0000000000000811.
90. Münzel, P.; Marx, D.; Köchel, H.; Schauer, A.; Bock, K. W. Genomic alterations of the c-myc protooncogene in relation to the overexpression of c-erbB2 and Ki-67 in human breast and cervix carcinomas. *Journal of Cancer Research and Clinical Oncology.* **1991**, *117*(6), 603–607. https://doi.org/10.1007/bf01613296.
91. Porta-Pardo, E.; Valencia, A.; Godzik, A. Understanding oncogenicity of cancer driver genes and mutations in the cancer genomics era. *FEBS Letters.* **2020**, *594*(24), 4233–4246. https://doi.org/10.1002/1873-3468.13781.
92. Ou, Z.; Lin, S.; Qiu, J.; Ding, W.; Ren, P.; Chen, D.; Wang, J.; Tong, Y.; Wu, D.; Chen, A.; Deng, Y.; Cheng, M.; Peng, T.; Lu, H.; Yang, H.; Wang, J.; Jin, X.; Ma, D.; Xu, X.; Wu, P. Single-Nucleus RNA sequencing and spatial transcriptomics reveal the immunological microenvironment of cervical squamous cell carcinoma. *Advanced Science*. **2022**, *9*(29). https://doi.org/10.1002/advs.202203040.
93. Martínez-Rodríguez, F.; Limones-González, J. E.; Mendoza-Almanza, B.; Esparza-Ibarra, E. L.; Gallegos-Flores, P. I.; Ayala-Luján, J. L.; Godina-González, S.; Salinas, E.; Mendoza-Almanza, G. Understanding Cervical Cancer through Proteomics. *Cells*. **2021**, *10*(8), 1854. https://doi.org/10.3390/cells10081854.
94. Nakagawa, H.; Fujita, M. Whole genome sequencing analysis for cancer genomics and precision medicine. *Cancer Science*. **2018**, *109*(3), 513–522. https://doi.org/10.1111/cas.13505.
95. Kukurba, K. R.; Montgomery, S. B. RNA sequencing and analysis. *Cold Spring Harbor Protocols*, **2015,** (11), pdb.top084970. https://doi.org/10.1101/pdb.top084970.
96. Coussens, L.M.; Werb, Z. Inflammation and cancer. *Nature* **2002**, *420*, 860–867. https://doi.org/10.1038/nature01322.
97. Emily, S.; Sigmund, A.W. Chronic inflammation and cancer. *Oncology* **2002**, *16*, 217–222. https://pubmed.ncbi.nlm.nih.gov/11866137/, accessed on 1 January 2022.
98. Nitin, S.; Deepak, B.; Jagadish, P. R.; Pankaj, B. P.; Savita, S. T.; Veena, B. P. Inflammation and cancer. *Ann. Afr. Med.* **2019**, *18*, 121–126. https://doi.org/10.4103/aam.aam_56_18.

99. Goldszmid, R.S.; Trinchieri, G. The price of immunity. *Nat. Immunol*. **2012**, 13, 932–938. https://doi.org/10.1038/ni.2422.
100. Ospelt, C.; Gay, S. TLRs and chronic inflammation. *Int. J. Biochem. Cell Biol*. **2010**, 42, 495–505. https://doi.org/10.1016/j.biocel.2009.10.010.
101. Newton, K.; Dixit, V. M. Signaling in Innate Immunity and Inflammation. *Cold Spring Harb. Erspectives Biol*. **2012**, 4, a006049. https://doi.org/10.1101/cshperspect.a006049.
102. Pradere, J. P.; Dapito, D. H.; Schwabe, R. F. The Yin and Yang of Toll-like receptors in cancer. *Oncogene*. **2013**, *33*, 3485–3495. https://doi.org/10.1038/onc.2013.302.
103. Kawai, T.; Akira, S. TLR signaling. *Semin. Immunol*. **2007**, 19, 24–32. https://doi.org/10.1016/j.smim.2006.12.004.
104. Kawasaki, T.; Kawai, T. Toll-Like Receptor Signaling Pathways. *Front. Immunol*. **2014**, 5, 461. https://doi.org/10.3389/fimmu.2014.00461.
105. Takeda, K.; Akira, S. TLR signaling pathways. *Semin. Immunol*. **2004**, 16, 3–9. https://doi.org/10.1016/j.smim.2003.10.003.
106. Lu, Y. C.; Yeh, W. C.; Ohashi, P.S. LPS/TLR4 signal transduction pathway. *Cytokine*. **2008**, 42, 145–151. https://doi.org/10.1016/j.cyto.2008.01.006.
107. Cen, X.; Liu, S.; Cheng, K. The Role of Toll-Like Receptor in Inflammation and Tumor Immunity. *Front. Pharmacol*. **2018**, 9, 878. https://doi.org/10.3389/fphar.2018.00878.
108. Ou, L.; Lin, S.; Song, B.; Liu, J.; Lai, R.; Shao, L. The mechanisms of graphene-based materials-induced programmed cell death: A review of apoptosis, autophagy, and programmed necrosis. *Int. J. Nanomed*. **2017**, 12, 6633–6646. https://doi.org/10.2147/ijn.s140526.
109. Zhao, X. L.; Lin, Y.; Jiang, J.; Tang, Z.; Yang, S.; Lu, L.; Liang, Y.; Liu, X.; Tan, J.; Hu, X.G.; et al. High-mobility group box 1 released by autophagic cancer-associated fibroblasts maintains the stemness of luminal breast cancer cells. *J. Pathol*. **2017**, 243, 376–389. https://doi.org/10.1002/path.4958.
110. Urban-Wojciuk, Z.; Khan, M.M.; Oyler, B.L.; Fåhraeus, R.; Marek-Trzonkowska, N.; Nita-Lazar, A.; Hupp, T.R.; Goodlett, D.R. The Role of TLRs in Anti-cancer Immunity and Tumor Rejection. *Front. Immunol.* **2019**, 10, 2388. https://doi.org/10.3389/fimmu.2019.02388.
111. Keshavarz, A.; Pourbagheri-Sigaroodi, A.; Zafari, P.; Bagheri, N.; Ghaffari, S.H.; Bashash, D. Toll-like receptors (TLRs) in cancer; with an extensive focus on TLR agonists and antagonists. *IUBMB Life*. **2020**, 73, 10–25. https://doi.org/10.1002/iub.2412.
112. Shi, M.; Chen, X.; Ye, K.; Yao, Y.; Li, Y. Application potential of toll-like receptors in cancer immunotherapy. *Medicine*. 2016, 95, e3951. https://doi.org/10.1097/md.0000000000003951.
113. Huang, L.; Xu, H.; Peng, G. TLR-mediated metabolic reprogramming in the tumor microenvironment: Potential novel strategies for cancer immunotherapy. *Cell. Mol. Immunol*. **2018**, 15, 428–437. https://doi.org/10.1038/cmi.2018.4.
114. Cheng, B.; Yuan, W. E.; Su, J.; Liu, Y.; Chen, J. Recent advances in small molecule based cancer immunotherapy. *Eur. J. Med. Chem*. **2018**, 157, 582–598. https://doi.org/10.1016/j.ejmech.2018.08.028.
115. Braunstein, M. J.; Kucharczyk, J.; Adams, S. Targeting Toll-Like Receptors for Cancer Therapy. *Target. Oncol*. **2018**, 13, 583–598. https://doi.org/10.1007/s11523-018-0589-7.

116. Farooq, M.; Batool, M.; Kim, M. S.; Choi, S. Toll-Like Receptors as a Therapeutic Target in the Era of Immunotherapies. *Front. Cell Dev. Biol*. **2021**, 9, 756315. https://doi.org/10.3389/fcell.2021.756315.
117. Pahlavanneshan, S.; Sayadmanesh, A.; Ebrahimiyan, H.; Basiri, M. Toll-Like Receptor-Based Strategies for Cancer Immunotherapy. *J. Immunol. Res*. **2021**, 2021, 9912188. https://doi.org/10.1155/2021/9912188.
118. Qureshi, S. T.; Larivière, L.; Leveque, G.; Clermont, S.; Moore, K. J.; Gros, P.; Malo, D. Endotoxin-tolerant Mice Have Mutations in Toll-like Receptor 4 (Tlr4). *J. Exp. Med*. **1999**, 189, 615–625. https://doi.org/10.1084/jem.189.4.615.
119. Arbour, N. C.; Lorenz, E.; Schutte, B. C.; Zabner, J.; Kline, J. N.; Jones, M.; Frees, K.; Watt, J. L.; Schwartz, D. A. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat. Genet*. **2000**, 25, 187–191. https://doi.org/10.1038/76048.
120. O'Neill, L. A. J.; Bryant, C. E.; Doyle, S. L. Therapeutic Targeting of Toll-Like Receptors for Infectious and Inflammatory Diseases and Cancer. *Pharmacol. Rev*. **2009**, 61, 177–197. https://doi.org/10.1124/pr.109.001073.
121. Miller, S. I.; Ernst, R. K.; Bader, M. W. LPS, TLR4 and infectious disease diversity. *Nat. Rev. Microbiol*. **2005**, 3, 36–46. https://doi.org/10.1038/nrmicro1068.
122. Olejnik, J.; Hume, A. J.; Mühlberger, E. Toll-like receptor 4 in acute viral infection: Too much of a good thing. *PLoS Pathog*. **2018**, 14, e1007390. https://doi.org/10.1371/journal.ppat.1007390.
123. Shirey, K. A.; Blanco, J. C. G.; Vogel, S. N. Targeting TLR4 Signaling to Blunt Viral-Mediated Acute Lung Injury. *Front. Immunol*. **2021**, 12, 705080. https://doi.org/10.3389/fimmu.2021.705080.
124. Aguirre-García, M.; Rojas-Bernabé, A.; Gómez-García, A. R.; Escalona-Montaño, A. TLR-Mediated Host Immune Response to Parasitic Infectious Diseases. In Toll-Like Receptors; Rezaeli, N., Ed.; *IntechOpen*: London, UK, **2020**, pp. 37–61. https://doi.org/10.5772/intechopen.84679.
125. Li, H.; Sun, B. Toll-like receptor 4 in atherosclerosis. *J. Cell. Mol. Med*. **2007**, 11, 88–95. https://doi.org/10.1111/j.1582-4934.2007.00011.x.
126. Roshan, M. H. K.; Tambo, A.; Pace, N. P. The Role of TLR2, TLR4, and TLR9 in the Pathogenesis of Atherosclerosis. *Int. J. Inflamm*. **2016**, 2016, 1532832. https://doi.org/10.1155/2016/1532832.
127. Zeng, X.; Guo, R.; Dong, M.; Zheng, J.; Lin, H.; Lu, H. Contribution of TLR4 signaling in intermittent hypoxia-mediated atherosclerosis progression. *J. Transl. Med*. **2018**, 16, 106. https://doi.org/10.1186/s12967-018-1479-6.
128. Li, H.; Jiao, Y.; Xie, M. Paeoniflorin Ameliorates Atherosclerosis by Suppressing TLR4-Mediated NF-B Activation. *Inflammation*. **2017**, 40, 2042–2051. https://doi.org/10.1007/s10753-017-0644-z.
129. Zakeri, A.; Russo, M. Dual Role of Toll-like Receptors in Human and Experimental Asthma Models. *Front. Immunol.* **2018**, 9, 1027. https://doi.org/10.3389/fimmu.2018.01027.
130. Crespo-Lessmann, A.; Mateus, E.; Vidal, S.; Ramos-Barbón, D.; Torrejón, M.; Giner, J.; Soto, L.; Juárez, C.; Plaza, V. Expression of toll-like receptors 2 and 4 in subjects with asthma by total serum IgE level. *Respir. Res.* **2016**, 17, 41. https://doi.org/10.1186/s12931-016-0355-2.
131. Zhao, J.; Shang, H.; Cao, X.; Huang, Y.; Fang, X.; Zhang, S.; Xie, M.; Xie, J.; Liu, X. Association of polymorphisms in TLR2 and TLR4 with asthma risk. *Medicine*. **2017**, 96, e7909. https://doi.org/10.1097/md.0000000000007909.

132. Li, Z.; Mao, X.; Liu, Q.; Song, H.; He, B.; Shi, P.; Zhang, Q.; Li, X.; Wang, J. Functional variations of the TLR4 gene in association with chronic obstructive pulmonary disease and pulmonary tuberculosis. *BMC Pulm. Med*. **2019**, 19, 184. https://doi.org/10.1186/s12890-019-0939-y.
133. Liu, M.; Xie, J.; Sun, Y. TLR4/MyD88/NF-B-Mediated Inflammation Contributes to Cardiac Dysfunction in Rats of PTSD. *Cell. Mol. Neurobiol*. **2020**, 40, 1029–1035. https://doi.org/10.1007/s10571-020-00791-9.
134. Jia, S. J.; Niu, P. P.; Cong, J. Z.; Zhang, B. K.; Zhao, M. TLR4 signaling: A potential therapeutic target in ischemic coronary artery disease. *Int. Immunopharmacol*. **2014**, 23, 54–59. https://doi.org/10.1016/j.intimp.2014.08.011.
135. Yu, L.; Feng, Z. The Role of Toll-Like Receptor Signaling in the Progression of Heart Failure. *Mediat. Inflamm*. **2018**, 2018, 9874109. https://doi.org/10.1155/2018/9874109.
136. De Vicente, L. G.; Pinto, A. P.; da Rocha, A. L.; Pauli, J. R.; de Moura, L. P.; Cintra, D. E.; Ropelle, E. R.; da Silva, A. S. Role of TLR4 in physical exercise and cardiovascular diseases. *Cytokine*. **2020**, 136, 155273. https://doi.org/10.1016/j.cyto.2020.155273.
137. Cheng, Y.; Zhu, Y.; Huang, X.; Zhang, W.; Han, Z.; Liu, S. Association between TLR2 and TLR4 Gene Polymorphisms and the Susceptibility to Inflammatory Bowel Disease: A Meta-Analysis. *PLoS ONE*. **2015**, 10, e0126803. https://doi.org/10.1371/journal.pone.0126803.
138. Dejban, P.; Nikravangolsefid, N.; Chamanara, M.; Dehpour, A.; Rashidian, A. The role of medicinal products in the treatment of inflammatory bowel diseases (IBD) through inhibition of TLR4/NF-kappaB pathway. *Phytother. Res*. **2020**, 35, 835–845. https://doi.org/10.1002/ptr.6866.
139. Lu, Y.; Li, X.; Liu, S.; Zhang, Y.; Zhang, D. Toll-like Receptors and Inflammatory Bowel Disease. *Front. Immunol*. **2018**, 9, 72. https://doi.org/10.3389/fimmu.2018.00072.
140. Sghaier, I.; Zidi, S.; Mouelhi, L.; Ghazoueni, E.; Brochot, E.; Almawi, W.; Loueslati, B. TLR3 and TLR4 SNP variants in the liver disease resulting from hepatitis B virus and hepatitis C virus infection. *Br. J. Biomed. Sci*. **2018**, 76, 35–41. https://doi.org/10.1080/09674845.2018.1547179.
141. Kapil, S.; Duseja, A.; Sharma, B. K.; Singla, B.; Chakraborti, A.; Das, A.; Ray, P.; Dhiman, R. K.; Chawla, Y. Genetic polymorphism in CD14gene, aco-receptor of TLR4 associated with non-alcoholic fatty liver disease. *World J. Gastroenterol*. **2016**, 22, 9346. https://doi.org/10.3748/wjg.v22.i42.9346.
142. Weber, S. N.; Bohner, A.; Dapito, D. H.; Schwabe, R. F.; Lammert, F. TLR4 Deficiency Protects against Hepatic Fibrosis and Diethylnitrosamine-Induced Pre-Carcinogenic Liver Injury in Fibrotic Liver. *PLoS ONE*. **2016**, 11, e0158819. https://doi.org/10.1371/journal.pone.0158819.
143. Hritz, I.; Mandrekar, P.; Velayudham, A.; Catalano, D.; Dolganiuc, A.; Kodys, K.; Kurt-Jones, E.; Szabo, G. The critical role of toll-like receptor (TLR) 4 in alcoholic liver disease is independent of the common TLR adapter MyD88. *Hepatology*. **2008**, 48, 1224–1231. https://doi.org/10.1002/hep.22470.
144. Santos-Martins, M.; Sameiro-Faria, M.; Ribeiro, S.; Rocha-Pereira, P.; Nascimento, H.; Reis, F.; Miranda, V.; Quintanilha, A.; Belo, L.; Beirão, I.; et al. TLR4 and TLR9 Polymorphisms Effect on Inflammatory Response in End-Stage Renal Disease Patients. *Eur. J. Inflamm*. **2014**, 12, 521–529. https://doi.org/10.1177/1721727x1401200314.

145. Dessing, M. C.; Kers, J.; Damman, J.; Leuvenink, H. G. D.; van Goor, H.; Hillebrands, J. L.; Hepkema, B. G.; Snieder, H.; van den Born, J.; de Borst, M. H.; et al. Toll-Like Receptor Family Polymorphisms Are Associated with Primary Renal Diseases but Not with Renal Outcomes Following Kidney Transplantation. *PLoS ONE*. **2015**, 10, e0139769. https://doi.org/10.1371/journal.pone.0139769.
146. Taha, I. M.; Abdu Allah, A. M.; Abd El Gayed, E. M. Expression of toll-like receptor 4 and its connection with type 2 diabetes mellitus. *Cell. Mol. Biol*. **2018**, 64, 15–20. https://doi.org/10.14715/cmb/2018.64.13.4.
147. Wang, L.; Wang, J.; Fang, J.; Zhou, H.; Liu, X.; Su, S. B. High glucose induces and activates Toll-like receptor 4 in endothelial cells of diabetic retinopathy. *Diabetol. Metab. Syndr*. **2015**, 7, 89. https://doi.org/10.1186/s13098-015-0086-4.
148. Wang, Z.; Ni, X.; Zhang, L.; Sun, L.; Zhu, X.; Zhou, Q.; Yang, Z.; Yuan, H. Toll-Like Receptor 4 and Inflammatory Micro Environment of Pancreatic Islets in Type-2 Diabetes Mellitus: A Therapeutic Perspective. Diabetes Metab. Syndr. Obes. *Targets Ther*. **2020**, 13, 4261–4272. https://doi.org/10.2147/dmso.s279104.
149. Huang, Q. Q.; Pope, R. M. Therole of Toll-like receptors in rheumatoid arthritis. *Curr. Rheumatol. Rep*. **2009**, 11, 357–364. https://doi.org/10.1007/s11926-009-0051-z.
150. Dai, Q.; Li, Y.; Wang, M.; Li, Y.; Li, J. TlR2 and TlR4 are involved in the treatment of rheumatoid arthritis synovial fibroblasts with a medicated serum of asarinin through inhibition of Th1/Th17 cytokines. *Exp. Ther. Med*. **2020**, 19, 3009–3016. https://doi.org/10.3892/etm.2020.8557.
151. Huang, N. Q.; Jin, H.; Zhou, S. Y.; Shi, J. S.; Jin, F. TLR4 is a link between diabetes and Alzheimer's disease. *Behav. Brain Res*. **2017**, 316, 234–244. https://doi.org/10.1016/j.bbr.2016.08.047.
152. Yang, J.; Wise, L.; Fukuchi, K. I. TLR4 Cross-Talk With NLRP3 Inflammasome and Complement Signaling Pathwaysin Alzheimer's Disease. *Front. Immunol*. **2020**, 11, 724. https://doi.org/10.3389/fimmu.2020.00724.
153. Miron, J.; Picard, C.; Lafaille-Magnan, M.; Savard, M.; Labonté, A.; Breitner, J.; Rosa-Neto, P.; Auld, D.; Poirier, J. Association of TLR4 with Alzheimer's disease risk and presymptomatic biomarkers of inflammation. *Alzheimers Dement*. **2019**, 15, 951–960. https://doi.org/10.1016/j.jalz.2019.03.012.
154. Paudel, Y. N.; Angelopoulou, E.; Piperi, C.; Othman, I.; Aamir, K.; Shaikh, M. F. Impact of HMGB1, RAGE, and TLR4 in Alzheimer's Disease (AD): From Risk Factors to Therapeutic Targeting. *Cells*. **2020**, 9, 383. https://doi.org/10.3390/cells9020383.
155. Perez-Pardo, P.; Dodiya, H. B.; Engen, P. A.; Forsyth, C. B.; Huschens, A. M.; Shaikh, M.; Voigt, R. M.; Naqib, A.; Green, S. J.; Kordower, J. H.; et al. Role of TLR4 in the gut-brain axis in Parkinson's disease: A translational study from men to mice. *Gut*. **2018**, 68, 829–843. https://doi.org/10.1136/gutjnl-2018-316844.
156. Zhao, J.; Han, X.; Xue, L.; Zhu, K.; Liu, H.; Xie, A. Association of TLR4 gene polymorphisms with sporadic Parkinson's disease in a HanChinese population. *Neurol. Sci*. **2015**, 36, 1659–1665. https://doi.org/10.1007/s10072-015-2227-9.
157. Kouli, A.; Horne, C.; Williams-Gray, C. Toll-like receptors and their therapeutic potential in Parkinson's disease and synucleinopathies. *Brain Behav. Immun*. **2019**, 81, 41–51. https://doi.org/10.1016/j.bbi.2019.06.042.
158. Campolo, M.; Paterniti, I.; Siracusa, R.; Filippone, A.; Esposito, E.; Cuzzocrea, S. TLR4 absence reduces neuroinflammation and inflammasome activation in Parkinson's diseases in vivo model. *Brain Behav. Immun*. **2019**, 76, 236–247. https://doi.org/10.1016/j.bbi.2018.12.003.

159. Obadia, N.; Andrade, G.; Leardini-Tristão, M.; Albuquerque, L.; Garcia, C.; Lima, F.; Daleprane, J.; Castro-Faria-Neto, H. C.; Tibiriçá, E.; Estato, V. TLR4 mutation protects neurovascular function and cognitive decline in high-fat diet-fed mice. *J. Neuroin f lamm*. **2022**, 19, 104. https://doi.org/10.1186/s12974-022-02465-3.
160. Asadzadeh Manjili, F.; Yousefi-Ahmadipour, A.; Kazemi Arababadi, M. The roles played by TLR4 in the pathogenesis of multiple sclerosis; A systematic review article. *Immunol. Lett*. **2020**, 220, 63–70. https://doi.org/10.1016/j.imlet.2020.02.004.
161. Oliveira, I.; Gomes, R.; Gomides, L.; dos Santos, J.; Carneiro, M.; Ribeiro-Dias, F.; Diniz, D. Interferon-Beta Treatment Differentially Alters TLR2 and TLR4-Dependent Cytokine Production in Multiple Sclerosis Patients. *Neuroimmunomodulation*. **2019**, 26, 77–83. https://doi.org/10.1159/000495787.
162. Oliveira, J.; Busson, M.; Etain, B.; Jamain, S.; Hamdani, N.; Boukouaci, W.; Amokrane, K.; Bennabi, M.; Le Guen, E.; Dargél, A. A.; et al. Polymorphism of Toll-like receptor 4 gene in bipolar disorder. *J. Affect. Disord*. **2014**, 152–154, 395–402. https://doi.org/10.1016/j.jad.2013.09.043.
163. Oblak, A.; Jerala, R. Toll-Like Receptor 4 Activation in Cancer Progression and Therapy. *Clin. Dev. Immunol*. **2011**, 1–12, 609579. https://doi.org/10.1155/2011/609579.
164. Ran, S.; Bhattarai, N.; Patel, R.; Volk-Draper, L. TLR4-Induced Inflammation Is a Key Promoter of Tumor Growth, Vascularization, and Metastasis. In Translational Studies on Inflammation; Nunes, A.C.F., Ed.; *IntechOpen: London, UK*. **2020**, pp. 133–167. https://doi.org/10.5772/intechopen.85195.
165. Hao, B.; Chen, Z.; Bi, B.; Yu, M.; Yao, S.; Feng, Y.; Yu, Y.; Pan, L.; Di, D.; Luo, G.; et al. Role of TLR4 as a prognostic factor for survival in various cancers oncotarget: A meta-analysis. *Oncotarget*. **2018**, 9, 13088–13099. https://doi.org/10.18632/oncotarget.24178.
166. Jiang, N.; Xie, F.; Chen, L.; Chen, F.; Sui, L. The effect of TLR4 on the growth and local inflammatory microenvironment of HPV-related cervical cancer in vivo. *Infect. Agents Cancer*. **2020**, 15, 10–12. https://doi.org/10.1186/s13027-020-0279-9.
167. Wang, Y.; Weng, Y.; Shi, Y.; Xia, X.; Wang, S.; Duan, H. Expression and Functional Analysis of Toll-like Receptor 4 in Human Cervical Carcinoma. J. Membr. Biol. 2014, 247, 591–599. https://doi.org/10.1007/s00232-014-9675-7.
168. Zhang, H.; Zhang, S. Theexpression of Foxp3 and TLR4 in cervical cancer: Association with immune escape and clinical pathology. *Arch. Gynecol. Obstet*. **2016**, 295, 705–712. https://doi.org/10.1007/s00404-016-4277-5.
169. Pappa, K.; Kontostathi, G.; Lygirou, V.; Zoidakis, J.; Anagnou, N. Novel structural approaches concerning HPV proteins: Insight into targeted therapies for cervical cancer (Review). *Oncol. Rep*. **2018**, 39, 1547–1554. https://doi.org/10.3892/or.2018.6257.
170. Yang, X.; Cheng, Y.; Li, C. The role of TLRs in cervical cancer with HPV infection: A review. *Signal Transduct. Target. Ther*. 2**017**, 2, 17055. https://doi.org/10.1038/sigtrans.2017.55.
171. Zhang,K.; Zhou, B.; Wang, Y.; Rao, L.; Zhang, L. The TLR4 gene polymorphisms and susceptibility to cancer: A systematic review and meta-analysis. *Eur. J. Cancer*. **2013**, 49, 946–954. https://doi.org/10.1016/j.ejca.2012.09.022.
172. Ding, L.; Jiang, Q.; Li, G.; Shen, J.; Du, J.; Lu, X.; Xiong, X. Comprehensive assessment of association between TLR4 gene polymorphisms and cancer risk: A systematic meta-analysis. *Oncotarget*. **2017**, 8, 100593–100602. https://doi.org/10.18632/oncotarget.21543.

173. Kutikhin, A. G. Impact of Toll-like receptor 4 polymorphisms on risk of cancer. Hum. Immunol. 2011, 72, 193–206. https://doi.org/10.1016/j.humimm.2010.11.003.
174. Pandey, N.; Chauhan, A.; Jain, N. TLR4 Polymorphisms and Expression in Solid Cancers. *Mol. Diagn. Ther*. **2018**, 22, 683–702. https://doi.org/10.1007/s40291-018-0361-9.
175. Chauhan, A.; Pandey, N.; Desai, A.; Raithatha, N.; Patel, P.; Choxi, Y.; Kapadia, R.; Khandelwal, R.; Jain, N. Association of TLR4 and TLR9 gene polymorphisms and haplotypes with cervicitis susceptibility. *PLoS ONE*. **2019**, 14, e0220330. https://doi.org/10.1371/journal.pone.0220330.
176. Huang, C.; Li, Z.; Zhong, X.; Wang, Y.; Ye, X.; Jing, L.; Huang, S.; Yin, Q.; Miao, Z.; Zhou, Z.; et al. Association between polymorphisms in TLR4 gene targeted by microRNA-140 and cervical precancerous lesion in south Chinese women: A case control study. *Genet. Mol. Res*. **2017**, 16, gmr16039831. https://doi.org/10.4238/gmr16039831.
177. Nath, N.; Mishra, P.; Panda, A.K.; Mishra, R. Polymorphisms and haplotypes of TLR4, TLR9 and CYP1A1 genes possibly interfere with high-risk human papillomavirus infection and cervical cancer susceptibility in Jharkhand, India. *Int. Immunopharmacol*. **2020**, 88, 106925. https://doi.org/10.1016/j.intimp.2020.106925.
178. Pandey, N. O.; Chauhan, A. V.; Raithatha, N. S.; Patel, P. K.; Khandelwal, R.; Desai, A. N.; Choxi, Y.; Kapadia, R. S.; Jain, N. D. Association of TLR4 and TLR9 polymorphisms and haplotypes with cervical cancer susceptibility. *Sci. Rep*. **2019**, 9, 9729. https://doi.org/10.1038/s41598-019-46077-z.
179. Pandey, N.; Chauhan, A.; Raithatha, N.; Patel, P.; Khandelwal, R.; Desai, A.; Choxi, Y.; Kapadia, R.; Jain, N. Influence of TLR4 and TLR9 polymorphisms and haplotypes on multiple hrHPV infections and HPV16 copy number in cervical cancer and cervicitis. *Microb. Pathog*. **2021**, 159, 105149. https://doi.org/10.1016/j.micpath.2021.105149.
180. Lifsted, T.; Voyer, T. L.; Williams, M.; Muller, W. J.; Klein-Szanto, A. J.; Buetow, K. H.; Hunter, K. W. Identification of inbred mouse strains harboring genetic modifiers of mammary tumor age of onset and metastatic progression. *Int. J. Cancer*. **1998**, 77, 640–644. https://doi.org/10.1002/(sici)1097-0215(19980812)77:4.
181. Hunter, K. W.; Welch, D. R.; Liu, E. T. Genetic background is an important determinant of metastatic potential. *Nat. Genet.* **2003**, 34, 23–24. https://doi.org/10.1038/ng0503-23b.
182. Steeg, P. S. Metastasis suppressors alter the signal transduction of cancer cells. *Nat. Rev. Cancer*. **2003**, 3, 55–63. https://doi.org/10.1038/nrc967.
183. Hunter, K. W. Allelic diversity in the host genetic background may be an important determinant in tumor metastatic dissemination. *Cancer Lett.* **2003**, 200, 97–105. https://doi.org/10.1016/s0304-3835(03)00420-8.
184. Schadt, E. E.; Monks, S. A.; Drake, T. A.; Lusis, A. J.; Che, N.; Colinayo, V.; Ruff, T. G.; Milligan, S. B.; Lamb, J.; Cavet, G.; et al. Genetics of gene expression surveyed in maize, mouse and man. *Nature*. **2003**, 422, 297–302. https://doi.org/10.1038/nature01434.
185. Yang, H.; Crawford, N.P.; Lukes, L.; Finney, R.; Lancaster, M.; Hunter, K.W. Metastasis predictive signature profiles pre-exist in normal tissues. *Clin. Exp. Metastasis*. **2005**, 22, 593–603. https://doi.org/10.1007/s10585-005-6244-6.
186. Zhou, S.; Peng, F. Patterns of metastases in cervical cancer: a population-based study. *International journal of clinical and experimental pathology*. **2020**, 13(7), 1615–1623. PMID: 32782680; PMCID: PMC7414489.

187. Yin, Z.; Tang, H.; Li, L.; Ni, J.; Yuan, S.; Lou, H.; Chen, M. Impact of sites versus number of metastases on survival of patients with organ metastasis from newly diagnosed cervical cancer. *Cancer Management and Research*. **2019**, Volume 11, 7759–7766. https://doi.org/10.2147/cmar.s203037.
188. Zhang, H.; Liu, J.; Dang, Q.; Wang, X.; Chen, J.; Lin, X.; Yang, N.; Du, J.; Shi, H.; Liu, Y. Han, J. Ribosomal protein RPL5 regulates colon cancer cell proliferation and migration through MAPK/ERK signaling pathway. *BMC Molecular and Cell Biology*. **2022**, *23*(1). https://doi.org/10.1186/s12860-022-00448-z.
189. Fancello, L.; Kampen, K. R.; Hofman, I. J.; Verbeeck, J.; De Keersmaecker, K. The ribosomal protein gene RPL5 is a haploinsufficient tumor suppressor in multiple cancer types. *Oncotarget*. **2017**, *8*(9), 14462–14478. https://doi.org/10.18632/oncotarget.14895.
190. Hsu, Y.; Wang, P.; Chang, C. Functional gene clusters in global pathogenesis of clear cell carcinoma of the ovary discovered by integrated analysis of transcriptomes. *International Journal of Environmental Research and Public Health*. **2020**, *17*(11), 3951. https://doi.org/10.3390/ijerph17113951.
191. Zhang, X.; Hong, S.; Yu, C.; Shen, X.; Sun, F.; Yang, J. Comparative analysis between high-grade serous ovarian cancer and healthy ovarian tissues using single-cell RNA sequencing. *Frontiers in Oncology*. **2023**, *13:1148628*. https://doi.org/10.3389/fonc.2023.1148628.
192. Chen, J.; Lei, C.; Zhang, H.; Huang, X.; Yang, Y.; Liu, J.; Jia, Y.; Shi, H.; Zhang, Y.; Zhang, J.; Du, J. RPL11 promotes non-small cell lung cancer cell proliferation by regulating endoplasmic reticulum stress and cell autophagy. *BMC Molecular and Cell Biology*. **2023**, *24*(1). https://doi.org/10.1186/s12860-023-00469-2.
193. Rao, Z.; Shen, J.; Wang, J.; Zhang, Z.; Zhou, J.; Zhu, J.; Chen, J.; Chen, W.; Wang, H. The role of PICT1 in RPL11/Mdm2/p53 pathway-regulated inhibition of cell growth induced by topoisomerase IIα inhibitor against cervical cancer cell line. *Biochemical Pharmacology*. **2022**, *201*, 115098. https://doi.org/10.1016/j.bcp.2022.115098.
194. Lin, Z.; Peng, R.; Sun, Y.; Zhang, L.; Zhang, Z. Identification of ribosomal protein family in triple-negative breast cancer by bioinformatics analysis. *Bioscience Reports*. **2021**, *41*(1). https://doi.org/10.1042/bsr20200869.
195. Yang, M.; Zhou, Y.; Deng, H.; Zhou, H.; Cheng, S.; Zhang, D.; He, X.; Mai, L.; Chen, Y.; Chen, J. Ribosomal protein L23 drives the metastasis of hepatocellular carcinoma via upregulating MMP9. *Frontiers in Oncology*. **2021**, *11*. https://doi.org/10.3389/fonc.2021.779748.
196. Kang, H.; Choi, M. C.; Kim, S.; Jeong, J.; Kwon, A.; Kim, T.; Kim, G.; Joo, W. D.; Park, H.; Lee, C.; Song, S. H.; Jung, S. G.; Hwang, S.; An, H. J. USP19 and RPL23 as candidate prognostic markers for Advanced-Stage High-Grade Serous ovarian carcinoma. *Cancers*. **2021**, *13*(16), 3976. https://doi.org/10.3390/cancers13163976.
197. Meng, X.; Tackmann, N. R.; Liu, S.; Yang, J.; Dong, J.; Wu, C.; Cox, A. D.; Zhang, Y. RPL23 links oncogenic RAS signaling to P53-Mediated Tumor Suppression. *Cancer Research*. **2016**, *76*(17), 5030–5039. https://doi.org/10.1158/0008-5472.can-15-3420.
198. Kumar, R.; Iden, M.; Tsaih, S.; Schmidt, R.; Ojesina, A. I.; Rader, J. S. Deciphering the divergent transcriptomic landscapes of cervical cancer cells grown in 3D and 2D cell culture systems. *Frontiers in Cell and Developmental Biology*. **2024**, *12*. https://doi.org/10.3389/fcell.2024.1413882.
199. Zhang, M.; Zhang, J.; Yan, W.; Chen, X. p73 expression is regulated by ribosomal protein RPL26 through mRNA translation and protein stability. *Oncotarget*. **2016**, *7*(48), 78255–78268. https://doi.org/10.18632/oncotarget.13126.

200. Elhamamsy, A. R.; Metge, B. J.; Alsheikh, H. A.; Shevde, L. A.; Samant, R. S. Ribosome biogenesis: a central player in cancer metastasis and therapeutic resistance. *Cancer Research*. **2022**, *82*(13), 2344–2353. https://doi.org/10.1158/0008-5472.can-21-4087.
201. Hu, Y.; Kang, C.; Zhao, J.; Nie, Y.; Zheng, L.; Li, H.; Li, X.; Wang, Q.; Qiu, Y. LncRNA PLAC2 down-regulates RPL36 expression and blocks cell cycle progression in glioma through a mechanism involving STAT1. *Journal of Cellular and Molecular Medicine*. **2017**, *22*(1), 497–510. https://doi.org/10.1111/jcmm.13338.
202. Cao, X.; Khitun, A.; Luo, Y.; Na, Z.; Phoodokmai, T.; Sappakhaw, K.; Olatunji, E.; Uttamapinant, C.; Slavoff, S. A. Alt-RPL36 downregulates the PI3K-AKT-mTOR signaling pathway by interacting with TMEM24. *Nature Communications*. **2021**, *12*(1). https://doi.org/10.1038/s41467-020-20841-6.
203. Lancaster, M.; Rouse, J.; Hunter, K. W. Modifiers of mammary tumor progression and metastasis on mouse Chromosomes 7, 9, and 17. *Mamm. Genome*. **2005**, 16, 120–126. https://doi.org/10.1007/s00335-004-2432-y.
204. Crawford, N. P.; Walker, R. C.; Lukes, L.; Officewala, J. S.; Williams, R. W.; Hunter, K. W. The Diasporin Pathway: A tumor progression-related transcriptional network that predicts breast cancer survival. *Clin. Exp. Metastasis*. **2008**, 25, 357–369. https://doi.org/10.1007/s10585-008-9146-6.
205. Park, Y.; Zhao, X.; Lesueur, F.; Lowy, D. R.; Lancaster, M.; Pharoah, P. D.; Qian, X.; Hunter, K. W. Sipa1 is a candidate for underlying the metastasis efficiency modifier locus Mtes1. *Nat. Genet.* **2005**, *37*, 1055–1062. https://doi.org/10.1038/ng1635.
206. Crawford, N. P.; Ziogas, A.; Peel, D.; Hess, J.; Anton-Culver, H.; Hunter, K. W. Germline polymorphisms in SIPA1 are associated with metastasis and other indicators of poor prognosis in breast cancer. *Breast Cancer Res.* **2006**, *8*, R16. https://doi.org/10.1186/bcr1389.
207. Crawford, N. P.; Yang, H.; Mattaini, K.; Hunter, K. W. The metastasis efficiency modifier ribosomal RNA processing 1 Homolog B (RRP1B) is a chromatin-associated factor. *J. Biol. Chem.* **2009**, *284*, 28660–28673. https://doi.org/10.1074/jbc.m109.023457.
208. Lee, M.; Dworkin, A. M.; Lichtenberg, J.; Patel, S. J.; Trivedi, N.; Gildea, D.; Bodine, D.M.; Crawford, N.P. Metastasis-Associated Protein Ribosomal RNA Processing 1 Homolog B (RRP1B) Modulates Metastasis through Regulation of Histone Methylation. *Mol. Cancer Res.* **2014**, *12*, 1818–1828. https://doi.org/10.1158/1541-7786.mcr-14-0167.
209. Lee, M.; Dworkin, A. M.; Gildea, D.; Trivedi, N.; Moorhead, G. B. G.; Crawford, N. P. RRP1B is a metastasis modifier that regulates the expression of alternative mRNA isoforms through interactions with SRSF1. *Oncogene.* **2013**, 33(14), 1818–1827. https://doi.org/10.1038/onc.2013.133.
210. Felgueiras, J.; Jerónimo, C.; Fardilha, M. Protein phosphatase 1 in tumorigenesis: Is it worth a closer look? *Biochim. Biophys. Acta (BBA)-Rev. Cancer* **2020**, *1874*, 188433. https://doi.org/10.1016/j.bbcan.2020.188433.
211. Srivastava, G.; Bajaj, R.; Kumar, G. S.; Gaudreau-Lapierre, A.; Nicolas, H.; Chamousset, D.; Kreitler, D.; Peti, W.; Trinkle-Mulcahy, L.; Page, R. The ribosomal RNA processing 1B:protein phosphatase 1 holoenzyme reveals non-canonical PP1 interaction motifs. *Cell Rep.* **2022**, *41*, 111726. https://doi.org/10.1016/j.celrep.2022.111726.

212. Chamousset, D.; De Wever, V.; Moorhead, G. B. G.; Chen, Y.; Boisvert, F.; Lamond, A. I.; Trinkle-Mulcahy, L. RRP1B Targets PP1 to Mammalian Cell Nucleoli and Is Associated with Pre-60S Ribosomal Subunits. *Mol. Biol. Cell* **2010**, *21*, 4212–4226. https://doi.org/10.1091/mbc.e10-04-0287.
213. Paik, J. C.; Wang, B.; Liu, K.; Lue, J. K.; Lin, W. Regulation of E2F1-induced apoptosis by the nucleolar protein RRP1B. *J. Biol. Chem.* **2010**, *285*, 6348–6363. https://doi.org/10.1074/jbc.m109.072074.
214. Grisendi, S.; Mecucci, C.; Falini, B.; Pandolfi, P. P. Nucleophosmin and cancer. *Nat. Rev. Cancer* **2006**, *6*, 493–505. https://doi.org/10.1038/nrc1885.
215. Okuwaki, M.; Saito, S.; Hirawake-Mogi, H.; Nagata, K. The interaction between nucleophosmin/NPM1 and the large ribosomal subunit precursors contribute to maintaining the nucleolar structure. *Biochim. Biophys. Acta (BBA)-Mol. Cell Res.* **2021**, *1868*, 118879. https://doi.org/10.1016/j.bbamcr.2020.118879.
216. Crawford, N. P.; Alsarraj, J.; Lukes, L.; Walker, R. C.; Officewala, J. S.; Yang, H. H.; Lee, M. P.; Ozato, K.; Hunter, K. W. Bromodomain 4 activation predicts breast cancer survival. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6380–6385. https://doi.org/10.1073/pnas.0710331105.
217. Alsarraj, J.; Faraji, F.; Geiger, T.; Mattaini, K.; Williams, M.; Wu, J. J.; Ha, N.; Merlino, T.; Walker, R. C.; Bosley, A. D.; et al. BRD4 Short Isoform Interacts with RRP1B, SIPA1 and Components of the LINC Complex at the Inner Face of the Nuclear Membrane. *PLoS ONE* **2013**, *8*, e80746. https://doi.org/10.1371/journal.pone.0080746.
218. Donati, B.; Lorenzini, E.; Ciarrocchi, A. BRD4 and Cancer: Going beyond transcriptional regulation. *Mol. Cancer* **2018**, *17*, 164. https://doi.org/10.1186/s12943-018-0915-9.
219. Chiang, S. K.; Chang, W. C.; Chen, S.; Chang, L. DOCK1 Regulates Growth and Motility through the RRP1B-Claudin-1 Pathway in Claudin-Low Breast Cancer Cells. *Cancers* **2019**, *11*, 1762. https://doi.org/10.3390/cancers11111762.
220. Chang, J.; Wang, F.; Chapin, W.; Huang, R. S. Identification of MicroRNAs as Breast Cancer Prognosis Markers through the Cancer Genome Atlas. *PLoS ONE* **2016**, *11*, e0168284. https://doi.org/10.1371/journal.pone.0168284.
221. Hattori, M.; Tsukamoto, N.; Nur-E-Kamal, M. S. A.; Rubinfeld, B.; Iwai, K.; Kubota, H.; Maruta, H.; Minato, N. Molecular Cloning of a Novel Mitogen-Inducible Nuclear Protein with a Ran GTPase-Activating Domain That Affects Cell Cycle Progression. *Molecular and Cellular Biology.* **1995**, *15*(1), 552–560. https://doi.org/10.1128/mcb.15.1.552.
222. Kurachi, H.; Wada, Y.; Tsukamoto, N.; Maeda, M.; Kubota, H.; Hattori, M.; Iwai, K.; Minato, N. Human SPA-1 gene product selectively expressed in lymphoid tissues is a specific GTPASE-activating protein for RAP1 and RAP2. *Journal of Biological Chemistry.* **1997**, *272*(44), 28081–28088. https://doi.org/10.1074/jbc.272.44.28081.
223. Hattori M: SIPA1 (signal-induced proliferation-associated 1). Atlas of Genetics and Cytogenetics in Oncology and Haematology, 2010. https://atlasgeneticsoncology.org/gene/46282/sipa1-(signal-induced-proliferation-associated-1) (accessed on 7 August 2024).
224. Xiao, P.; Dolinska, M.; Sandhow, L.; Kondo, M.; Johansson, A.; Bouderlique, T.; Zhao, Y.; Li, X.; Dimitriou, M.; Rassidakis, G. Z.; Hellström-Lindberg, E.; Minato, N.; Walfridsson, J.; Scadden, D. T.; Sigvardsson, M.; Qian, H. (). Sipa1 deficiency–induced bone marrow niche alterations lead to the initiation of myeloproliferative neoplasm. *Blood Advances.* 2018, *2*(5), 534–548. https://doi.org/10.1182/bloodadvances.2017013599.

225. Xu, Y.; Ikeda, S.; Sumida, K.; Yamamoto, R.; Tanaka, H.; Minato, N. Sipa1 deficiency unleashes a host-immune mechanism eradicating chronic myelogenous leukemia-initiating cells. *Nature Communications.* **2018**, *9*(1). https://doi.org/10.1038/s41467-018-03307-8.
226. Guo, L.; Zhang, W.; Zhang, X.; Wang, J.; Nie, J.; Jin, X.; Ma, Y.; Shi, W.; Zhou, X.; Zhang, Y.; Xu, Y.; Tanaka, Y.; Yuan, J.; Liao, X.; Yang, G.; Su, L. A novel transcription factor SIPA1: identification and verification in triple-negative breast cancer. *Oncogene.* **2023**, *42*(35), 2641–2654. https://doi.org/10.1038/s41388-023-02787-3.
227. Alsarraj, J.; Faraji, F.; Geiger, T. R.; Mattaini, K. R.; Williams, M.; Wu, J.; Ha, N.; Merlino, T.; Walker, R. C.; Bosley, A. D.; Xiao, Z.; Andresson, T.; Esposito, D.; Smithers, N.; Lugo, D.; Prinjha, R.; Day, A.; Crawford, N. P. S.; Ozato, K.; Hunter, K. W. BRD4 Short Isoform Interacts with RRP1B, SIPA1 and Components of the LINC Complex at the Inner Face of the Nuclear Membrane. *PLoS ONE.* **2013**, *8*(11), e80746. https://doi.org/10.1371/journal.pone.0080746.
228. Farina, A.; Hattori, M.; Qin, J.; Nakatani, Y.; Minato, N.; Ozato, K. Bromodomain protein BRD4 binds to GTPASe-Activating SPA-1, modulating its activity and subcellular localization. *Molecular and Cellular Biology.* **2004**, *24*(20), 9059–9069. https://doi.org/10.1128/mcb.24.20.9059-9069.2004.
229. Su, L.; Hattori, M.; Moriyama, M.; Murata, N.; Harazaki, M.; Kaibuchi, K.; Minato, N. AF-6 Controls Integrin-mediated Cell Adhesion by Regulating Rap1 Activation through the Specific Recruitment of Rap1GTP and SPA-1. *Journal of Biological Chemistry.* **2003**, *278*(17), 15232–15238. https://doi.org/10.1074/jbc.m211888200.
230. Zhang, Y.; Gong, Y.; Hu, D.; Zhu, P.; Wang, N.; Zhang, Q.; Wang, M.; Aldeewan, A.; Xia, H.; Qu, X.; Ring, B. Z.; Minato, N.; Su, L. Nuclear SIPA1 activates integrin β1 promoter and promotes invasion of breast cancer cells. *Oncogene.* **2014**, *34*(11), 1451–1462. https://doi.org/10.1038/onc.2014.36.
231. Feng, L.; Weng, J.; Yao, C.; Wang, R.; Wang, N.; Zhang, Y.; Tanaka, Y.; Su, L. Extracellular Vesicles Derived from SIPA1high Breast Cancer Cells Enhance Macrophage Infiltration and Cancer Metastasis through Myosin-9. *Biology.* **2022**, *11*(4), 543. https://doi.org/10.3390/biology11040543.
232. Yi, S.; Li, G. The association of SIPA1 gene polymorphisms with breast cancer risk: evidence from published studies. *Tumor Biology.* **2014**, *35*(1), 441–445. https://doi.org/10.1007/s13277-013-1061-z.
233. Yao, C.; Weng, J.; Feng, L.; Zhang, W.; Xu, Y.; Zhang, P.; Tanaka, Y.; Su, L. SIPA1 enhances aerobic glycolysis through HIF-2A pathway to promote breast cancer metastasis. *Frontiers in Cell and Developmental Biology.* **2022**, *9*. https://doi.org/10.3389/fcell.2021.779169.
234. Wang, N.; Weng, J.; Xia, J.; Zhu, Y.; Chen, Q.; Hu, D.; Zhang, X.; Sun, R.; Feng, J.; Minato, N.; Gong, Y.; Su, L. SIPA1 enhances SMAD2/3 expression to maintain stem cell features in breast cancer cells. *Stem Cell Research.* **2020**, *49*, 102099. https://doi.org/10.1016/j.scr.2020.102099.
235. Park, Y.; Zhao, X.; Lesueur, F.; Lowy, D. R.; Lancaster, M.; Pharoah, P.; Qian, X.; Hunter, K. W. Sipa1 is a candidate for underlying the metastasis efficiency modifier locus Mtes1. *Nature Genetics.* **2005**, *37*(10), 1055–1062. https://doi.org/10.1038/ng1635.
236. Noda, Y.; Horikawa, S.; Furukawa, T.; Hirai, K.; Katayama, Y.; Asai, T.; Kuwahara, M.; Katagiri, K.; Kinashi, T.; Hattori, M.; Minato, N.; Sasaki, S. Aquaporin-2 trafficking is regulated by PDZ-domain containing protein SPA-1. *FEBS Letters.* **2004**, *568*(1–3), 139–145. https://doi.org/10.1016/j.febslet.2004.05.021.

237. Shimizu, Y.; Hamazaki, Y.; Hattori, M.; Doi, K.; Terada, N.; Kobayashi, T.; Toda, Y.; Yamasaki, T.; Inoue, T.; Kajita, Y.; Maeno, A.; Kamba, T.; Mikami, Y.; Kamoto, T.; Yamada, T.; Kanno, T.; Yoshikawa, K.; Ogawa, O.; Minato, N.; Nakamura, E. SPA-1 controls the invasion and metastasis of human prostate cancer. *Cancer Science.* **2011**, *102*(4), 828–836. https://doi.org/10.1111/j.1349-7006.2011.01876.x.
238. Liu, C., Jiang, W., Hargest, R., & Martin, T. (2019). SIPA1 is a modulator of HGF induced tumour metastasis via the regulation of tight junctions in lung adenocarcinoma cells. *Annals of Oncology*, *30*, v22–v23. https://doi.org/10.1093/annonc/mdz238.079.
239. Horitani, S.; Ueda, Y.; Kamioka, Y.; Kondo, N.; Ikeda, Y.; Naganuma, M.; Kinashi, T. The critical role of Rap1-GAPs Rasa3 and Sipa1 in T cells for pulmonary transit and egress from the lymph nodes. *Frontiers in Immunology.* **2023**, *14*. https://doi.org/10.3389/fimmu.2023.1234747.
240. Liu, C.; Jiang, W.; Zhang, L.; Hargest, R.; Martin, T. A. SIPA1 is a modulator of HGF/MET induced tumour metastasis via the regulation of tight Junction-Based cell to cell barrier function. *Cancers.* **2021**, *13*(7), 1747. https://doi.org/10.3390/cancers 13071747.
241. Li, J.; Wang, J.; Liu, C.; Ma, D.; Ma, J. Dynamic relationship between SIPA1 gene and protein expression and the development of gastric cancer. *Genetics and Molecular Research.* **2017**, *16*(1). https://doi.org/10.4238/gmr16019271.
242. Ji, K.; Ye, L.; Toms, A. M.; Hargest, R.; Martin, T. A.; Ruge, F.; Ji, J.; Jiang, W. G. Expression of signal-induced proliferation-associated gene 1 (SIPA1), a RapGTPase-activating protein, is increased in colorectal cancer and has diverse effects on functions of colorectal cancer cells. *Cancer genomics & proteomics.* **2012**, *9*(5), 321–327.
243. Li, Y.; Wang, M.; Jiang, L.; Jia, J.; Pan, F.; Li, W.; Wang, B.; Huang, K.; Luo, J. SIPA1 promotes epithelial-mesenchymal transition in colorectal cancer through STAT3 activation. *Heliyon.* **2024**, *10*(14), e34527. https://doi.org/10.1016/j.heliyon.2024.e34527.
244. Gao, H.; Liu, C.; Benedikt, J.; Jiang, W. G.; Martin, T. A. PTH-93 AF6 (Afadin/MLLT4) and tight junctional regulating SIPA1, expression and clinical/prognostic value in colorectal cancer. *Gut*. 2021. https://doi.org/10.1136/gutjnl-2021-bsg.296.
245. Takahara, T., Kasamatsu, A., Yamatoji, M., Iyoda, M., Kasama, H., Saito, T., Takeuchi, S., Endo-Sakamoto, Y., Shiiba, M., Tanzawa, H., & Uzawa, K. (2017). SIPA1 promotes invasion and migration in human oral squamous cell carcinoma by ITGB1 and MMP7. *Experimental Cell Research*, *352*(2), 357–363. https://doi.org/10.1016/j.yexcr.2017.02.026.
246. Zhang, P.; Wang, X. Suppression of SIPA-1 expression may reduce bladder cancer invasion and metastasis via the downregulation of E-cadherin and ZO-1. *Experimental and Therapeutic Medicine.* **2015**, *11*(1), 213–217. https://doi.org/10.3892/etm.2015.2891.
247. Mathieu, V.; Pirker, C.; Schmidt, W. M.; Spiegl-Kreinecker, S.; Lötsch, D.; Heffeter, P.; Hegedus, B.; Grusch, M.; Kiss, R.; Berger, W. Aggressiveness of human melanoma xenograft models is promoted by aneuploidy-driven gene expression deregulation. *Oncotarget.* **2012**, *3*(4), 399–413. https://doi.org/10.18632/oncotarget.473.
248. Du, Y.; Li, S.; Zhou, T.; Zhao, J.; Liu, J. SIPA1 boosts migration and proliferation, and blocks apoptosis of glioma by activating the phosphorylation of the FAK signaling pathway. *Journal of Medical Biochemistry.* **2022**, *41*(1), 108–114. https://doi.org/10.5937/jomb0-32903.

249. Schwartz, S.; Meshorer, E.; Ast, G. Chromatin organization marks exon-intron structure. *Nature Structural & Molecular Biology.* **2009**, *16*(9), 990–995. https://doi.org/10.1038/nsmb.1659.
250. Luco, R. F.; Pan, Q.; Tominaga, K.; Blencowe, B. J.; Pereira-Smith, O. M.; Misteli, T. Regulation of alternative splicing by Histone modifications. *Science.* **2010**, *327*(5968), 996–1000. https://doi.org/10.1126/science.1184208.
251. Braunschweig, U.; Gueroussov, S.; Plocik, A. M.; Graveley, B. R.; Blencowe, B. J. Dynamic Integration of Splicing within Gene Regulatory Pathways. *Cell.* **2013**, *152*(6), 1252–1269. https://doi.org/10.1016/j.cell.2013.02.034.
252. Kornblihtt, A. R.; Schor, I. E.; Alló, M.; Dujardin, G.; Petrillo, E.; Muñoz, M. J. Alternative splicing: a pivotal step between eukaryotic transcription and translation. *Nature Reviews Molecular Cell Biology.* **2013**, *14*(3), 153–165. https://doi.org/10.1038/nrm3525.
253. Lin, S.; Fu, X. SR proteins and related factors in alternative splicing. *Advances in Experimental Medicine and Biology.* **2007**, 107–122. https://doi.org/10.1007/978-0-387-77374-2_7.
254. Shepard, P. J.; Hertel, K. J. The SR protein family. *Genome Biology.* **2009**, *10*(10), 242. https://doi.org/10.1186/gb-2009-10-10-242.
255. Zhou, Z.; Fu, X. Regulation of splicing by SR proteins and SR protein-specific kinases. *Chromosoma.* **2013**, *122*(3), 191–207. https://doi.org/10.1007/s00412-013-0407-z.
256. Erkelenz, S.; Mueller, W. F.; Evans, M. S.; Busch, A.; Schöneweis, K.; Hertel, K. J.; Schaal, H. Position-dependent splicing activation and repression by SR and hnRNP proteins rely on common mechanisms. *RNA.* **2012**, *19*(1), 96–102. https://doi.org/10.1261/rna.037044.112.
257. David, C. J.; Manley, J. L. Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged. *Genes & Development.* **2010**, *24*(21), 2343–2364. https://doi.org/10.1101/gad.1973010.
258. Ghigna, C.; Giordano, S.; Shen, H.; Benvenuto, F.; Castiglioni, F.; Comoglio, P. M.; Green, M. R.; Riva, S.; Biamonti, G. Cell Motility Is Controlled by SF2/ASF through Alternative Splicing of the Ron Protooncogene. *Molecular Cell.* **2005**, *20*(6), 881–890. https://doi.org/10.1016/j.molcel.2005.10.026.
259. Anczuków, O.; Rosenberg, A. Z.; Akerman, M.; Das, S.; Zhan, L.; Karni, R.; Muthuswamy, S. K.; Krainer, A. R. The splicing factor SRSF1 regulates apoptosis and proliferation to promote mammary epithelial cell transformation. *Nature Structural & Molecular Biology.* **2012**, *19*(2), 220–228. https://doi.org/10.1038/nsmb.2207.
260. Paz, S.; Ritchie, A.; Mauer, C.; Caputi, M. The RNA binding protein SRSF1 is a master switch of gene expression and regulation in the immune system. *Cytokine & Growth Factor Reviews.* **2021**, *57*, 19–26. https://doi.org/10.1016/j.cytogfr.2020.10.008.
261. Müller-McNicoll, M.; Botti, V.; De Jesus Domingues, A. M.; Brandl, H.; Schwich, O. D.; Steiner, M. C.; Curk, T.; Poser, I.; Zarnack, K.; Neugebauer, K. M. SR proteins are NXF1 adaptors that link alternative RNA processing to mRNA export. *Genes & Development.* **2016**, *30*(5), 553–566. https://doi.org/10.1101/gad.276477.115.
262. Das, S.; Krainer, A. R. Emerging functions of SRSF1, splicing factor and oncoprotein, in RNA metabolism and cancer. *Molecular Cancer Research.* **2014**, *12*(9), 1195–1204. https://doi.org/10.1158/1541-7786.mcr-14-0131.
263. Valacca, C.; Bonomi, S.; Buratti, E.; Pedrotti, S.; Baralle, F. E.; Sette, C.; Ghigna, C.; Biamonti, G. Sam68 regulates EMT through alternative splicing–activated nonsense-mediated mRNA decay of the SF2/ASF proto-oncogene. *The Journal of Cell Biology.* **2010**, *191*(1), 87–99. https://doi.org/10.1083/jcb.201001073.

264. Fregoso, O. I.; Das, S.; Akerman, M.; Krainer, A. R. Splicing-Factor Oncoprotein SRSF1 Stabilizes p53 via RPL5 and Induces Cellular Senescence. *Molecular Cell.* **2013**, *50*(1), 56–66. https://doi.org/10.1016/j.molcel.2013.02.001.
265. Huang, Y.; Steitz, J. A. Splicing factors SRP20 and 9G8 promote the nucleocytoplasmic export of mRNA. *Molecular Cell.* **2001**, *7*(4), 899–905. https://doi.org/10.1016/s1097-2765(01)00233-7.
266. Michlewski, G.; Sanford, J. R.; Cáceres, J. F. The splicing factor SF2/ASF regulates translation initiation by enhancing phosphorylation of 4E-BP1. *Molecular Cell.* **2008**, *30*(2), 179–189. https://doi.org/10.1016/j.molcel.2008.03.013.
267. Maslon, M. M.; Heras, S. R.; Bellora, N.; Eyras, E.; Cáceres, J. F. The translational landscape of the splicing factor SRSF1 and its role in mitosis. *eLife.* **2014**, *3*. https://doi.org/10.7554/elife.02028.
268. Lai, M.; Tarn, W. Hypophosphorylated ASF/SF2 binds TAP and is present in messenger ribonucleoproteins. *Journal of Biological Chemistry.* **2004**, *279*(30), 31745–31749. https://doi.org/10.1074/jbc.c400173200.
269. Hargous, Y.; Hautbergue, G. M.; Tintaru, A. M.; Skrisovska, L.; Golovanov, A. P.; Stevenin, J.; Lian, L. Y.; Wilson, S. A.; Allain, F. H. T. Molecular basis of RNA recognition and TAP binding by the SR proteins SRp20 and 9G8. *The EMBO Journal.* **2006**, *25*(21), 5126–5137. https://doi.org/10.1038/sj.emboj.7601385.
270. Tintaru, A. M.; Hautbergue, G. M.; Hounslow, A. M.; Hung, M.; Lian, L.; Craven, C. J.; Wilson, S. A. Structural and functional analysis of RNA and TAP binding to SF2/ASF. *EMBO Reports.* **2007**, *8*(8), 756–762. https://doi.org/10.1038/sj.embor.7401031.
271. Zhou, H.; Bulek, K.; Li, X.; Herjan, T.; Yu, M.; Qian, W.; Wang, H.; Zhou, G.; Chen, X.; Yang, H.; Hong, L.; Zhao, J.; Qin, L.; Fukuda, K.; Flotho, A.; Gao, J.; Dongre, A.; Carman, J. A.; Kang, Z.; Li, X. IRAK2 directs stimulus-dependent nuclear export of inflammatory mRNAs. *eLife.* **2017**, *6*. https://doi.org/10.7554/elife.29630.
272. Colwill, K.; Pawson, T.; Andrews, B.; Prasad, J.; Manley, J. L.; Bell, J. C.; Duncan, P. I. The Clk/Sty protein kinase phosphorylates SR splicing factors and regulates their intranuclear distribution. *EMBO J.* **1996,** Jan 15;15(2):265-75. PMID: 8617202; PMCID: PMC449941.
273. Koizumi, J.; Okamoto, Y.; Onogi, H.; Mayeda, A.; Krainer, A. R.; Hagiwara, M. The Subcellular Localization of SF2/ASF Is Regulated by Direct Interaction with SR Protein Kinases (SRPKs). *Journal of Biological Chemistry.* **1999**, *274*(16), 11125–11131. https://doi.org/10.1074/jbc.274.16.11125.
274. Rossi, F.; Labourier, E.; Forné, T.; Divita, G.; Derancourt, J.; Riou, J. F.; Antoine, E.; Cathala, G.; Brunel, C.; Tazi, J. Specific phosphorylation of SR proteins by mammalian DNA topoisomerase I. *Nature.* **1996**, *381*(6577), 80–82. https://doi.org/10.1038/381080a0.
275. Novoyatleva, T.; Heinrich, B.; Tang, Y.; Benderska, N.; Butchbach, M. E.; Lorson, C. L.; Lorson, M. A.; Ben-Dov, C.; Fehlbaum, P.; Bracco, L.; Burghes, A. H.; Bollen, M.; Stamm, S. Protein phosphatase 1 binds to the RNA recognition motif of several splicing factors and regulates alternative pre-mRNA processing. *Human Molecular Genetics.* **2007**, *17*(1), 52–70. https://doi.org/10.1093/hmg/ddm284.
276. Sun, S.; Zhang, Z.; Sinha, R.; Karni, R.; Krainer, A. R. SF2/ASF autoregulation involves multiple layers of post-transcriptional and translational control. *Nature Structural & Molecular Biology.* **2010**, *17*(3), 306–312. https://doi.org/10.1038/nsmb.1750.

277. Wu, H.; Sun, S.; Tu, K.; Gao, Y.; Xie, B.; Krainer, A. R.; Zhu, J. A Splicing-Independent function of SF2/ASF in MicroRNA processing. *Molecular Cell.* **2010**, *38*(1), 67–77. https://doi.org/10.1016/j.molcel.2010.02.021.
278. Loomis, R. J.; Naoe, Y.; Parker, J. B.; Savic, V.; Bozovsky, M. R.; Macfarlan, T.; Manley, J. L.; Chakravarti, D. Chromatin Binding of SRp20 and ASF/SF2 and Dissociation from Mitotic Chromosomes Is Modulated by Histone H3 Serine 10 Phosphorylation. *Molecular Cell.* **2009**, *33*(4), 450–461. https://doi.org/10.1016/j.molcel.2009.02.003.
279. Kanehiro, Y.; Todo, K.; Negishi, M.; Fukuoka, J.; Gan, W.; Hikasa, T.; Kaga, Y.; Takemoto, M.; Magari, M.; Li, X.; Manley, J. L.; Ohmori, H.; Kanayama, N. Activation-induced cytidine deaminase (AID)-dependent somatic hypermutation requires a splice isoform of the serine/arginine-rich (SR) protein SRSF1. *Proceedings of the National Academy of Sciences.* **2012**, *109*(4), 1216–1221. https://doi.org/10.1073/pnas.1120368109.
280. Kawaguchi, Y.; Nariki, H.; Kawamoto, N.; Kanehiro, Y.; Miyazaki, S.; Suzuki, M.; Magari, M.; Tokumitsu, H.; Kanayama, N. SRSF1-3 contributes to diversification of the immunoglobulin variable region gene by promoting accumulation of AID in the nucleus. *Biochemical and Biophysical Research Communications.* **2017**, *485*(2), 261–266. https://doi.org/10.1016/j.bbrc.2017.02.097.
281. Karni, R.; De Stanchina, E.; Lowe, S. W.; Sinha, R.; Mu, D.; Krainer, A. R. The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nature Structural & Molecular Biology.* **2007**, *14*(3), 185–193. https://doi.org/10.1038/nsmb1209.
282. Steiner, D. F.; Thomas, M. F.; Hu, J. K.; Yang, Z.; Babiarz, J. E.; Allen, C. D.; Matloubian, M.; Blelloch, R.; Ansel, K. M. MicroRNA-29 regulates T-Box transcription factors and interferon-Γ production in helper T cells. *Immunity.* **2011**, *35*(2), 169–181. https://doi.org/10.1016/j.immuni.2011.07.009.
283. Botta, C.; Cucè, M.; Pitari, M. R.; Caracciolo, D.; Gullà, A.; Morelli, E.; Riillo, C.; Biamonte, L.; Cantafio, M. E. G.; Prabhala, R.; Mignogna, C.; Di Vito, A.; Altomare, E.; Amodio, N.; Di Martino, M. T.; Correale, P.; Rossi, M.; Giordano, A.; Munshi, N. C.; Tassone, P. MiR-29b antagonizes the pro-inflammatory tumor-promoting activity of multiple myeloma-educated dendritic cells. *Leukemia.* **2017**, *32*(4), 1003–1015. https://doi.org/10.1038/leu.2017.336.
284. Ma, L.; Teruya-Feldstein, J.; Weinberg, R. A. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature.* **2007**, *449*(7163), 682–688. https://doi.org/10.1038/nature06174.
285. Das, S.; Anczuków, O.; Akerman, M.; Krainer, A. R. Oncogenic splicing factor SRSF1 is a critical transcriptional target of MYC. *Cell Reports.* **2012**, *1*(2), 110–117. https://doi.org/10.1016/j.celrep.2011.12.001.
286. Anczuków, O.; Rosenberg, A. Z.; Akerman, M.; Das, S.; Zhan, L.; Karni, R.; Muthuswamy, S. K.; Krainer, A. R. The splicing factor SRSF1 regulates apoptosis and proliferation to promote mammary epithelial cell transformation. *Nature Structural & Molecular Biology.* **2012**, *19*(2), 220–228. https://doi.org/10.1038/nsmb.2207.
287. Gout, S.; Brambilla, E.; Boudria, A.; Drissi, R.; Lantuejoul, S.; Gazzeri, S.; Eymin, B. Abnormal expression of the Pre-mRNA splicing regulators SRSF1, SRSF2, SRPK1 and SRPK2 in non small cell lung carcinoma. *PLoS ONE.* **2012**, *7*(10), e46539. https://doi.org/10.1371/journal.pone.0046539.
288. Shultz, J. C.; Goehe, R. W.; Murudkar, C. S.; Wijesinghe, D. S.; Mayton, E. K.; Massiello, A.; Hawkins, A. J.; Mukerjee, P.; Pinkerman, R. L.; Park, M. A.; Chalfant, C. E. SRSF1 regulates the alternative splicing of Caspase 9 via a novel intronic splicing

enhancer affecting the chemotherapeutic sensitivity of Non–Small cell lung cancer cells. *Molecular Cancer Research.* **2011**, *9*(7), 889–900. https://doi.org/10.1158/1541-7786.mcr-11-0061.

289. Wang, J.; Liu, T.; Wang, M.; Lv, W.; Wang, Y.; Jia, Y.; Zhang, R.; Liu, L. SRSF1-dependent alternative splicing attenuates BIN1 expression in non-small cell lung cancer. *Journal of Cellular Biochemistry.* **2019**, *121*(2), 946–953. https://doi.org/10.1002/jcb.29366.
290. Martínez-Terroba, E.; Ezponda, T.; Bértolo, C.; Sainz, C.; Remírez, A.; Agorreta, J.; Garmendia, I.; Behrens, C.; Pio, R.; Wistuba, I. I.; Montuenga, L. M.; Pajares, M. J. The oncogenic RNA-binding protein SRSF1 regulates LIG1 in non-small cell lung cancer. *Laboratory Investigation.* **2018**, *98*(12), 1562–1574. https://doi.org/10.1038/s41374-018-0128-2.
291. Chang, H.; Lin, J. SRSF1 and RBM4 differentially modulate the oncogenic effect of HIF-1α in lung cancer cells through alternative splicing mechanism. *Biochimica Et Biophysica Acta (BBA) – Molecular Cell Research.* **2019**, *1866*(12), 118550. https://doi.org/10.1016/j.bbamcr.2019.118550.
292. Chen, L.; Luo, C.; Shen, L.; Liu, Y.; Wang, Q.; Zhang, C.; Guo, R.; Zhang, Y.; Xie, Z.; Wei, N.; Wu, W.; Han, J.; Feng, Y. SRSF1 Prevents DNA Damage and Promotes Tumorigenesis through Regulation of DBF4B Pre-mRNA Splicing. *Cell Reports.* **2017**, *21*(12), 3406–3413. https://doi.org/10.1016/j.celrep.2017.11.091.
293. Sheng, J.; Zhao, J.; Xu, Q.; Wang, L.; Zhang, W. Zhang, Y. Bioinformatics analysis of SRSF1-controlled gene networks in colorectal cancer. *Oncology Letters.* **2017**. https://doi.org/10.3892/ol.2017.6900.
294. Liu, H.; Gong, Z.; Li, K.; Zhang, Q.; Xu, Z.; Xu, Y. SRPK1/2 and PP1α exert opposite functions by modulating SRSF1-guided MKNK2 alternative splicing in colon adenocarcinoma. *Journal of Experimental & Clinical Cancer Research.* **2021**, *40*(1). https://doi.org/10.1186/s13046-021-01877-y.
295. Akalay, I.; Tan, T. Z.; Kumar, P.; Janji, B.; Mami-Chouaib, F.; Charpy, C.; Vielh, P.; Larsen, A. K.; Thiery, J. P.; Sabbah, M.; Chouaib, S. Targeting WNT1-inducible signaling pathway protein 2 alters human breast cancer cell susceptibility to specific lysis through regulation of KLF-4 and miR-7 expression. *Oncogene.* **2014**, *34*(17), 2261–2271. https://doi.org/10.1038/onc.2014.151.
296. Du, J.; Luo, Y.; Zhang, S.; Wang, B.; Chen, C.; Zhu, G.; Zhu, P.; Cai, C.; Wan, J.; Cai, J.; Chen, S.; Dai, Z.; Zhu, W. Splicing factor SRSF1 promotes breast cancer progression via oncogenic splice switching of PTPMT1. *Journal of Experimental & Clinical Cancer Research.* **2021**, *40*(1). https://doi.org/10.1186/s13046-021-01978-8.
297. Vaklavas, C.; Blume, S. W.; Grizzle, W. E. Hallmarks and determinants of oncogenic translation revealed by ribosome profiling in models of breast cancer. *Translational Oncology.* **2020**, *13*(2), 452–470. https://doi.org/10.1016/j.tranon.2019.12.002.
298. Oh, J.; Pradella, D.; Shao, C.; Li, H.; Choi, N.; Ha, J.; Ruggiero, S.; Fu, X.; Zheng, X.; Ghigna, C.; Shen, H. Widespread alternative splicing changes in metastatic breast cancer cells. *Cells.* **2021**, *10*(4), 858. https://doi.org/10.3390/cells10040858.
299. Gao, X.; Dai, C.; Huang, S.; Tang, J.; Chen, G.; Li, J.; Zhu, Z.; Zhu, X.; Zhou, S.; Gao, Y.; Hou, Z.; Fang, Z.; Xu, C.; Wang, J.; Wu, D.; Sharifi, N.; Li, Z. Functional silencing of HSD17B2 in prostate cancer promotes disease progression. *Clinical Cancer Research.* **2019**, *25*(4), 1291–1301. https://doi.org/10.1158/1078-0432.ccr-18-2392.

300. Guan, X.; Sun, D.; Lu, E.; Urrutia, J. A.; Reiter, R. E.; Rettig, M.; Evans, C. P.; Lara, P.; Gleave, M.; Beer, T. M.; Thomas, G. V.; Huang, J.; Aggarwal, R. R.; Quigley, D. A.; Foye, A.; Chen, W. S.; Youngren, J.; Weinstein, A. S.; Stuart, J. M.; Alumkal, J. J. Copy Number Loss of 17q22 Is Associated with Enzalutamide Resistance and Poor Prognosis in Metastatic Castration-Resistant Prostate Cancer. *Clinical Cancer Research.* **2020**, *26*(17), 4616–4624. https://doi.org/10.1158/1078-0432.ccr-19-2303.
301. Broggi, G.; Lo Giudice, A.; Di Mauro, M.; Asmundo, M. G.; Pricoco, E.; Piombino, E.; Caltabiano, R.; Morgia, G.; Russo, G. I. SRSF-1 and microvessel density immunohistochemical analysis by semi-automated tissue microarray in prostate cancer patients with diabetes (DIAMOND study). *The Prostate.* **2021**, *81*(12), 882–892. https://doi.org/10.1002/pros.24185.
302. Amin, E. M.; Oltean, S.; Hua, J.; Gammons, M. V.; Hamdollah-Zadeh, M.; Welsh, G. I.; Cheung, M.; Ni, L.; Kase, S.; Rennel, E. S.; Symonds, K. E.; Nowak, D. G.; Royer-Pokora, B.; Saleem, M. A.; Hagiwara, M.; Schumacher, V. A.; Harper, S. J.; Hinton, D. R.; Bates, D. O.; Ladomery, M. R. WT1 mutants reveal SRPK1 to be a downstream angiogenesis target by altering VEGF splicing. *Cancer Cell.* **2011**, *20*(6), 768–780. https://doi.org/10.1016/j.ccr.2011.10.016.
303. Mavrou, A.; Brakspear, K.; Hamdollah-Zadeh, M.; Damodaran, G.; Babaei-Jadidi, R.; Oxley, J.; Gillatt, D. A.; Ladomery, M. R.; Harper, S. J.; Bates, D. O.; Oltean, S. Serine–arginine protein kinase 1 (SRPK1) inhibition as a potential novel targeted therapeutic strategy in prostate cancer. *Oncogene.* **2014**, *34*(33), 4311–4319. https://doi.org/10.1038/onc.2014.360.
304. Stella, M.; Falzone, L.; Caponnetto, A.; Gattuso, G.; Barbagallo, C.; Battaglia, R.; Mirabella, F.; Broggi, G.; Altieri, R.; Certo, F.; Caltabiano, R.; Barbagallo, G. M. V.; Musumeci, P.; Ragusa, M.; Di Pietro, C.; Libra, M.; Purrello, M.; Barbagallo, D. Serum Extracellular Vesicle-Derived CIRCHIPK3 and CIRCSMARCA5 are two novel diagnostic biomarkers for glioblastoma multiforme. *Pharmaceuticals.* **2021**, *14*(7), 618. https://doi.org/10.3390/ph14070618.
305. Broggi, G.; Salvatorelli, L.; Barbagallo, D.; Certo, F.; Altieri, R.; Tirrò, E.; Massimino, M.; Vigneri, P.; Guadagno, E.; Maugeri, G.; D'Agata, V.; Musumeci, G.; Ragusa, M.; Barbagallo, G. M. V.; Russo, D.; Caltabiano, R. Diagnostic utility of the immunohistochemical expression of serine and arginine rich splicing Factor 1 (SRSF1) in the differential diagnosis of adult gliomas. *Cancers.* **2021**, *13*(9), 2086. https://doi.org/10.3390/cancers13092086.
306. Barbagallo, D.; Caponnetto, A.; Barbagallo, C.; Battaglia, R.; Mirabella, F.; Brex, D.; Stella, M.; Broggi, G.; Altieri, R.; Certo, F.; Caltabiano, R.; Barbagallo, G. M. V.; Anfuso, C. D.; Lupo, G.; Ragusa, M.; Di Pietro, C.; Hansen, T. B.; Purrello, M. The GAUGAA Motif Is Responsible for the Binding between circSMARCA5 and SRSF1 and Related Downstream Effects on Glioblastoma Multiforme Cell Migration and Angiogenic Potential. *International Journal of Molecular Sciences.* **2021**, *22*(4), 1678. https://doi.org/10.3390/ijms22041678.
307. Ye, L.; Xu, K.; Bai, G.; Yuan, J.; Ran, F. SRSF1 induces glioma progression and has a potential diagnostic application in grading primary glioma. *Oncology Letters.* **2023**, *26*(2). https://doi.org/10.3892/ol.2023.13934.
308. Li, S.; Huang, X.; Zheng, S.; Zhang, W.; Liu, F.; Cao, Q. High expression of SRSF1 facilitates osteosarcoma progression and unveils its potential mechanisms. *BMC Cancer.* **2024**, *24*(1). https://doi.org/10.1186/s12885-024-12346-y.

309. Mole, S.; Faizo, A. A. A., Hernandez-Lopez, H.; Griffiths, M.; Stevenson, A.; Roberts, S.; Graham, S. V. Human papillomavirus type 16 infection activates the host serine arginine protein kinase 1 (SRPK1) – splicing factor axis. *Journal of General Virology.* **2020**, *101*(5), 523–532. https://doi.org/10.1099/jgv.0.001402.
310. Dong, M.; Dong, Z.; Zhu, X.; Zhang, Y.; Song, L. Long non-coding RNA MIR205HG regulates KRT17 and tumor processes in cervical cancer via interaction with SRSF1. *Experimental and Molecular Pathology.* **2019**, *111*, 104322. https://doi.org/10.1016/j.yexmp.2019.104322.
311. Francies, F. Z.; Bassa, S.; Chatziioannou, A.; Kaufmann, A. M.; Dlamini, Z. Splicing Genomics Events in Cervical Cancer: Insights for phenotypic stratification and biomarker Potency. *Genes.* **2021**, *12*(2), 130. https://doi.org/10.3390/genes12020130.
312. Kim, Y.; Kim, B.; Ryu, J.; Lee, G.; Kim, H.; Choi, K.; Ryu, J.; Na, K.; Park, M.; So, H.; Cho, J.; Park, D. HNRNPA1, a splicing regulator, is an effective target protein for cervical cancer detection: comparison with conventional tumor markers. *International Journal of Gynecological Cancer.* **2017**, *27*(2), 326–331. https://doi.org/10.1097/igc.0000000000000868.
313. Erdem, M.; Özgül, I.; Erson-Bensan, A. E. HNRNPA1 (heterogeneous nuclear ribonucleoprotein A1). *Atlas of Genetics and Cytogenetics in Oncology and Haematology.* **2019**, *6*. https://doi.org/10.4267/2042/70455.
314. Erdem, M.; Özgül, I.; Erson-Bensan, A. E. HNRNPA1 (heterogeneous nuclear ribonucleoprotein A1). *Atlas of Genetics and Cytogenetics in Oncology and Haematology.* **2019**, *6*. https://doi.org/10.4267/2042/70455.
315. Shen, L.; Li, Y.; Hu, G.; Huang, Y.; Song, X.; Yu, S.; Xu, X. MIR155HG Knockdown Inhibited the Progression of Cervical Cancer by Binding SRSF1. *OncoTargets and Therapy.* **2020**, *Volume 13*, 12043–12054. https://doi.org/10.2147/ott.s267594.
316. Dalenc, F.; Drouet, J.; Ader, I.; Delmas, C.; Rochaix, P.; Favre, G.; Cohen-Jonathan, E.; Toulas, C. Increased expression of a COOH-truncated nucleophosmin resulting from alternative splicing is associated with cellular resistance to ionizing radiation in HeLa cells. *International Journal of Cancer.* **2002**, *100*(6), 662–668. https://doi.org/10.1002/ijc.10558.
317. Bergsma, A. J.; Van Der Wal, E.; Broeders, M.; Van Der Ploeg, A. T.; Pijnappel, W. P. Alternative splicing in genetic diseases: improved diagnosis and novel treatment options. *International Review of Cell and Molecular Biology.* **2018**, 85–141. https://doi.org/10.1016/bs.ircmb.2017.07.008.
318. Eddy, S. R. Non-coding RNA genes and the modern RNA world. *Nature Reviews Genetics*. **2001**, *2*(12), 919–929. https://doi.org/10.1038/35103511.
319. Mercer, T. R.; Dinger, M. E.; Mattick, J. S. Long non-coding RNAs: insights into functions. *Nature Reviews Genetics*. **2009**, *10*(3), 155–159. https://doi.org/10.1038/nrg2521.
320. Nie, L.; Wu, H. J.; Hsu, J. M.; Chang, S. S.; LaBaff, A. M.; Li, C. W.; Wang, Y.; Hsu, J. L.; Hung, M. C. Long non-coding RNAs: versatile master regulators of gene expression and crucial players in cancer. *PubMed*. **2012**, *4*(2), 127–150. https://pubmed.ncbi.nlm.nih.gov/22611467.
321. Hajjari, M.; Khoshnevisan, A.; Shin, Y. K. Molecular function and regulation of long non-coding RNAs: paradigms with potential roles in cancer. *Tumor Biology*. **2014**, *35*(11), 10645–10663. https://doi.org/10.1007/s13277-014-2636-z.
322. Kurokawa, R. Long noncoding RNA as a regulator for transcription. *Progress in Molecular and Subcellular Biology*. **2010**, 29–41. https://doi.org/10.1007/978-3-642-16502-3_2.

323. Wapinski, O.; Chang, H. Y. Long noncoding RNAs and human disease. *Trends in Cell Biology*. **2011**, *21*(6), 354–361. https://doi.org/10.1016/j.tcb.2011.04.001.
324. Chew, G.; Pauli, A.; Rinn, J. L.; Regev, A.; Schier, A. F.; & Valen, E. Ribosome profiling reveals resemblance between long non-coding RNAs and 5′ leaders of coding RNAs. *Development*. **2013**, *140*(13), 2828–2834. https://doi.org/10.1242/dev.098343.
325. Fatica, A.; Bozzoni, I. Long non-coding RNAs: new players in cell differentiation and development. *Nature Reviews Genetics*. **2013**, *15*(1), 7–21. https://doi.org/10.1038/nrg3606.
326. Mercer, T. R.; Mattick, J. S. Structure and function of long noncoding RNAs in epigenetic regulation. *Nature Structural & Molecular Biology*. **2013**, *20*(3), 300–307. https://doi.org/10.1038/nsmb.2480.
327. Eades, G.; Zhang, Y.; Li, Q.; Xia, J.; Yao, Y.; & Zhou, Q. Long non-coding RNAs in stem cells and cancer. *World Journal of Clinical Oncology*. **2014**, *5*(2), 134. https://doi.org/10.5306/wjco.v5.i2.134.
328. Rinn, J. L. LNCRNAs: linking RNA to chromatin. *Cold Spring Harbor Perspectives in Biology*. 2014, *6*(8), a018614. https://doi.org/10.1101/cshperspect.a018614.
329. Flynn, R. A.; Chang, H. Y. Long noncoding RNAs in Cell-Fate programming and reprogramming. *Cell Stem Cell*. **2014**, *14*(6), 752–761. https://doi.org/10.1016/j.stem.2014.05.014.
330. Li, Y.; Syed, J.; Sugiyama, H. RNA-DNA triplex formation by long noncoding RNAs. *Cell Chemical Biology*. **2016**, *23*(11), 1325–1333. https://doi.org/10.1016/j.chembiol.2016.09.011.
331. Kuo, C.; Hänzelmann, S.; Cetin, N. S.; Frank, S.; Zajzon, B.; Derks, J.; Akhade, V. S.; Ahuja, G.; Kanduri, C.; Grummt, I.; Kurian, L.; Costa, I. G. Detection of RNA–DNA binding sites in long noncoding RNAs. *Nucleic Acids Research*. **2019**, *47*(6), e32. https://doi.org/10.1093/nar/gkz037.
332. Blank-Giwojna, A.; Postepska-Igielska, A.; Grummt, I. LNCRNA KHPS1 activates a poised enhancer by Triplex-Dependent recruitment of epigenomic regulators. *Cell Reports*. **2019**, *26*(11), 2904-2915.e4. https://doi.org/10.1016/j.celrep.2019.02.059.
333. Maldonado, R.; Schwartz, U.; Silberhorn, E.; Längst, G. Nucleosomes stabilize SSRNA-DSDNA triple helices in human cells. *Molecular Cell*. **2019**, *73*(6), 1243-1254.e6. https://doi.org/10.1016/j.molcel.2019.01.007.
334. Postepska-Igielska, A.; Giwojna, A.; Gasri-Plotnitsky, L.; Schmitt, N.; Dold, A.; Ginsberg, D.; Grummt, I. LNCRNA KHPS1 regulates expression of the proto-oncogene SPHK1 via Triplex-Mediated changes in chromatin structure. *Molecular Cell*. **2015**, *60*(4), 626–636. https://doi.org/10.1016/j.molcel.2015.10.001.
335. Sun, Q.; Csorba, T.; Skourti-Stathaki, K.; Proudfoot, N. J.; & Dean, C. R-Loop stabilization represses antisense transcription at the Arabidopsis FLC locus. *Science*. **2013**, *340*(6132), 619–621. https://doi.org/10.1126/science.1234848.
336. Tan-Wong, S. M.; Dhir, S.; Proudfoot, N. J. R-Loops Promote Antisense Transcription across the Mammalian Genome. *Molecular Cell*. **2019**, *76*(4), 600-616.e6. https://doi.org/10.1016/j.molcel.2019.10.002.
337. Niehrs, C.; Luke, B. Regulatory R-loops as facilitators of gene expression and genome stability. *Nature Reviews Molecular Cell Biology*. **2020**, *21*(3), 167–178. https://doi.org/10.1038/s41580-019-0206-3.
338. Arab, K.; Karaulanov, E.; Musheev, M.; Trnka, P., Schäfer; A., Grummt, I.; Niehrs, C. GADD45A binds R-loops and recruits TET1 to CpG island promoters. *Nature Genetics*. **2019**, *51*(2), 217–223. https://doi.org/10.1038/s41588-018-0306-6.

339. Ariel, F.; Lucero, L.; Christ, A.; Mammarella, M. F.; Jegu, T.; Veluchamy, A.; Mariappan, K.; Latrasse, D.; Blein, T.; Liu, C.; Benhamed, M.; Crespi, M. R-Loop Mediated trans Action of the APOLO Long Noncoding RNA. *Molecular Cell*. **2020**, *77*(5), 1055-1065.e4. https://doi.org/10.1016/j.molcel.2019.12.015.
340. Keskin, H.; Shen, Y.; Huang, F.; Patel, M., Yang; T., Ashley, K.; Mazin, A. V.; Storici, F. Transcript-RNA-templated DNA recombination and repair. *Nature*. **2014**, *515*(7527), 436–439. https://doi.org/10.1038/nature13682.
341. Keskin, H.; Meers, C.; Storici, F. Transcript RNA supports precise repair of its own DNA gene. *RNA Biology*. **2015**, *13*(2), 157–165. https://doi.org/10.1080/15476286.2015.1116676.
342. Wei, L.; Nakajima, S.; Böhm, S.; Bernstein, K. A.; Shen, Z.; Tsang, M.; Levine, A. S.; Lan, L. DNA damage during the G0/G1 phase triggers RNA-templated, Cockayne syndrome B-dependent homologous recombination. *Proceedings of the National Academy of Sciences*. **2015**, *112*(27). https://doi.org/10.1073/pnas.1507105112.
343. Chakraborty, A.; Tapryal, N.; Venkova, T.; Horikoshi, N.; Pandita, R. K.; Sarker, A. H.; Sarkar, P. S.; Pandita, T. K.; Hazra, T. K. Classical non-homologous end-joining pathway utilizes nascent RNA for error-free double-strand break repair of transcribed genes. *Nature Communications*. **2016**, *7*(1). https://doi.org/10.1038/ncomms13049.
344. Michelini, F.; Pitchiaya, S.; Vitelli, V.; Sharma, S.; Gioia, U.; Pessina, F.; Cabrini, M.; Wang, Y.; Capozzo, I.; Iannelli, F.; Matti, V.; Francia, S.; Shivashankar, G. V.; Walter, N. G.; Di Fagagna, F. D. Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks. *Nature Cell Biology*. **2017**, *19*(12), 1400–1411. https://doi.org/10.1038/ncb3643.
345. Hung, T.; Wang, Y.; Lin, M. F.; Koegel, A. K.; Kotake, Y.; Grant, G. D.; Horlings, H. M.; Shah, N.; Umbricht, C.; Wang, P.; Wang, Y.; Kong, B.; Langerød, A.; Børresen-Dale, A. L.; Kim, S. K.; Van De Vijver, M.; Sukumar, S.; Whitfield, M. L.; Kellis, M.; Chang, H. Y. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. *Nature Genetics*. **2011**, *43*(7), 621–629. https://doi.org/10.1038/ng.848.
346. Chaudhary, R.; Gryder, B.; Woods, W. S.; Subramanian, M.; Jones, M. F., Li; X. L.; Jenkins, L. M.; Shabalina, S. A.; Mo, M., Dasso, M.; Yang, Y.; Wakefield, L. M.; Zhu, Y.; Frier, S. M.; Moriarity, B. S.; Prasanth, K. V.; Perez-Pinera, P.; Lal, A. Prosurvival long noncoding RNA PINCR regulates a subset of p53 targets in human colorectal cancer cells by binding to Matrin 3. *eLife*. **2017**, *6*. https://doi.org/10.7554/elife.23244.
347. Sharma, V.; Khurana, S.; Kubben, N.; Abdelmohsen, K.; Oberdoerffer, P.; Gorospe, M.; Misteli, T. A BRCA 1-interacting lnc RNA regulates homologous recombination. *EMBO Reports*. **2015**, *16*(11), 1520–1534. https://doi.org/10.15252/embr.201540437.
348. Lee, S.; Kopp, F.; Chang, T.; Sataluri, A.; Chen, B.; Sivakumar, S.; Yu, H.; Xie, Y.; Mendell, J. T. Noncoding RNA NORAD regulates genomic stability by sequestering PUMILIO proteins. *Cell*. **2016**, *164*(1–2), 69–80. https://doi.org/10.1016/j.cell.2015.12.017.
349. Munschauer, M.; Nguyen, C. T.; Sirokman, K.; Hartigan, C. R.; Hogstrom, L.; Engreitz, J. M.; Ulirsch, J. C.; Fulco, C. P.; Subramanian, V.; Chen, J.; Schenone, M.; Guttman, M.; Carr, S. A.; Lander, E. S. The NORAD lncRNA assembles a topoisomerase complex critical for genome stability. *Nature*. **2018**, *561*(7721), 132–136. https://doi.org/10.1038/s41586-018-0453-z.
350. Tichon, A.; Perry, R. B.; Stojic, L; Ulitsky, I. SAM68 is required for regulation of Pumilio by the NORAD long noncoding RNA. *Genes & Development*. **2018**, *32*(1), 70–78. https://doi.org/10.1101/gad.309138.117.

351. Bharti, S. K.; Khan, I.; Banerjee, T.; Sommers, J. A.; Wu, Y.; Brosh, R. M. Molecular functions and cellular roles of the ChlR1 (DDX11) helicase defective in the rare cohesinopathy Warsaw breakage syndrome. *Cellular and Molecular Life Sciences*. **2014**, *71*(14), 2625–2639. https://doi.org/10.1007/s00018-014-1569-4.
352. Seila, A. C.; Calabrese, J. M.; Levine, S. S.; Yeo, G. W.; Rahl, P. B.; Flynn, R. A.; Young, R. A.; Sharp, P. A. Divergent Transcription from Active Promoters. *Science*. **2008**, *322*(5909), 1849–1851. https://doi.org/10.1126/science.1162253.
353. Luo, S.; Lu, J. Y.; Liu, L.; Yin, Y.; Chen, C.; Han, X.; Wu, B.; Xu, R.; Liu, W.; Yan, P.; Shao, W.; Lu, Z.; Li, H.; Na, J.; Tang, F.; Wang, J.; Zhang, Y. E.; Shen, X. Divergent LNCRNAs regulate gene expression and lineage differentiation in pluripotent cells. *Cell Stem Cell*. **2016**, *18*(5), 637–652. https://doi.org/10.1016/j.stem.2016.01.024.
354. Gil, N.; Ulitsky, I. Regulation of gene expression by cis-acting long non-coding RNAs. *Nature Reviews Genetics*. **2019**, *21*(2), 102–117. https://doi.org/10.1038/s41576-019-0184-5.
355. Wutz, A. Gene silencing in X-chromosome inactivation: advances in understanding facultative heterochromatin formation. *Nature Reviews Genetics*. **2011**, *12*(8), 542–553. https://doi.org/10.1038/nrg3035.
356. Sahakyan, A.; Yang, Y.; & Plath, K. The role of XIST in X-Chromosome dosage compensation. *Trends in Cell Biology*. **2018**, *28*(12), 999–1013. https://doi.org/10.1016/j.tcb.2018.05.005.
357. Jeon, Y.; Lee, J. T. YY1 tethers XIST RNA to the inactive X nucleation Center. *Cell*. **2011**, *146*(1), 119–133. https://doi.org/10.1016/j.cell.2011.06.026.
358. McHugh, C. A.; Chen, C.; Chow, A.; Surka, C. F.; Tran, C.; McDonel, P.; Pandya-Jones, A.; Blanco, M.; Burghard, C.; Moradian, A.; Sweredoski, M. J.; Shishkin, A. A.; Su, J., Lander, E. S.; Hess, S.; Plath, K.; Guttman, M. The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3. *Nature*. **2015**, *521*(7551), 232–236. https://doi.org/10.1038/nature14443.
359. Pintacuda, G.; Wei, G.; Roustan, C.; Kirmizitas, B. A.; Solcan, N.; Cerase, A.; Castello, A.; Mohammed, S.; Moindrot, B.; Nesterova, T. B.; Brockdorff, N. HNRNPK recruits PCGF3/5-PRC1 to the XIST RNA B-Repeat to establish Polycomb-Mediated chromosomal silencing. *Molecular Cell*. **2017**, *68*(5), 955-969.e10. https://doi.org/10.1016/j.molcel.2017.11.013.
360. Colognori, D.; Sunwoo, H.; Kriz, A. J.; Wang, C.; Lee, J. T. Xist Deletional Analysis Reveals an Interdependency between Xist RNA and Polycomb Complexes for Spreading along the Inactive X. *Molecular Cell*. **2019**, *74*(1), 101-117.e10. https://doi.org/10.1016/j.molcel.2019.01.015.
361. Engreitz, J. M.; Pandya-Jones, A.; McDonel, P.; Shishkin, A.; Sirokman, K.; Surka, C.; Kadri, S.; Xing, J.; Goren, A.; Lander, E. S.; Plath, K.; Guttman, M. The XIST LNCRNA exploits Three-Dimensional genome architecture to spread across the X chromosome. *Science*. **2013**, *341*(6147). https://doi.org/10.1126/science.1237973.
362. Giorgetti, L.; Lajoie, B. R.; Carter, A. C.; Attia, M.; Zhan, Y.; Xu, J.; Chen, C. J.; Kaplan, N.; Chang, H. Y.; Heard, E.; Dekker, J. Structural organization of the inactive X chromosome in the mouse. *Nature*. **2016**, *535*(7613), 575–579. https://doi.org/10.1038/nature18589.
363. Pandya-Jones, A.; Markaki, Y.; Serizay, J.; Chitiashvili, T.; Leon, W. R. M.; Damianov, A.; Chronis, C.; Papp, B.; Chen, C.; McKee, R.; Wang, X.; Chau, A.; Sabri, S.; Leonhardt, H.; Zheng, S.; Guttman, M.; Black, D. L.; Plath, K. A protein assembly mediates Xist localization and gene silencing. *Nature*. **2020**, *587*(7832), 145–151. https://doi.org/10.1038/s41586-020-2703-0.

364. Beckedorff, F. C.; Ayupe, A. C.; Crocci-Souza, R.; Amaral, M. S.; Nakaya, H. I.; Soltys, D. T.; Menck, C. F. M.; Reis, E. M.; Verjovski-Almeida, S. The Intronic long noncoding RNA ANRASSF1 recruits PRC2 to the RASSF1A promoter, reducing the expression of RASSF1A and increasing cell proliferation. *PLoS Genetics*. **2013**, *9*(8), e1003705. https://doi.org/10.1371/journal.pgen.1003705.
365. Herzog, V. A.; Lempradl, A.; Trupke, J.; Okulski, H.; Altmutter, C.; Ruge, F.; Boidol, B.; Kubicek, S.; Schmauss, G.; Aumayr, K.; Ruf, M.; Pospisilik, A.; Dimond, A.; Senergin, H. B.; Vargas, M. L.; Simon, J. A.; Ringrose, L. A strand-specific switch in noncoding transcription switches the function of a Polycomb/Trithorax response element. *Nature Genetics*. **2014**, *46*(9), 973–981. https://doi.org/10.1038/ng.3058.
366. Alecki, C.; Chiwara, V.; Sanz, L. A.; Grau, D.; Pérez, O. A.; Boulier, E. L.; Armache, K.; Chédin, F.; Francis, N. J. RNA-DNA strand exchange by the Drosophila Polycomb complex PRC2. *Nature Communications*. **2020**, *11*(1). https://doi.org/10.1038/s41467-020-15609-x.
367. Csorba, T.; Questa, J. I.; Sun, Q.; Dean, C. Antisense COOLAIR mediates the coordinated switching of chromatin states at FLC during vernalization. *Proceedings of the National Academy of Sciences*. **2014**, *111*(45), 16160–16165. https://doi.org/10.1073/pnas.1419030111.
368. Rosa, S.; Duncan, S.; Dean, C. Mutually exclusive sense–antisense transcription at FLC facilitates environmentally induced gene repression. *Nature Communications*. **2016**, *7*(1). https://doi.org/10.1038/ncomms13031.
369. Thebault, P.; Boutin, G.; Bhat, W.; Rufiange, A.; Martens, J.; Nourani, A. Transcription regulation by the noncoding RNA SRG1 requires SPT2-Dependent chromatin deposition in the wake of RNA polymerase II. *Molecular and Cellular Biology*. **2011**, *31*(6), 1288–1300. https://doi.org/10.1128/mcb.01083-10.
370. Latos, P. A.; Pauler, F. M.; Koerner, M. V.; Şenergin, H. B.; Hudson, Q. J.; Stocsits, R. R.; Allhoff, W.; Stricker, S. H.; Klement, R. M.; Warczok, K. E.; Aumayr, K.; Pasierbek, P.; Barlow, D. P. AIRN transcriptional overlap, but not its LNCRNA products, induces imprinted IGF2R silencing. *Science*. **2012**, *338*(6113), 1469–1472. https://doi.org/10.1126/science.1228110.
371. Santoro, F.; Pauler, F. M. Silencing by the imprinted Airn macro lncRNA. *Cell Cycle*. **2013**, *12*(5), 711–712. https://doi.org/10.4161/cc.23860.
372. Stojic, L.; Niemczyk, M.; Orjalo, A.; Ito, Y.; Ruijter, A. E. M.; Uribe-Lewis, S.; Joseph, N.; Weston, S.; Menon, S.; Odom, D. T.; Rinn, J.; Gergely, F.; Murrell, A. Transcriptional silencing of long noncoding RNA GNG12-AS1 uncouples its transcriptional and product-related functions. *Nature Communications*. **2016**, *7*(1). https://doi.org/10.1038/ncomms10406.
373. Rom, A.; Melamed, L.; Gil, N.; Goldrich, M. J.; Kadir, R.; Golan, M.; Biton, I.; Perry, R. B.; Ulitsky, I. Regulation of CHD2 expression by the Chaserr long noncoding RNA gene is essential for viability. *Nature Communications*. **2019**, *10*(1). https://doi.org/10.1038/s41467-019-13075-8.
374. Li, W.; Notani, D.; Ma, Q.; Tanasa, B.; Nunez, E.; Chen, A. Y.; Merkurjev, D.; Zhang, J.; Ohgi, K.; Song, X.; Oh, S.; Kim, H.; Glass, C. K.; Rosenfeld, M. G.. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature*. **2013**, *498*(7455), 516–520. https://doi.org/10.1038/nature12210.

375. Hon, C.; Ramilowski, J. A.; Harshbarger, J.; Bertin, N.; Rackham, O. J. L.; Gough, J.; Denisenko, E.; Schmeier, S.; Poulsen, T. M.; Severin, J.; Lizio, M.; Kawaji, H.; Kasukawa, T.; Itoh, M.; Burroughs, M. A.; Noma, S.; Quelen, S. D., Alam, T.; Medvedeva, Y. A.; Forrest, A. R. R. An atlas of human long non-coding RNAs with accurate 5′ ends. *Nature*. **2017**, *543*(7644), 199–204. https://doi.org/10.1038/nature21374.
376. Jiao, W.; Chen, Y.; Song, H.; Li, D.; Mei, H.; Yang, F.; Fang, E.; Wang, X.; Huang, K.; Zheng, L.; Tong, Q. HPSE enhancer RNA promotes cancer progression through driving chromatin looping and regulating hnRNPU/p300/EGR1/HPSE axis. *Oncogene*. **2018**, *37*(20), 2728–2745. https://doi.org/10.1038/s41388-018-0128-0.
377. Kim, Y. J.; Xie, P.; Cao, L.; Zhang, M. Q.; Kim, T. H. Global transcriptional activity dynamics reveal functional enhancer RNAs. *Genome Research*. **2018**, *28*(12), 1799–1811. https://doi.org/10.1101/gr.233486.117.
378. Marques, A. C.; Hughes, J.; Graham, B.; Kowalczyk, M. S.; Higgs, D. R.; Ponting, C. P. Chromatin signatures at transcriptional start sites separate two equally populated yet distinct classes of intergenic long noncoding RNAs. *Genome Biology*. **2013**, *14*(11), R131. https://doi.org/10.1186/gb-2013-14-11-r131.
379. Gil, N.; Ulitsky, I. Production of Spliced Long Noncoding RNAs Specifies Regions with Increased Enhancer Activity. *Cell Systems*. **2018**, *7*(5), 537-547.e3. https://doi.org/10.1016/j.cels.2018.10.009.
380. Tan, J. Y.; Biasini, A.; Young, R. S.; Marques, A. C. Splicing of enhancer-associated lincRNAs contributes to enhancer activity. *Life Science Alliance*. **2020**, *3*(4), e202000663. https://doi.org/10.26508/lsa.202000663.
381. Amaral, P. P.; Leonardi, T.; Han, N.; Viré, E.; Gascoigne, D. K.; Arias-Carrasco, R.; Büscher, M.; Pandolfini, L.; Zhang, A.; Pluchino, S.; Maracaja-Coutinho, V.; Nakaya, H. I.; Hemberg, M.; Shiekhattar, R.; Enright, A. J.; Kouzarides, T. Genomic positional conservation identifies topological anchor point RNAs linked to developmental loci. *Genome Biology*. **2018**, *19*(1). https://doi.org/10.1186/s13059-018-1405-5.
382. Fanucchi, S.; Mhlanga, M. M. Enhancer-Derived LNCRNAs regulate genome architecture: fact or fiction? *Trends in Genetics*. **2017**, *33*(6), 375–377. https://doi.org/10.1016/j.tig.2017.03.004.
383. Van Steensel, B.; Furlong, E. E. M. The role of transcription in shaping the spatial organization of the genome. *Nature Reviews Molecular Cell Biology*. **2019**, *20*, 327–337. https://doi.org/10.1038/s41580-019-0114-6.
384. Grossi, E.; Raimondi, I.; Goñi, E.; González, J.; Marchese, F. P.; Chapaprieta, V.; Martín-Subero, J. I.; Guo, S.; Huarte, M. A lncRNA-SWI/SNF complex crosstalk controls transcriptional activation at specific promoter regions. *Nature Communications*. **2020**, *11*(936). https://doi.org/10.1038/s41467-020-14623-3.
385. Tomita, S.; Abdalla, M. O. A.; Fujiwara, S.; Matsumori, H.; Maehara, K.; Ohkawa, Y.; Iwase, H.; Saitoh, N.; Nakao, M. A cluster of noncoding RNAs activates the ESR1 locus during breast cancer adaptation. *Nature Communications*. **2015**, *6*(6966). https://doi.org/10.1038/ncomms7966.
386. Anderson, K. M.; Anderson, D. M.; McAnally, J. R.; Shelton, J. M.; Bassel-Duby, R.; Olson, E. N. Transcription of the non-coding RNA upperhand controls Hand2 expression and heart development. *Nature*. **2016**, *539*(7629), 433–436. https://doi.org/10.1038/nature20128.

387. Dao, L. T. M.; Galindo-Albarrán, A. O.; Castro-Mondragon, J. A.; Andrieu-Soler, C.; Medina-Rivera, A.; Souaid, C.; Charbonnier, G.; Griffon, A.; Vanhille, L.; Stephen, T.; Alomairi, J.; Martin, D.; Torres, M.; Fernandez, N.; Soler, E.; Van Helden, J.; Puthier, D.; Spicuglia, S. Genome-wide characterization of mammalian promoters with distal enhancer functions. *Nature Genetics*. **2017**, *49*(7), 1073–1081. https://doi.org/10.1038/ng.3884.
388. Paralkar, V. R.; Taborda, C. C.; Huang, P.; Yao, Y.; Kossenkov, A. V.; Prasad, R.; Luan, J.; Davies, J. O.; Hughes, J. R.; Hardison, R. C.; Blobel, G. A.; Weiss, M. J. Unlinking an lncRNA from Its Associated cis Element. *Molecular Cell*. **2016**, *62*(1), 104–110. https://doi.org/10.1016/j.molcel.2016.02.029.
389. Engreitz, J. M.; Haines, J. E.; Perez, E. M.; Munson, G.; Chen, J.; Kane, M.; McDonel, P. E.; Guttman, M.; Lander, E. S. Local regulation of gene expression by lncRNA promoters, transcription and splicing. *Nature*. **2016**, *539*(7629), 452–455. https://doi.org/10.1038/nature20149.
390. Yin, Y.; Yan, P.; Lu, J.; Song, G.; Zhu, Y.; Li, Z.; Zhao, Y.; Shen, B.; Huang, X.; Zhu, H.; Orkin, S. H.; Shen, X. Opposing Roles for the lncRNA Haunt and Its Genomic Locus in Regulating HOXA Gene Activation during Embryonic Stem Cell Differentiation. *Cell Stem Cell*. **2015**, *16*(5), 504–516. https://doi.org/10.1016/j.stem.2015.03.007.
391. Hartford, C. C. R.; Lal, A. When long noncoding becomes protein coding. *Molecular and Cellular Biology*. **2020**, *40*(6). https://doi.org/10.1128/mcb.00528-19.
392. Yin, Q.; Yang, L.; Zhang, Y.; Xiang, J.; Wu, Y.; Carmichael, G. G.; Chen, L. Long Noncoding RNAs with snoRNA Ends. *Molecular Cell*. **2012**, *48*(2), 219–230. https://doi.org/10.1016/j.molcel.2012.07.033.
393. Wu, H.; Yin, Q.; Luo, Z.; Yao, R.; Zheng, C.; Zhang, J.; Xiang, J.; Yang, L.; Chen, L. Unusual Processing Generates SPA LncRNAs that Sequester Multiple RNA Binding Proteins. *Molecular Cell*. **2016**, *64*(3), 534–548. https://doi.org/10.1016/j.molcel.2016.10.007.
394. Yap, K.; Mukhina, S.; Zhang, G.; Tan, J. S.; Ong, H. S.; Makeyev, E. V. A short tandem Repeat-Enriched RNA assembles a nuclear compartment to control alternative splicing and promote cell survival. *Molecular Cell*. **2018**, *72*(3), 525-540.e13. https://doi.org/10.1016/j.molcel.2018.08.041.
395. Barrios, N. R.; Legascue, M. F.; Benhamed, M.; Ariel, F.; Crespi, M. Splicing regulation by long noncoding RNAs. *Nucleic Acids Research*. **2018**, *46*(5), 2169–2184. https://doi.org/10.1093/nar/gky095.
396. Lee, S.; Kopp, F.; Chang, T.; Sataluri, A.; Chen, B.; Sivakumar, S.; Yu, H.; Xie, Y.; Mendell, J. T. Noncoding RNA NORAD regulates genomic stability by sequestering PUMILIO proteins. *Cell*. **2016**, *164*(1–2), 69–80. https://doi.org/10.1016/j.cell.2015.12.017.
397. Guo, C.; Ma, X.; Xing, Y.; Zheng, C.; Xu, Y.; Shan, L.; Zhang, J.; Wang, S.; Wang, Y.; Carmichael, G. G.; Yang, L.; Chen, L. Distinct processing of LNCRNAs contributes to non-conserved functions in stem cells. *Cell*. **2020**, *181*(3), 621-636.e22. https://doi.org/10.1016/j.cell.2020.03.006.
398. Grelet, S.; Link, L. A.; Howley, B.; Obellianne, C.; Palanisamy, V.; Gangaraju, V. K.; Diehl, J. A.; Howe, P. H. A regulated PNUTS mRNA to lncRNA splice switch mediates EMT and tumour progression. *Nature Cell Biology*. **2017**, *19*(9), 1105–1115. https://doi.org/10.1038/ncb3595.

399. Gong, C.; Maquat, L. E. lncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3′ UTRs via Alu elements. *Nature*. **2011**, *470*(7333), 284–288. https://doi.org/10.1038/nature09701.
400. Kretz, M.; Siprashvili, Z.; Chu, C.; Webster, D. E.; Zehnder, A.; Qu, K.; Lee, C. S.; Flockhart, R. J.; Groff, A. F.; Chow, J.; Johnston, D.; Kim, G. E.; Spitale, R. C.; Flynn, R. A.; Zheng, G. X. Y.; Aiyer, S.; Raj, A.; Rinn, J. L.; Chang, H. Y.; Khavari, P. A. Control of somatic tissue differentiation by the long non-coding RNA TINCR. *Nature*. **2012**, *493*(7431), 231–235. https://doi.org/10.1038/nature11661.
401. Wang, J.; Gong, C.; Maquat, L. E. Control of myogenesis by rodent SINE-containing lncRNAs. *Genes & Development*. **2013**, *27*(7), 793–804. https://doi.org/10.1101/gad.212639.112.
402. Carrieri, C.; Cimatti, L.; Biagioli, M.; Beugnet, A.; Zucchelli, S.; Fedele, S.; Pesce, E.; Ferrer, I.; Collavin, L.; Santoro, C.; Forrest, A. R. R.; Carninci, P.; Biffo, S.; Stupka, E.; Gustincich, S. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. *Nature*. **2012**, *491*(7424), 454–457. https://doi.org/10.1038/nature11508.
403. Salmena, L.; Poliseno, L.; Tay, Y.; Kats, L.; Pandolfi, P. P. A CERNA hypothesis: the rosetta stone of a hidden RNA language? *Cell*. **2011**, *146*(3), 353–358. https://doi.org/10.1016/j.cell.2011.07.014.
404. Denzler, R.; Agarwal, V.; Stefano, J.; Bartel, D. P.; Stoffel, M. Assessing the ceRNA Hypothesis with Quantitative Measurements of miRNA and Target Abundance. *Molecular Cell*. **2014**, *54*(5), 766–776. https://doi.org/10.1016/j.molcel.2014.03.045.
405. Bosson, A. D.; Zamudio, J. R.; Sharp, P. A. Endogenous MIRNA and target concentrations determine susceptibility to potential CERNA competition. *Molecular Cell*. **2014**, *56*(3), 347–359. https://doi.org/10.1016/j.molcel.2014.09.018.
406. Fatima, F.; Nawaz, M. Vesiculated Long Non-Coding RNAs: Offshore Packages Deciphering Trans-Regulation between Cells, Cancer Progression and Resistance to Therapies. *Non-Coding RNA*. **2017**, *3*(1), 10. https://doi.org/10.3390/ncrna3010010.
407. Zhao, Y.; Sun, L.; Wang, R. R.; Hu, J.; Cui, J. The effects of mitochondria-associated long noncoding RNAs in cancer mitochondria: New players in an old arena. *Critical Reviews in Oncology/Hematology*. **2018**, *131*, 76–82. https://doi.org/10.1016/j.critrevonc.2018.08.005.
408. Vendramin, R.; Verheyden, Y.; Ishikawa, H.; Goedert, L.; Nicolas, E.; Saraf, K.; Armaos, A.; Ponti, R. D.; Izumikawa, K.; Mestdagh, P.; Lafontaine, D. L. J.; Tartaglia, G. G.; Takahashi, N.; Marine, J.; Leucci, E. SAMMSON fosters cancer cell fitness by concertedly enhancing mitochondrial and cytosolic translation. *Nature Structural & Molecular Biology*. **2018**, *25*(11), 1035–1046. https://doi.org/10.1038/s41594-018-0143-4.
409. Geisler, S.; Coller, J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. *Nature Reviews Molecular Cell Biology*. **2013**, *14*(11), 699–712. https://doi.org/10.1038/nrm3679.
410. De Goede, O. M.; Nachun, D. C.; Ferraro, N. M.; Gloudemans, M. J.; Rao, A. S.; Smail, C.; Eulalio, T. Y.; Aguet, F.; Ng, B.; Xu, J.; Barbeira, A. N.; Castel, S. E.; Kim-Hellmuth, S.; Park, Y.; Scott, A. J.; Strober, B. J.; Brown, C. D.; Wen, X.; Hall, I. M.; Volpi, S. Population-scale tissue transcriptomics maps long non-coding RNAs to complex disease. *Cell*. **2021**, *184*(10), 2633-2648.e19. https://doi.org/10.1016/j.cell.2021.03.050.

411. Bartonicek, N.; Clark, M. B.; Quek, X. C.; Torpy, J. R.; Pritchard, A. L.; Maag, J. L. V.; Gloss, B. S.; Crawford, J.; Taft, R. J.; Hayward, N. K.; Montgomery, G. W.; Mattick, J. S.; Mercer, T. R.; Dinger, M. E. Intergenic disease-associated regions are abundant in novel transcripts. *Genome Biology*. **2017**, *18*(1). https://doi.org/10.1186/s13059-017-1363-3.
412. Kelley, R. L.; Meller, V. H.; Gordadze, P. R.; Roman, G.; Davis, R. L.; Kuroda, M. I. Epigenetic Spreading of the Drosophila Dosage Compensation Complex from roX RNA Genes into Flanking Chromatin. *Cell*. **1999**, *98*(4), 513–522. https://doi.org/10.1016/s0092-8674(00)81979-0.
413. Doherty, A. S.; Mann, M. R.; Tremblay, K. D.; Bartolomei, M. S.; Schultz, R. M. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo1. *Biology of Reproduction*. **2000**, *62*(6), 1526–1535. https://doi.org/10.1095/biolreprod62.6.1526.
414. Sleutels, F.; Zwart, R.; Barlow, D. P. The non-coding Air RNA is required for silencing autosomal imprinted genes. Nature. **2002**, 415(6873), 810–813. https://doi.org/10.1038/415810a.
415. Thakur, N.; Tiwari, V. K.; Thomassin, H.; Pandey, R. R.; Kanduri, M.; Göndör, A.; Grange, T.; Ohlsson, R.; Kanduri, C. An antisense RNA regulates the bidirectional silencing property of the KCNQ1 imprinting control region *Molecular and Cellular Biology*. **2004**, 24(18), 7855–7862. https://doi.org/10.1128/mcb.24.18.7855-7862.2004.
416. Li, P.; Tao, Z.; Dean, C. Phenotypic evolution through variation in splicing of the noncoding RNA COOLAIR. *Genes & Development*. **2015**, *29*(7), 696–701. https://doi.org/10.1101/gad.258814.115.
417. Gao, F.; Cai, Y., Kapranov, P.; Xu, D. Reverse-genetics studies of lncRNAs – what we have learnt and paths forward. *Genome Biology*. **2020**, *21*(1). https://doi.org/10.1186/s13059-020-01994-5.
418. Andergassen, D.; Rinn, J. L. From genotype to phenotype: genetics of mammalian long non-coding RNAs in vivo. *Nature Reviews Genetics*. **2021**, *23*(4), 229–243. https://doi.org/10.1038/s41576-021-00427-8.
419. Statello, L.; Guo, C.; Chen, L.; Huarte, M. Gene regulation by long non-coding RNAs and its biological functions. *Nature Reviews Molecular Cell Biology*. **2020**, *22*(2), 96–118. https://doi.org/10.1038/s41580-020-00315-9.
420. Carlevaro-Fita, J.; Lanzós, A.; Feuerbach, L.; Hong, C.; Mas-Ponte, D.; Pedersen, J. S.; Abascal, F.; Amin, S. B.; Bader, G. D.; Barenboim, J.; Beroukhim, R.; Bertl, J.; Boroevich, K. A.; Brunak, S.; Campbell, P. J.; Carlevaro-Fita, J.; Chakravarty, D.; Chan, C. W. Y.; Chen, K.; Von Mering, C. Cancer LncRNA Census reveals evidence for deep functional conservation of long noncoding RNAs in tumorigenesis. *Communications Biology*. **2020**, *3*(1). https://doi.org/10.1038/s42003-019-0741-7.
421. Sparber, P.; Filatova, A.; Khantemirova, M.; Skoblov, M. The role of long non-coding RNAs in the pathogenesis of hereditary diseases. *BMC Medical Genomics*. **2019**, *12*(S2). https://doi.org/10.1186/s12920-019-0487-6.
422. Cai, P.; Otten, A. B.; Cheng, B.; Ishii, M. A.; Zhang, W.; Huang, B.; Qu, K.; Sun, B. K. A genome-wide long noncoding RNA CRISPRi screen identifies PRANCR as a novel regulator of epidermal homeostasis. *Genome Research*. **2019**, *30*(1), 22–34. https://doi.org/10.1101/gr.251561.119.
423. Horlbeck, M. A.; Liu, S. J.; Chang, H. Y.; Lim, D. A.; Weissman, J. S. Fitness effects of CRISPR/Cas9-targeting of long noncoding RNA genes. *Nature Biotechnology*. **2020**, *38*(5), 573–576. https://doi.org/10.1038/s41587-020-0428-0.

424. Fanucchi, S.; Fok, E. T.; Dalla, E.; Shibayama, Y.; Börner, K.; Chang, E. Y.; Stoychev, S.; Imakaev, M.; Grimm, D.; Wang, K. C.; Li, G.; Sung, W.; Mhlanga, M. M. Immune genes are primed for robust transcription by proximal long noncoding RNAs located in nuclear compartments. *Nature Genetics*. **2018**, *51*(1), 138–150. https://doi.org/10.1038/s41588-018-0298-2.
425. Chen, L.; Zhu, Q.; Kaufmann, K. Long non-coding RNAs in plants: emerging modulators of gene activity in development and stress responses. *Planta*. **2020**, *252*(5). https://doi.org/10.1007/s00425-020-03480-5.
426. Huarte, M.; Guttman, M.; Feldser, D.; Garber, M.; Koziol, M. J.; Kenzelmann-Broz, D.; Khalil, A. M.; Zuk, O.; Amit, I.; Rabani, M.; Attardi, L. D.; Regev, A.; Lander, E. S.; Jacks, T.; Rinn, J. L. A Large Intergenic Noncoding RNA Induced by p53 Mediates Global Gene Repression in the p53 Response. *Cell*. **2010**, *142*(3), 409–419. https://doi.org/10.1016/j.cell.2010.06.040.
427. Rothschild, G.; Zhang, W.; Lim, J.; Giri, P. K.; Laffleur, B. Chen, Y.; Fang, M.; Chen, Y.; Nair, L.; Liu, Z.; Deng, H.; Hammarström, L.; Wang, J.; Basu, U. Noncoding RNA transcription alters chromosomal topology to promote isotype-specific class switch recombination. *Science Immunology*. **2020**, *5*(44). https://doi.org/10.1126/sciimmunol.aay5864.
428. Zhang, P.; Cao, L.; Fan, P.; Mei, Y.; Wu, M. LncRNA-MIF, a c-Myc-activated long non-coding RNA, suppresses glycolysis by promoting Fbxw7-mediated c-Myc degradation. *EMBO Reports*. **2016**, *17*(8), 1204–1220. https://doi.org/10.15252/embr.201642067.
429. Zheng, X.; Han, H.; Liu, G.; Ma, Y.; Pan, R.; Sang, L.; Li, R.; Yang, L.; Marks, J. R.; Wang, W.; Lin, A. LncRNA wires up Hippo and Hedgehog signaling to reprogramme glucose metabolism. *The EMBO Journal*. **2017**, *36*(22), 3325–3335. https://doi.org/10.15252/embj.201797609.
430. Wierzbicki, A. T.; Blevins, T.; Swiezewski, S. (). Long noncoding RNAs in plants. *Annual Review of Plant Biology*. **2021**, *72*(1), 245–271. https://doi.org/10.1146/annurev-arplant-093020-035446.
431. Wu, E.; Guo, X.; Teng, X.; Zhang, R.; Li, F.; Cui, Y.; Zhang, D.; Liu, Q.; Luo, J.; Wang, J.; Chen, R. Discovery of Plasma Membrane-Associated RNAs through APEX-seq. *Cell Biochemistry and Biophysics*. **2021**, *79*(4), 905–917. https://doi.org/10.1007/s12013-021-00991-0.
432. Chen, Y.; Qi, F.; Gao, F.; Cao, H.; Xu, D.; Salehi-Ashtiani, K.; Kapranov, P. Hovlinc is a recently evolved class of ribozyme found in human lncRNA. *Nature Chemical Biology*. **2021**, *17*(5), 601–607. https://doi.org/10.1038/s41589-021-00763-0.
433. Lekka, E.; Hall, J. Noncoding RNAs in disease. *FEBS Letters*. **2018**, *592*(17), 2884–2900. https://doi.org/10.1002/1873-3468.13182.
434. Hajjari, M.; Khoshnevisan, A.; Shin, Y. K. Long non-coding RNAs in hematologic malignancies: road to translational research. *Frontiers in Genetics*. **2013**, *4*. https://doi.org/10.3389/fgene.2013.00250.
435. Du, Z.; Fei, T.; Verhaak, R. G. W.; Su, Z.; Zhang, Y.; Brown, M.; Chen, Y.; Liu, X. S. Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer. *Nature Structural & Molecular Biology*. **2013**, *20*(7), 908–913. https://doi.org/10.1038/nsmb.2591.
436. Martens-Uzunova, E. S.; Böttcher, R.; Croce, C. M.; Jenster, G.; Visakorpi, T.; Calin, G. A. Long noncoding RNA in prostate, bladder, and kidney cancer. *European Urology*. **2014**, *65*(6), 1140–1151. https://doi.org/10.1016/j.eururo.2013.12.003.

437. Qiu, M.; Hu, J.; Yin, R.; Xu, L. Long noncoding RNA: an emerging paradigm of cancer research. *Tumor Biology*. **2013**, *34*(2), 613–620. https://doi.org/10.1007/s13277-013-0658-6.
438. Walsh, A. L.; Tuzova, A. V.; Bolton, E. M.; Lynch, T. H.; Perry, A. S. Long noncoding RNAs and prostate carcinogenesis: the missing 'linc'? *Trends in Molecular Medicine*. **2014**, *20*(8), 428–436. https://doi.org/10.1016/j.molmed.2014.03.005.
439. Gao, Y.; Wang, P.; Wang, Y.; Ma, X.; Zhi, H.; Zhou, D.; Li, X.; Fang, Y.; Shen, W.; Xu, Y.; Shang, S.; Wang, L.; Wang, L.; Ning, S.; Li, X. Lnc2Cancer v2.0: updated database of experimentally supported long non-coding RNAs in human cancers. *Nucleic Acids Research.* **2018**, *47*(D1), D1028–D1033. https://doi.org/10.1093/nar/gky1096.
440. Sánchez, Y.; Segura, V.; Marín-Béjar, O.; Athie, A.; Marchese, F. P.; González, J.; Bujanda, L.; Guo, S.; Matheu, A.; Huarte, M. Genome-wide analysis of the human p53 transcriptional network unveils a lncRNA tumour suppressor signature. *Nature Communications*. **2014**, *5*(1). https://doi.org/10.1038/ncomms6812.
441. Hart, J. R.; Roberts, T. C.; Weinberg, M. S.; Morris, K. V.; Vogt, P. K. MYC regulates the non-coding transcriptome. *Oncotarget*. **2014**, *5*(24), 12543–12554. https://doi.org/10.18632/oncotarget.3033.
442. Zhou, Y.; Zhang, X.; Klibanski, A. MEG3 noncoding RNA: a tumor suppressor. *Journal of Molecular Endocrinology*. **2012**, *48*(3), R45–R53. https://doi.org/10.1530/jme-12-0008.
443. Uroda, T.; Anastasakou, E.; Rossi, A.; Teulon, J.; Pellequer, J.; Annibale, P.; Pessey, O.; Inga, A.; Chillón, I.; Marcia, M. Conserved Pseudoknots in lncRNA MEG3 Are Essential for Stimulation of the p53 Pathway. *Molecular Cell*. **2019**, *75*(5), 982-995.e9. https://doi.org/10.1016/j.molcel.2019.07.025.
444. Sweta, S.; Dudnakova, T.; Sudheer, S.; Baker, A. H.; Bhushan, R. Importance of long non-coding RNAs in the development and disease of skeletal muscle and cardiovascular lineages. *Frontiers in Cell and Developmental Biology*. **2019**, *7*. https://doi.org/10.3389/fcell.2019.00228.
445. Hobuß, L.; Bär, C.; Thum, T. Long non-coding RNAs: at the heart of cardiac dysfunction? *Frontiers in Physiology*. **2019**, *10*. https://doi.org/10.3389/fphys.2019.00030.
446. Sun, L.; Lin, J. D. Function and mechanism of long noncoding RNAs in adipocyte biology. *Diabetes*. **2019**, *68*(5), 887–896. https://doi.org/10.2337/dbi18-0009.
447. Chen, Y. G.; Satpathy, A. T.; Chang, H. Y. Gene regulation in the immune system by long noncoding RNAs. *Nature Immunology*. **2017**, *18*(9), 962–972. https://doi.org/10.1038/ni.3771.
448. Briggs, J. A.; Wolvetang, E. J.; Mattick, J. S.; Rinn, J. L.; Barry, G. Mechanisms of long non-coding RNAs in mammalian nervous system development, plasticity, disease, and evolution. *Neuron*. **2015**, *88*(5), 861–877. https://doi.org/10.1016/j.neuron.2015.09.045.
449. Sauvageau, M.; Goff, L. A.; Lodato, S.; Bonev, B.; Groff, A. F.; Gerhardinger, C.; Sanchez-Gomez, D. B.; Hacisuleyman, E.; Li, E., Spence, M.; Liapis, S. C.; Mallard, W.; Morse, M.; Swerdel, M. R.; D'Ecclessis, M. F.; Moore, J. C.; Lai, V.; Gong, G.; Yancopoulos, G. D.; Rinn, J. L. Multiple knockout mouse models reveal lincRNAs are required for life and brain development. *eLife*. **2013**, *2*. https://doi.org/10.7554/elife.01749.
450. Wei, C.; Luo, T.; Zou, S.; Wu, A. The role of long noncoding RNAs in central nervous system and neurodegenerative diseases. *Frontiers in Behavioral Neuroscience*. **2018**, *12*. https://doi.org/10.3389/fnbeh.2018.00175.

451. Perry, R. B.; Hezroni, H.; Goldrich, M. J.; Ulitsky, I. Regulation of neuroregeneration by long noncoding RNAs. *Molecular Cell*. **2018**, *72*(3), 553-567.e5. https://doi.org/10.1016/j.molcel.2018.09.021.
452. Faghihi, M. A.; Zhang, M.; Huang, J., Modarresi, F.; Van Der Brug, M. P.; Nalls, M. A., Cookson, M. R.; St-Laurent, G.; Wahlestedt, C. Evidence for natural antisense transcript-mediated inhibition of microRNA function. *Genome Biology*. **2010**, *11*(5). https://doi.org/10.1186/gb-2010-11-5-r56.
453. Luo, M.; Jeong, M.; Sun, D.; Park, H. J.; Rodriguez, B. A.; Xia, Z.; Yang, L.; Zhang, X.; Sheng, K.; Darlington, G. J.; Li, W.; Goodell, M. A. Long Non-Coding RNAs control hematopoietic stem cell function. *Cell Stem Cell*. **2015**, *16*(4), 426–438. https://doi.org/10.1016/j.stem.2015.02.002.
454. Wang, K. C.; Yang, Y. W.; Liu, B.; Sanyal, A.; Corces-Zimmerman, R.; Chen, Y.; Lajoie, B. R.; Protacio, A.; Flynn, R. A.; Gupta, R. A.; Wysocka, J.; Lei, M.; Dekker, J.; Helms, J. A.; Chang, H. Y. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature*. **2011**, *472*(7341), 120–124. https://doi.org/10.1038/nature09819.
455. Hadjicharalambous, N.; Lindsay, N. Long Non-Coding RNAs and the innate immune response. *Non-Coding RNA*. **2019**, *5*(2), 34. https://doi.org/10.3390/ncrna5020034.
456. Peng, X.; Gralinski, L.; Armour, C. D.; Ferris, M. T.; Thomas, M. J.; Proll, S.; Bradel-Tretheway, B. G.; Korth, M. J.; Castle, J. C.; Biery, M. C.; Bouzek, H. K.; Haynor, D. R.; Frieman, M. B.; Heise, M. T.; Raymond, C. K.; Baric, R. S.; Katze, M. G. Unique signatures of long noncoding RNA expression in response to virus infection and altered innate immune signaling. *mBio*. **2010**, *1*(5). https://doi.org/10.1128/mbio.00206-10.
457. Kambara, H.; Niazi, F.; Kostadinova, L.; Moonka, D. K.; Siegel, C. T.; Post, A. B.; Carnero, E.; Barriocanal, M.; Fortes, P.; Anthony, D. D.; Valadkhan, S. Negative regulation of the interferon response by an interferon-induced long non-coding RNA. *Nucleic Acids Research*. **2014**, *42*(16), 10668–10680. https://doi.org/10.1093/nar/gku713.
458. Lee, J.; Mendell, J. T. Antisense-Mediated Transcript knockdown triggers premature transcription termination. *Molecular Cell*. **2020**, *77*(5), 1044-1054.e3. https://doi.org/10.1016/j.molcel.2019.12.011.
459. Lai, F.; Damle, S. S.; Ling, K. K.; Rigo, F. Directed RNASE H Cleavage of nascent transcripts causes transcription termination. *Molecular Cell*. **2020**, *77*(5), 1032-1043.e4. https://doi.org/10.1016/j.molcel.2019.12.029.
460. Dhuri, K.; Bechtold, C.; Quijano, E.; Pham, H.; Gupta, A.; Vikram, A.; Bahal, R. Antisense oligonucleotides: an emerging area in drug discovery and development. *Journal of Clinical Medicine*. **2020**, *9*(6), 2004. https://doi.org/10.3390/jcm9062004.
461. Warner, K. D.; Hajdin, C. E.; Weeks, K. M. Principles for targeting RNA with drug-like small molecules. *Nature Reviews Drug Discovery*. **2018**, *17*(8), 547–558. https://doi.org/10.1038/nrd.2018.93.
462. Perez-Pinera, P.; Kocak, D. D.; Vockley, C. M.; Adler, A. F.; Kabadi, A. M.; Polstein, L. R.; Thakore, P. I.; Glass, K. A.; Ousterout, D. G.; Leong, K. W.; Guilak, F.; Crawford, G. E.; Reddy, T. E.; Gersbach, C. A. RNA-guided gene activation by CRISPR-Cas9–based transcription factors. *Nature Methods*. **2013**, *10*(10), 973–976. https://doi.org/10.1038/nmeth.2600.
463. Abudayyeh, O. O.; Gootenberg, J. S.; Essletzbichler, P.; Han, S.; Joung, J.; Belanto, J. J.; Verdine, V.; Cox, D. B. T.; Kellner, M. J.; Regev, A.; Lander, E. S.; Voytas, D. F.; Ting, A. Y.; Zhang, F. RNA targeting with CRISPR–Cas13. *Nature*. **2017**, *550*(7675), 280–284. https://doi.org/10.1038/nature24049.

464. Walter, N. G. Are non-protein coding RNAs junk or treasure? *BioEssays*. **2024**, *46*(4). https://doi.org/10.1002/bies.202300201.
465. Uszczynska-Ratajczak, B.; Lagarde, J.; Frankish, A.; Guigó, R.; Johnson, R. Towards a complete map of the human long non-coding RNA transcriptome. *Nature Reviews Genetics*. **2018**, *19*(9), 535–548. https://doi.org/10.1038/s41576-018-0017-y.
466. Fang, S.; Zhang, L.; Guo, J.; Niu, Y.; Wu, Y.; Li, H.; Zhao, L.; Li, X.; Teng, X.; Sun, X.; Sun, L.; Zhang, M. Q.; Chen, R.; Zhao, Y. NONCODEV5: a comprehensive annotation database for long non-coding RNAs. *Nucleic Acids Research*. **2017**, *46*(D1), D308–D314. https://doi.org/10.1093/nar/gkx1107.
467. Mas-Ponte, D.; Carlevaro-Fita, J.; Palumbo, E.; Pulido, T. H.; Guigo, R.; Johnson, R. LncATLAS database for subcellular localization of long noncoding RNAs. *RNA*. **2017**, *23*(7), 1080–1087. https://doi.org/10.1261/rna.060814.117.
468. Ma, L.; Cao, J.; Liu, L.; Du, Q.; Li, Z.; Zou, D.; Bajic, V. B.; Zhang, Z. LncBook: a curated knowledgebase of human long non-coding RNAs. *Nucleic Acids Research*. **2018**, *47*(D1), D128–D134. https://doi.org/10.1093/nar/gky960.
469. Seifuddin, F.; Singh, K.; Suresh, A.; Judy, J. T.; Chen, Y.; Chaitankar, V.; Tunc, I.; Ruan, X.; Li, P.; Chen, Y.; Cao, H.; Lee, R. S.; Goes, F. S.; Zandi, P. P.; Jafri, M. S.; Pirooznia, M. lncRNAKB, a knowledgebase of tissue-specific functional annotation and trait association of long noncoding RNA. *Scientific Data*. **2020**, *7*(1). https://doi.org/10.1038/s41597-020-00659-z.
470. Kopp, F.; Mendell, J. T. Functional classification and experimental dissection of long noncoding RNAs. *Cell*. **2018**, *172*(3), 393–407. https://doi.org/10.1016/j.cell.2018.01.011.
471. Wu, P.; Mo, Y.; Peng, M.; Tang, T.; Zhong, Y.; Deng, X.; Xiong, F.; Guo, C.; Wu, X.; Li, Y.; Li, X.; Li, G.; Zeng, Z.; Xiong, W. Emerging role of tumor-related functional peptides encoded by lncRNA and circRNA. *Molecular Cancer*. **2020**, *19*(1). https://doi.org/10.1186/s12943-020-1147-3.
472. Wright, B. W.;Yi, Z.; Weissman, J. S.; Chen, J. The dark proteome: translation from noncanonical open reading frames. *Trends in Cell Biology*. **2022**, *32*(3), 243–258. https://doi.org/10.1016/j.tcb.2021.10.010.
473. Gonzàlez-Porta, M.; Frankish, A.; Rung, J.; Harrow, J.; Brazma, A. Transcriptome analysis of human tissues and cell lines reveals one dominant transcript per gene. *Genome Biology*. **2013**, *14*(7), R70. https://doi.org/10.1186/gb-2013-14-7-r70.
474. Williamson, L.; Saponaro, M.; Boeing, S.; East, P.; Mitter, R.; Kantidakis, T.; Kelly, G. P.; Lobley, A.; Walker, J.; Spencer-Dene, B.; Howell, M.; Stewart, A.; Svejstrup, J. Q. UV Irradiation Induces a Non-coding RNA that Functionally Opposes the Protein Encoded by the Same Gene. *Cell*. **2017**, *168*(5), 843-855.e13. https://doi.org/10.1016/j.cell.2017.01.019.
475. Patop, I. L.; Wüst, S.; Kadener, S. Past, present, and future of circ RNA s. *The EMBO Journal*. **2019**, *38*(16). https://doi.org/10.15252/embj.2018100836.
476. Fueyo, R., Judd, J.; Feschotte, C.; Wysocka, J. Roles of transposable elements in the regulation of mammalian transcription. *Nature Reviews Molecular Cell Biology*. **2022**, *23*(7), 481–497. https://doi.org/10.1038/s41580-022-00457-y.
477. Sanbonmatsu, K. Getting to the bottom of lncRNA mechanism: structure–function relationships. *Mammalian Genome*. **2021**, *33*(2), 343–353. https://doi.org/10.1007/s00335-021-09924-x.
478. Cao, H.; Kapranov, P. Methods to Analyze the Non-Coding RNA Interactome – Recent Advances and Challenges. *Frontiers in Genetics*. **2022**, *13*. https://doi.org/10.3389/fgene.2022.857759.

479. Mattick, J. S.; Amaral, P. P.; Carninci, P.; Carpenter, S.; Chang, H. Y.; Chen, L.; Chen, R.; Dean, C.; Dinger, M. E.; Fitzgerald, K. A.; Gingeras, T. R.; Guttman, M.; Hirose, T.; Huarte, M.; Johnson, R.; Kanduri, C.; Kapranov, P.; Lawrence, J. B.; Lee, J. T.; Wu, M. Long non-coding RNAs: definitions, functions, challenges and recommendations. *Nature Reviews Molecular Cell Biology*. **2023**, *24*(6), 430–447. https://doi.org/10.1038/s41580-022-00566-8.
480. Rinn, J. L.; Kertesz, M.; Wang, J. K.; Squazzo, S. L.; Xu, X.; Brugmann, S. A.; Goodnough, L. H.; Helms, J. A.; Farnham, P. J.; Segal, E.; Chang, H. Y. Functional demarcation of active and silent chromatin domains in human HOX LOCI by noncoding RNAs. *Cell*. **2007**, *129*(7), 1311–1323. https://doi.org/10.1016/j.cell.2007.05.022.
481. He, S.; Liu, S.; Zhu, H. The sequence, structure and evolutionary features of HOTAIR in mammals. *BMC Evolutionary Biology*. **2011**, *11*(1). https://doi.org/10.1186/1471-2148-11-102.
482. Davidovich, C.; Zheng, L.; Goodrich, K. J.; Cech, T. R. Promiscuous RNA binding by Polycomb repressive complex 2. *Nature Structural & Molecular Biology*. **2013**, *20*(11), 1250–1257. https://doi.org/10.1038/nsmb.2679.
483. Gupta, R. A.; Shah, N.; Wang, K. C.; Kim, J.; Horlings, H. M.; Wong, D. J.; Tsai, M.; Hung, T.; Argani, P.; Rinn, J. L.; Wang, Y.; Brzoska, P.; Kong, B.; Li, R.; West, R. B.; Van De Vijver, M. J.; Sukumar, S.; Chang, H. Y. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature*. **2010**, *464*(7291), 1071–1076. https://doi.org/10.1038/nature08975.
484. Wu, H.; Zeng, H.; Dong, A.; Li, F.; He, H.; Senisterra, G.; Seitova, A.; Duan, S.; Brown, P. J.; Vedadi, M.; Arrowsmith, C. H.; Schapira, M. Structure of the catalytic domain of EZH2 reveals conformational plasticity in cofactor and substrate binding sites and explains oncogenic mutations. *PLoS ONE*. **2013**, *8*(12), e83737. https://doi.org/10.1371/journal.pone.0083737.
485. Herz, H. M.; Shilatifard, A. The JARID2–PRC2 duality: Figure 1. *Genes & Development*. **2010**, *24*(9), 857–861. https://doi.org/10.1101/gad.1921610.
486. Pasini, D.; Cloos, P. A. C.; Walfridsson, J.; Olsson, L.; Bukowski, J. P.; Johansen, J. V.; Bak, M.; Tommerup, N.; Rappsilber, J.; Helin, K. JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. *Nature*. **2010**, *464*(7286), 306–310. https://doi.org/10.1038/nature08788.
487. Cifuentes-Rojas, C.; Hernandez, A. J.; Sarma, K.; Lee, J. T. Regulatory Interactions between RNA and Polycomb Repressive Complex 2. *Molecular Cell*. **2014**, *55*(2), 171–185. https://doi.org/10.1016/j.molcel.2014.05.009.
488. Tsai, M.; Manor, O.; Wan, Y.; Mosammaparast, N.; Wang, J. K.; Lan, F.; Shi, Y.; Segal, E.; Chang, H. Y. Long noncoding RNA as modular scaffold of Histone modification complexes. *Science*. **2010**, *329*(5992), 689–693. https://doi.org/10.1126/science.1192002.
489. Li, L.; Liu, B.; Wapinski, O. L.; Tsai, M.; Qu, K.; Zhang, J.; Carlson, J. C.; Lin, M.; Fang, F.; Gupta, R. A.; Helms, J. A.; Chang, H. Y. Targeted disruption of Hotair leads to homeotic transformation and gene derepression. *Cell Reports*. **2013**, *5*(1), 3–12. https://doi.org/10.1016/j.celrep.2013.09.003.
490. Lu, L.; Zhu, G.; Zhang, C.; Deng, Q.; Katsaros, D.; Mayne, S. T.; Risch, H. A.; Mu, L.; Canuto, E. M.; Gregori, G.; Benedetto, C.; Yu, H. Association of large noncoding RNA HOTAIR expression and its downstream intergenic CpG island methylation with survival in breast cancer. *Breast Cancer Research and Treatment*. **2012**, *136*(3), 875–883. https://doi.org/10.1007/s10549-012-2314-z.

491. Chiyomaru, T.; Fukuhara, S.; Saini, S.; Majid, S.; Deng, G.; Shahryari, V.; Chang, I.; Tanaka, Y.; Enokida, H.; Nakagawa, M.; Dahiya, R.; Yamamura, S. Long non-coding RNA HOTAIR is targeted and regulated by MIR-141 in human cancer cells. *Journal of Biological Chemistry*. **2014**, *289*(18), 12550–12565. https://doi.org/10.1074/jbc.m113.488593.
492. Yang, G.; Zhang, S.; Gao, F.; Liu, Z.; Lu, M.; Peng, S.; Zhang, T.; Zhang, F. Osteopontin enhances the expression of HOTAIR in cancer cells via IRF1. *Biochimica Et Biophysica Acta (BBA) – Gene Regulatory Mechanisms*. **2014**, *1839*(9), 837–848. https://doi.org/10.1016/j.bbagrm.2014.06.020.
493. Ma, M.; Li, C.; Zhang, Y.; Weng, M.; Zhang, M.; Qin, Y.; Gong, W.; Quan, Z. Long non-coding RNA HOTAIR, a c-Myc activated driver of malignancy, negatively regulates miRNA-130a in gallbladder cancer. *Molecular Cancer*. **2014**, *13*(1). https://doi.org/10.1186/1476-4598-13-156.
494. Bhan, A.; Hussain, I.; Ansari, K. I.; Bobzean, S. A.; Perrotti, L. I.; Mandal, S. S. Bisphenol-A and diethylstilbestrol exposure induces the expression of breast cancer associated long noncoding RNA HOTAIR in vitro and in vivo. *The Journal of Steroid Biochemistry and Molecular Biology*. **2014**, *141*, 160–170. https://doi.org/10.1016/j.jsbmb.2014.02.002.
495. Zhao, W.; Liang, Y. A. Y.; Xie. X. W. Role of HOTAIR long noncoding RNA in metastatic progression of lung cancer. *PubMed*. **2014**, *18*(13), 1930–1936. https://pubmed.ncbi.nlm.nih.gov/25010625.
496. Nakagawa, T.; Endo, H.; Yokoyama, M.; Abe, J.; Tamai, K.; Tanaka, N.; Sato, I.; Takahashi, S.; Kondo, T.; Satoh, K. Large noncoding RNA HOTAIR enhances aggressive biological behavior and is associated with short disease-free survival in human non-small cell lung cancer. *Biochemical and Biophysical Research Communications*. **2013**, *436*(2), 319–324. https://doi.org/10.1016/j.bbrc.2013.05.101.
497. Hajjari, M.; Salavaty, A. HOTAIR: an oncogenic long non-coding RNA in different cancers. *DOAJ (DOAJ: Directory of Open Access Journals)*. **2015**, *12*(1), 1–9. https://doi.org/10.7497/j.issn.2095-3941.2015.0006.
498. Cantile, M.; Di Bonito, M.; De Bellis, M. T.; Botti, G. Functional Interaction among lncRNA HOTAIR and MicroRNAs in Cancer and Other Human Diseases. *Cancers*. **2021**, *13*(3), 570. https://doi.org/10.3390/cancers13030570.
499. Chen, L.; Qian, X.; Wang, Z.; Zhou, X. The HOTAIR lncRNA: A remarkable oncogenic promoter in human cancer metastasis (Review). *Oncology Letters*. **2021**, *21*(4). https://doi.org/10.3892/ol.2021.12563.
500. Bhan, A.; Mandal, S. S. LncRNA HOTAIR: A master regulator of chromatin dynamics and cancer. *Biochimica Et Biophysica Acta (BBA) - Reviews on Cancer*. **2015**, *1856*(1), 151–164. https://doi.org/10.1016/j.bbcan.2015.07.001.
501. Cai, B.; Wu, Z.; Liao, K.; Zhang, S. Long noncoding RNA HOTAIR can serve as a common molecular marker for lymph node metastasis: a meta-analysis. *Tumor Biology*. **2014**, *35*(9), 8445–8450. https://doi.org/10.1007/s13277-014-2311-4.
502. Arshi, A.; Raeisi, F.; Mahmoudi, E.; Mohajerani, F.; Kabiri, H.; Fazel, R.; Zabihian-Langeroudi, M.; Jusic, A. A comparative study of HOTAIR expression in breast cancer patient tissues and cell lines. *PubMed*. **2020**, *22*(2), 178–184. https://doi.org/10.22074/cellj.2020.6543.
503. Raju, G. S. R.; Pavitra, E.; Bandaru, S. S.; Varaprasad, G. L.; Nagaraju, G. P.; Malla, R. R.; Huh, Y. S.; Han, Y. HOTAIR: a potential metastatic, drug-resistant and prognostic regulator of breast cancer. *Molecular Cancer*. **2023**, *22*(1). https://doi.org/10.1186/s12943-023-01765-3.

504. Yang, J.; Xu, S.; Wang, S.; Zou, X.; Duan, M.; Zhang, Q.; Wang, C. HOTAIR as a diagnostic and prognostic biomarker of gastrointestinal cancers: an updated meta-analysis and bioinformatics analysis based on TCGA data. *Bioscience Reports*. **2023**, *43*(3). https://doi.org/10.1042/bsr20222174.
505. Suwardjo, S.; Permana, K. G.; Aryandono, T.; Heriyanto, D.; Anwar, S. Long-Noncoding-RNA HOTAIR Upregulation is Associated with Poor Breast Cancer Outcome: A Systematic Review and Meta Analysis. *Asian Pacific Journal of Cancer Prevention*. **2024**, *25*(4), 1169–1182. https://doi.org/10.31557/apjcp.2024.25.4.1169.
506. Tang, Q.; Hann, S. S. HOTAIR: An oncogenic long Non-Coding RNA in human cancer. *Cellular Physiology and Biochemistry*. **2018**, *47*(3), 893–913. https://doi.org/10.1159/000490131.
507. Zhang, Q.; Huang, X.; Liao, J.; Dong, Y.; Zhu, J.; He, C.; Huang, J.; Tang, Y.; Wu, D.; Tian, J. LNCRNA HOTAIR promotes neuronal damage through facilitating NLRP3 Mediated-Pyroptosis activation in Parkinson's disease via regulation of MIR-326/ELAVL1 axis. *Cellular and Molecular Neurobiology*. **2020**, *41*(8), 1773–1786. https://doi.org/10.1007/s10571-020-00946-8.
508. Elhai, M.; Micheroli, R.; Houtman, M.; Mirrahimi, M.; Moser, L.; Pauli, C.; Bürki, K.; Laimbacher, A.; Kania, G.; Klein, K.; Schätzle, P.; Bertoncelj, M. F.; Edalat, S. G.; Keusch, L.; Khmelevskaya, A.; Toitou, M.; Geiss, C.; Rauer, T.; Sakkou, M., Ospelt, C. The long non-coding RNA HOTAIR contributes to joint-specific gene expression in rheumatoid arthritis. *Nature Communications*. **2023**, *14*(1). https://doi.org/10.1038/s41467-023-44053-w.
509. Ni, S.; Xu, W.; Liao, G.; Wang, Y.; Li, J. LNCRNA HOTAIR promotes LPS-Induced inflammation and apoptosis of cardiomyocytes via LIN28-Mediated PDCD4 stability. *Inflammation*. **2021**, *44*(4), 1452–1463. https://doi.org/10.1007/s10753-021-01431-0.
510. Skrypek, N.; Goossens, S.; De Smedt, E.; Vandamme, N.; Berx, G. Epithelial-to-Mesenchymal transition: Epigenetic reprogramming driving cellular plasticity. *Trends in Genetics*. **2017**, *33*(12), 943–959. https://doi.org/10.1016/j.tig.2017.08.004.
511. Nieto, M. A. The ins and outs of the epithelial to mesenchymal transition in health and disease. *Annual Review of Cell and Developmental Biology*. **2011**, *27*(1), 347–376. https://doi.org/10.1146/annurev-cellbio-092910-154036.
512. Syed, V. TGF-B signaling in cancer. *Journal of Cellular Biochemistry*. **2016**, *117*(6), 1279–1287. https://doi.org/10.1002/jcb.25496.
513. Peinado, H.; Olmeda, D.; Cano, A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nature Reviews. Cancer*. **2007**, *7*(6), 415–428. https://doi.org/10.1038/nrc2131.
514. Mani, S. A.; Yang, J.; Brooks, M.; Schwaninger, G.; Zhou, A.; Miura, N.; Kutok, J. L.; Hartwell, K.; Richardson, A. L.; Weinberg, R. A. Mesenchyme Forkhead 1 (FOXC2) plays a key role in metastasis and is associated with aggressive basal-like breast cancers. *Proceedings of the National Academy of Sciences*. **2007**, *104*(24), 10069–10074. https://doi.org/10.1073/pnas.0703900104.
515. David, C. J.; Huang, Y.; Chen, M.; Su, J.; Zou, Y.; Bardeesy, N.; Iacobuzio-Donahue, C. A.; Massagué, J. TGF-β Tumor Suppression through a Lethal EMT. *Cell*. **2016**, *164*(5), 1015–1030. https://doi.org/10.1016/j.cell.2016.01.009.
516. Wong, S. H. M.; Fang, C. M.; Chuah, L.,; Leong, C. O.; Ngai, S. C. E-cadherin: Its dysregulation in carcinogenesis and clinical implications. *Critical Reviews in Oncology/Hematology*. **2018**, *121*, 11–22. https://doi.org/10.1016/j.critrevonc.2017.11.010.

517. Hao, Y.; Baker, D.; Dijke, P. T. TGF-B-Mediated Epithelial-Mesenchymal Transition and Cancer metastasis. *International Journal of Molecular Sciences*. **2019**, *20*(11), 2767. https://doi.org/10.3390/ijms20112767.
518. Wu, K.; Xu, K.; Liu, K.; Huang, J.; Chen, J.; Zhang, J.; Zhang, N. Long noncoding RNA BC200 regulates cell growth and invasion in colon cancer. *The International Journal of Biochemistry & Cell Biology*. **2018**, *99*, 219–225. https://doi.org/10.1016/j.biocel.2018.04.001.
519. Dasgupta, P.; Kulkarni, P.; Majid, S.; Shahryari, V.; Hashimoto, Y.; Bhat, N. S.; Shiina, M.; Deng, G.; Saini, S.; Tabatabai, Z. L.; Yamamura, S.; Tanaka, Y.; Dahiya, R. MicroRNA-203 Inhibits Long Noncoding RNA HOTAIR and Regulates Tumorigenesis through Epithelial-to-mesenchymal Transition Pathway in Renal Cell Carcinoma. *Molecular Cancer Therapeutics*. **2018**, *17*(5), 1061–1069. https://doi.org/10.1158/1535-7163.mct-17-0925.
520. Hong, Q.; Li, O.; Zheng, W.; Xiao, W.; Zhang, L.; Wu, D.; Cai, G.; He, J. C.; Chen, X. LncRNA HOTAIR regulates HIF-1α/AXL signaling through inhibition of miR-217 in renal cell carcinoma. *Cell Death and Disease*. **2017**, *8*(5), e2772. https://doi.org/10.1038/cddis.2017.181.
521. Chen, X.; Wang, S.; Jiang, X.; Zhang, M.; Ding, Y. Long non-coding RNA HOTAIR: from pan-cancer analysis to colorectal cancer-related uridine metabolism. *Aging*. **2024**, *1;16(9):*7752-7773. https://doi.org/10.18632/aging.205781.
522. Zhang, H.; Cai, K.; Wang, J.; Wang, X.; Cheng, K.; Shi, F.; Jiang, L.; Zhang, Y.; Dou, J. MIR-7, inhibited indirectly by LinCRNA HOTAIR, directly inhibits SETDB1 and reverses the EMT of breast cancer stem cells by downregulating the STAT3 pathway. *Stem Cells*. **2014**, *32*(11), 2858–2868. https://doi.org/10.1002/stem.1795.
523. Gong, X.; Zhu, Z. Retracted: Long noncoding RNA HOTAIR contributes to progression in hepatocellular carcinoma by sponging MIR-217-5P. *Cancer Biotherapy and Radiopharmaceuticals*. **2020**, *35*(5), 387–396. https://doi.org/10.1089/cbr.2019.3070.
524. Wang, Y.; Gong, G.; Xu, J.; Zhang, Y.; Wu, S.; Wang, S. Long noncoding RNA HOTAIR promotes breast cancer development by targeting ZEB1 via sponging miR-601. *Cancer Cell International*. **2020**, *20*, 320. https://doi.org/10.1186/s12935-020-01410-9.
525. Wang, A.; Tan, P.; Zhuang, Y.; Zhang, X.; Yu, Z.; Li, L. Down-regulation of long non-coding RNA HOTAIR inhibits invasion and migration of oesophageal cancer cells via up-regulation of microRNA-204. *Journal of Cellular and Molecular Medicine*. **2019**, *23*(10), 6595–6610. https://doi.org/10.1111/jcmm.14502.
526. Wu, Y.; Zhang, L.; Zhang, L.; Wang, Y.; Li, H.; Ren, X.; Wei, F.; Yu, W.; Liu, T.; Wang, X.; Zhou, X.; Yu, J.; Hao, X. Long non-coding RNA HOTAIR promotes tumor cell invasion and metastasis by recruiting EZH2 and repressing E-cadherin in oral squamous cell carcinoma. *International Journal of Oncology*. **2015**, *46*(6), 2586–2594. https://doi.org/10.3892/ijo.2015.2976.
527. Ren, Y.; Jia, H.; Xu, Y.; Zhou, X.; Zhao, X.; Wang, Y.; Song, X.; Zhu, Z.; Sun, T.; Dou, Y.; Tian, W.; Zhao, X.; Kang, C.; Mei, M. Paracrine and epigenetic control of CAF-induced metastasis: the role of HOTAIR stimulated by TGF-ß1 secretion. *Molecular Cancer*. **2018**, *17*(1). https://doi.org/10.1186/s12943-018-0758-4.
528. Song, Y.; Wang, R.; Li, L.; Liu, X.; Wang, Y.; Wang, Q.; Zhang, Q. Long non-coding RNA HOTAIR mediates the switching of histone H3 lysine 27 acetylation to methylation to promote epithelial-to-mesenchymal transition in gastric cancer. *International Journal of Oncology*. **2018**, Jan; 54(1): 77-86. https://doi.org/10.3892/ijo.2018.4625.

529. Liu, M.; Zhang, H.; Li, Y.; Wang, R.; Li, Y.; Zhang, H.; Ren, D.; Liu, H.; Kang, C.; Chen, J. HOTAIR, a long noncoding RNA, is a marker of abnormal cell cycle regulation in lung cancer. *Cancer Science*. **2018**, *109*(9), 2717–2733. https://doi.org/10.1111/cas.13745.
530. Kim, H.; Yim, G.; Baek, S.; Kim, J.; Kim, Y. Long noncoding RNA HOTAIR is associated with human cervical cancer progression. *Gynecologic Oncology*. **2014**, *133*, 5. https://doi.org/10.1016/j.ygyno.2014.03.027.
531. Berrondo, C.; Flax, J.; Kucherov, V.; Siebert, A.; Osinski, T.; Rosenberg, A.; Fucile, C.; Richheimer, S.; Beckham, C. J. Expression of the Long Non-Coding RNA HOTAIR Correlates with Disease Progression in Bladder Cancer and Is Contained in Bladder Cancer Patient Urinary Exosomes. *PLoS ONE*. **2016**, *11*(1), e0147236. https://doi.org/10.1371/journal.pone.0147236.
532. Noorolyai, S.; Shajari, N.; Baghbani, E.; Sadreddini, S.; Baradaran, B. The relation between PI3K/AKT signalling pathway and cancer. *Gene*. **2019**, *698*, 120–128. https://doi.org/10.1016/j.gene.2019.02.076.
533. Petrulea, M. S.; Plantinga, T. S.; Smit, J. W.; Georgescu, C. E.; Netea-Maier, R. T. PI3K/Akt/mTOR: A promising therapeutic target for non-medullary thyroid carcinoma. *Cancer Treatment Reviews*. **2015**, *41*(8), 707–713. https://doi.org/10.1016/j.ctrv.2015.06.005.
534. Chen, J.; Lin, C.; Yong, W., Ye, Y.; Huang, Z. Calycosin and genistein induce apoptosis by inactivation of HOTAIR/P-AKT signaling pathway in human breast cancer MCF-7 cells. *Cellular Physiology and Biochemistry*. **2015**, *35*(2), 722–728. https://doi.org/10.1159/000369732.
535. Hales, E. C.; Taub, J. W.; Matherly, L. H. New insights into Notch1 regulation of the PI3K–AKT–mTOR1 signaling axis: Targeted therapy of γ-secretase inhibitor resistant T-cell acute lymphoblastic leukemia. *Cellular Signalling*. **2014**, *26*(1), 149–161. https://doi.org/10.1016/j.cellsig.2013.09.021.
536. Cheng, C.; Qin, Y.; Zhi, Q.; Wang, J.; Qin, C. Knockdown of long non-coding RNA HOTAIR inhibits cisplatin resistance of gastric cancer cells through inhibiting the PI3K/Akt and Wnt/β-catenin signaling pathways by up-regulating miR-34a. *International Journal of Biological Macromolecules*. **2018**, *107*, 2620–2629. https://doi.org/10.1016/j.ijbiomac.2017.10.154.
537. Li, E.; Zhao, Z.; Ma, B.; Zhang, J. Long noncoding RNA HOTAIR promotes the proliferation and metastasis of osteosarcoma cells through the AKT/mTOR signaling pathway. *Experimental and Therapeutic Medicine*. **2017**, *14, 5321-5328*. https://doi.org/10.3892/etm.2017.5248.
538. Li, Z.; Qian, J.; Li, J.; Zhu, C. Knockdown of lncRNA-HOTAIR downregulates the drug-resistance of breast cancer cells to doxorubicin via the PI3K/AKT/mTOR signaling pathway. *Experimental and Therapeutic Medicine*. **2019**, *18(1):435-442*. https://doi.org/10.3892/etm.2019.7629.
539. Pan, S.; Liu, Y.; Liu, Q.; Xiao, Y.; Liu, B.; Ren, X.; Qi, X.; Zhou, H.; Zeng, C.; Jia, L. HOTAIR/miR-326/FUT6 axis facilitates colorectal cancer progression through regulating fucosylation of CD44 via PI3K/AKT/mTOR pathway. *Biochimica Et Biophysica Acta (BBA) – Molecular Cell Research*. **2019**, *1866*(5), 750–760. https://doi.org/10.1016/j.bbamcr.2019.02.004.
540. Luan, W.; Li, R.; Liu, L.; Ni, X.; Shi, Y.; Xia, Y.; Wang, J.; Lu, F.; Xu, B. Long non-coding RNA HOTAIR acts as a competing endogenous RNA to promote malignant melanoma progression by sponging miR-152-3p. *Oncotarget*. **2017**, *8*(49), 85401–85414. https://doi.org/10.18632/oncotarget.19910.

541. Li, Z.; Luo, J. Epigenetic regulation of HOTAIR in advanced chronic myeloid leukemia. *Cancer Management and Research*. **2018**, *Volume 10*, 5349–5362. https://doi.org/10.2147/cmar.s166859.
542. Li, T.; Qin, Y.; Zhen, Z.; Shen, H.; Cong, T.; Schiferle, E.; Xiao, S. Long non-coding RNA HOTAIR/microRNA-206 sponge regulates STC2 and further influences cell biological functions in head and neck squamous cell carcinoma. *Cell Proliferation*. **2019**, *52*(5). https://doi.org/10.1111/cpr.12651.
543. Sadeghalvad, M.; Mansouri, K.; Mohammadi-Motlagh, H.; Noorbakhsh, F.; Mostafaie, A.; Alipour, S.; Rezaei, N. Long non-coding RNA HOTAIR induces the PI3K/AKT/mTOR signaling pathway in breast cancer cells. *Revista Da Associação Médica Brasileira*. **2022**, *68*(4), 456–462. https://doi.org/10.1590/1806-9282.20210966.
544. Itoh, N.; Ornitz, D. M. Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. *The Journal of Biochemistry*. **2010**, *149*(2), 121–130. https://doi.org/10.1093/jb/mvq121.
545. Ke, J.; Yao, Y.; Zheng, J.; Wang, P.; Liu, Y.; Ma, J.; Li, Z.; Liu, X.; Li, Z.; Wang, Z.; Xue, Y. Knockdown of long non-coding RNA HOTAIR inhibits malignant biological behaviors of human glioma cells via modulation of miR-326. *Oncotarget*. **2015**, *6*(26), 21934–21949. https://doi.org/10.18632/oncotarget.4290.
546. Clevers, H.; Nusse, R. WNT/B-Catenin signaling and disease. *Cell*. **2012**, *149*(6), 1192–1205. https://doi.org/10.1016/j.cell.2012.05.012.
547. Ochoa-Hernández, A. B.; Juárez-Vázquez, C. I.; Rosales-Reynoso, M. A.; Barros-Núñez, P. [WNT-β-catenin signaling pathway and its relationship with cancer]. *PubMed*. **2013**, *80*(4), 389–398. https://pubmed.ncbi.nlm.nih.gov/23374390.
548. Li, J.; Tian, H.; Yang, J.; Gong, Z. Long noncoding RNAs regulate cell growth, proliferation, and apoptosis. *DNA And Cell Biology*. **2016**, *35*(9), 459–470. https://doi.org/10.1089/dna.2015.3187.
549. Gao, J.; Li, J.; Du, J.; Li, X. Long non-coding RNA HOTAIR is a marker for hepatocellular carcinoma progression and tumor recurrence. *Oncology Letters*. **2016**, *11*(3), 1791–1798. https://doi.org/10.3892/ol.2016.4130.
550. Salmerón-Bárcenas, E.; Illades-Aguiar, B.; Del Moral-Hernández, O.; Ortega-Soto, A.; Hernández-Sotelo, D. HOTAIR knockdown decreased the activity WNT/B-Catenin signaling pathway and increased the mRNA levels of its negative regulators in HELA cells. *Cellular Physiology and Biochemistry*. **2019**, *53*(6), 948–960. https://doi.org/10.33594/000000188.
551. Liu, Y. W.; Sun, M.; Xia, R.; Zhang, E. B.; Liu, X. H.; Zhang, Z. H.; Xu, T. P.; De, W.; Liu, B. R.; Wang, Z. X. LincHOTAIR epigenetically silences miR34a by binding to PRC2 to promote the epithelial-to-mesenchymal transition in human gastric cancer. *Cell Death and Disease*. **2015**, *6*(7), e1802. https://doi.org/10.1038/cddis.2015.150.
552. Xiao, Z.; Qu, Z.; Chen, Z.; Fang, Z.; Zhou, K.; Huang, Z.; Guo, X.; Zhang, Y. LncRNA HOTAIR is a Prognostic Biomarker for the Proliferation and Chemoresistance of Colorectal Cancer via MiR-203a-3p-Mediated Wnt/ß-Catenin Signaling Pathway. *Cellular Physiology and Biochemistry*. **2018**, *46*(3), 1275–1285. https://doi.org/10.1159/000489110.
553. Ge, X.; Ma, H.; Zheng, X.; Ruan, H.; Liao, X.; Xue, W.; Chen, Y.; Zhang, Y.; Jia, W. HOTAIR, a prognostic factor in esophageal squamous cell carcinoma, inhibits WIF-1 expression and activates Wnt pathway. *Cancer Science*. **2013**, *104*(12), 1675–1682. https://doi.org/10.1111/cas.12296.

554. Olsson, A.; Dimberg, A.; Kreuger, J.; Claesson-Welsh, L. VEGF receptor signalling ? in control of vascular function. *Nature Reviews Molecular Cell Biology*. **2006**, *7*(5), 359–371. https://doi.org/10.1038/nrm1911.
555. Fu, W.; Lu, Y.; Hu, B.; Liang, W.; Zhu, X.; Yang, H.; Li, G.; Zhang, J. Long noncoding RNA hotair mediated angiogenesis in nasopharyngeal carcinoma by direct and indirect signaling pathways. *Oncotarget*. **2015**, *7*(4), 4712–4723. https://doi.org/10.18632/oncotarget.6731.
556. Burger, R. A. Role of vascular endothelial growth factor inhibitors in the treatment of gynecologic malignancies. *Journal of Gynecologic Oncology*. **2010**, *21*(1), 3. https://doi.org/10.3802/jgo.2010.21.1.3.
557. Liu, X.; Liu, Z.; Sun, M.; Liu, J.; Wang, Z.; De, W. The long non-coding RNA HOTAIR indicates a poor prognosis and promotes metastasis in non-small cell lung cancer. *BMC Cancer*. **2013**, *13*(1). https://doi.org/10.1186/1471-2407-13-464.
558. Kim, H. J.; Lee, D. W.; Yim, G. W.; Nam, E. J.; Kim, S.; Kim, S. W.; Kim, Y. T. Long non-coding RNA HOTAIR is associated with human cervical cancer progression. *International Journal of Oncology*. **2014**, *46*(2), 521–530. https://doi.org/10.3892/ijo.2014.2758.
559. Zhao, W.; Yuan, H.; Ren, X.; Huang, K.; Guo, Z. Association between expression of HOTAIR and invasiveness of gliomas, and its predictive value. *Advances in Clinical and Experimental Medicine*. **2019**, *28*(9), 1179–1183. https://doi.org/10.17219/acem/99527.
560. Flavahan, W. A.; Gaskell, E.; Bernstein, B. E. Epigenetic plasticity and the hallmarks of cancer. *Science*. **2017**, *357*(6348). https://doi.org/10.1126/science.aal2380.
561. Ding, C.; Cheng, S.; Yang, Z.; Lv, Z.; Xiao, H.; Du, C.; Peng, C.; Xie, H.; Zhou, L.; Wu, J.; Zheng, S. Long Non-Coding RNA HOTAIR promotes cell migration and invasion via Down-Regulation of RNA binding motif protein 38 in hepatocellular carcinoma cells. *International Journal of Molecular Sciences*. **2014**, *15*(3), 4060–4076. https://doi.org/10.3390/ijms15034060.
562. Fan, Q.; Yang, L.; Zhang, X.; Peng, X.; Wei, S.; Su, D.; Zhai, Z. Hua, X.; Li, H. The emerging role of exosome-derived non-coding RNAs in cancer biology. *Cancer Letters*. **2018**, *414*, 107–115. https://doi.org/10.1016/j.canlet.2017.10.040.
563. Ma, X.; Li, Z.; Li, T.; Zhu, L.; Li, Z.; Tian, N. Long non-coding RNA HOTAIR enhances angiogenesis by induction of VEGFA expression in glioma cells and transmission to endothelial cells via glioma cell derived-extracellular vesicles. *PubMed*. **2017**, *9*(11), 5012–5021. https://pubmed.ncbi.nlm.nih.gov/29218099.
564. Botti, G.; Scognamiglio, G.; Aquino, G.; Liguori, G.; Cantile, M. LNCRNA HOTAIR in tumor microenvironment: What role? *International Journal of Molecular Sciences*. **2019**, *20*(9), 2279. https://doi.org/10.3390/ijms20092279.
565. Wang, Y.; Yi, K.; Liu, X.; Tan, Y.; Jin, W.; Li, Y.; Zhou, J.; Wang, H.; Kang, C. HOTAIR Up-Regulation activates NF-KB to induce immunoescape in gliomas. *Frontiers in Immunology*. **2021**, *12:*785463. https://doi.org/10.3389/fimmu.2021.785463.
566. Yang, H.; Fu, H.; Xu, W.; Zhang, X. Exosomal non-coding RNAs: a promising cancer biomarker. *Clinical Chemistry and Laboratory Medicine (CCLM)*. **2016**, *54*(12), 1871–1879. https://doi.org/10.1515/cclm-2016-0029.
567. Tang, S.; Zheng, K.; Tang, Y.; Li, Z.; Zou, T.; Liu, D. Overexpression of serum exosomal HOTAIR is correlated with poor survival and poor response to chemotherapy in breast cancer patients. *Journal of Biosciences*. **2019**, *44*(2). https://doi.org/10.1007/s12038-019-9861-y.

568. Wang, J.; Zhou, Y.; Lu, J. Combined detection of serum exosomal miR-21 and HOTAIR as diagnostic and prognostic biomarkers for laryngeal squamous cell carcinoma. *Med Oncol*. **2014**, *31, 148*. https://doi.org/10.1007/s12032-014-0148-8.
569. Zhang, J.; Qiu, W.; Zhu, H.; Liu, H.; Sun, J.; Chen, Y.; Shen, H.; Qian, C.; Shen, Z. HOTAIR contributes to the carcinogenesis of gastric cancer via modulating cellular and exosomal miRNAs level. *Cell Death and Disease*. **2020**, *11*(9). https://doi.org/10.1038/s41419-020-02946-4.
570. Yao, Z.; Jia, C.; Tai, Y.; Liang, H.; Zhong, Z.; Xiong, Z.; Deng, M.; Zhang, Q. Serum exosomal long noncoding RNAs lnc-FAM72D-3 and lnc-EPC1-4 as diagnostic biomarkers for hepatocellular carcinoma. *Aging*. **2020**, *12*(12), 11843–11863. https://doi.org/10.18632/aging.103355.
571. Poulet, C.; Njock, M.; Moermans, C.; Louis, E.; Louis, R.; Malaise, M.; Guiot, J. Exosomal Long Non-Coding RNAs in lung diseases. *International Journal of Molecular Sciences*. **2020**, *21*(10), 3580. https://doi.org/10.3390/ijms21103580.
572. Zhu, C.; Wang, X.; Wang, Y.; Wang, K. Functions and underlying mechanisms of lncRNA HOTAIR in cancer chemotherapy resistance. *Cell Death Discovery*. **2022**, *8*(1). https://doi.org/10.1038/s41420-022-01174-3.
573. Peng, L.; Yuan, X.,; Jiang, B.; Tang, Z.; Li, G. LncRNAs: key players and novel insights into cervical cancer. *Tumor Biology*. **2015**, *37*(3), 2779–2788. https://doi.org/10.1007/s13277-015-4663-9.
574. Sharma, S.; Mandal, P.; Sadhukhan, T.; Chowdhury, R. R.; Mondal, N. R.; Chakravarty, B.; Chatterjee, T.; Roy, S.; Sengupta, S. Bridging Links between Long Noncoding RNA HOTAIR and HPV Oncoprotein E7 in Cervical Cancer Pathogenesis. *Scientific Reports*. **2015**, *5*(1). https://doi.org/10.1038/srep11724.
575. Huang, L.; Liao, L.; Liu, A.; Wu, J.; Cheng, X.; Lin, J.; Zheng, M. Overexpression of long noncoding RNA HOTAIR predicts a poor prognosis in patients with cervical cancer. *Archives of Gynecology and Obstetrics*. **2014**, *290*(4), 717–723. https://doi.org/10.1007/s00404-014-3236-2.
576. Liu, M.; Li, N. The diagnostic value of lncRNA HOTAIR for cervical carcinoma in vaginal discharge and serum. *Medicine*. **2023**, *102*(26), e34042. https://doi.org/10.1097/md.0000000000034042.
577. Zheng, P.; Xiong, Q.; Wu, Y.; Chen, Y.; Chen, Z.; Fleming, J.; Gao, D.; Bi, L.; Ge, F. Quantitative Proteomics Analysis Reveals Novel Insights into Mechanisms of Action of Long Noncoding RNA Hox Transcript Antisense Intergenic RNA (HOTAIR) in HeLa Cells*. *Molecular & Cellular Proteomics*. **2015**, *14*(6), 1447–1463. https://doi.org/10.1074/mcp.m114.043984.
578. Shi, L.; Zhang, D.; Han, H.; Zhang, L.; Li, S.; Yang, F.; He, C. HOTAIR knockdown impairs metastasis of cervical cancer cells by down-regulating metastasis-related genes. *Journal of Obstetrics and Gynaecology*. **2023**, *43*(1). https://doi.org/10.1080/01443615.2023.2181060.
579. Lee, M.; Kim, H. J.; Kim, S. W.; Park, S.; Chun, K.; Cho, N. H.; Song, Y. S.; Kim, Y. T. The long non-coding RNA HOTAIR increases tumour growth and invasion in cervical cancer by targeting the Notch pathway. *Oncotarget*. **2016**, *7*(28), 44558–44571. https://doi.org/10.18632/oncotarget.10065.
580. Sun, J.; Chu, H.; Ji, J.; Huo, G.; Song, Q.; Zhang, X. Long non-coding RNA HOTAIR modulates HLA-G expression by absorbing miR-148a in human cervical cancer. *International Journal of Oncology*. **2016**, *49*(3), 943–952. https://doi.org/10.3892/ijo.2016.3589.

581. Ji, F.; Wuerkenbieke, D.; He, Y.; Ding, Y.; Du, R. Long noncoding RNA HOTAIR: an oncogene in human cervical cancer interacting with MicroRNA-17-5P. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics*. **2018**, *26*(3), 353–361. https://doi.org/10.3727/096504017x15002869385155.
582. Buranjiang, G.; Abuduwanke, A.; Li, X.; Abulizi, G. LncRNA HOTAIR enhances RCC2 to accelerate cervical cancer progression by sponging miR-331-3p. *Clinical & Translational Oncology*. **2023**, *25*(6), 1650–1660. https://doi.org/10.1007/s12094-022-03059-4.
583. Liu, M.; Jia, J.; Wang, X.; Liu, Y.; Wang, C.; Fan, R. Long non-coding RNA HOTAIR promotes cervical cancer progression through regulating BCL2 via targeting miR-143-3p. *Cancer Biology & Therapy*. **2018**, *19*(5), 391–399. https://doi.org/10.1080/15384047.2018.1423921.
584. Zhou, Y.; Wang, Y.; Lin, M.; Wu, D.; Zhao, M. LncRNA HOTAIR promotes proliferation and inhibits apoptosis by sponging miR-214-3p in HPV16 positive cervical cancer cells. *Cancer Cell International*. **2021**, *21*(1). https://doi.org/10.1186/s12935-021-02103-7.
585. Zhang, D.; Zhou, X.; Zhang, J.; Zhou, Y.,; Ying, J.; Wu, G.; Qian, J. Propofol promotes cell apoptosis via inhibiting HOTAIR mediated mTOR pathway in cervical cancer. *Biochemical and Biophysical Research Communications*. **2015**, *468*(4), 561–567. https://doi.org/10.1016/j.bbrc.2015.10.129.
586. Zhang, L.; Qian, H.; Sha, M.; Luan, Z.; Lin, M.; Yuan, D.; Li, X.; Huang, J.; Ye, L. Downregulation of HOTAIR expression mediated Anti-Metastatic effect of artesunate on cervical cancer by inhibiting COX-2 expression. *PLoS ONE*. **2016**, *11*(10), e0164838. https://doi.org/10.1371/journal.pone.0164838.
587. Jing, L.; Yuan, W.; Ruofan, D.; Jinjin, Y.; Haifeng, Q. HOTAIR enhanced aggressive biological behaviors and induced radio-resistance via inhibiting p21 in cervical cancer. *Tumor Biology*. **2014**, *36*(5), 3611–3619. https://doi.org/10.1007/s13277-014-2998-2.
588. Luo, J.; Zhu, W.; Tang, Y.; Cao, H.; Zhou, Y.; Ji, R.; Zhou, X.; Lu, Z.; Yang, H.; Zhang, S.; Cao, J. Artemisinin derivative artesunate induces radiosensitivity in cervical cancer cells in vitro and in vivo. *Radiation Oncology*. **2014**, *9*(1). https://doi.org/10.1186/1748-717x-9-84.
589. Zhang, L.; Liu, Z.; Ye, J.; Sha, M.; Qian, H.; Bu, X.; Luan, Z.; Xu, X.; Huang, A.; Yuan, D.; Wu, Y.; Wang, X.; Wang, J.; Huang, J.; Ye, L. Artesunate exerts an anti-immunosuppressive effect on cervical cancer by inhibiting PGE2 production and Foxp3 expression. *Cell Biology International*. **2014**, *38*(5), 639–646. https://doi.org/10.1002/cbin.10244.
590. Li, S.; Xiong, Q.; Chen, M.; Wang, B.; Yang, X.; Yang, M.; Wang, Q.; Cui, Z.; Ge, F. Long noncoding RNA HOTAIR interacts with Y-Box Protein-1 (YBX1) to regulate cell proliferation. *Life Science Alliance*. **2021**, *4*(9), e202101139. https://doi.org/10.26508/lsa.202101139.
591. Li, J.; Wang, Y.; Yu, J.; Dong, R.; Qiu, H. A high level of circulating HOTAIR is associated with progression and poor prognosis of cervical cancer. *Tumor Biology*. **2014**, *36*(3), 1661–1665. https://doi.org/10.1007/s13277-014-2765-4.
592. Ji, P.; Diederichs, S.; Wang, W.; Böing, S.; Metzger, R.; Schneider, P. M.; Tidow, N.; Brandt, B.; Buerger, H.; Bulk, E.; Thomas, M.; Berdel, W. E.; Serve, H.; Müller-Tidow, C. MALAT-1, a novel noncoding RNA, and thymosin β4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene*. **2003**, *22*(39), 8031–8041. https://doi.org/10.1038/sj.onc.1206928.

593. Wilusz, J. E.; Freier, S. M.; Spector, D. L. 3′ End processing of a long Nuclear-Retained noncoding RNA yields a TRNA-like cytoplasmic RNA. *Cell*. **2008**, *135*(5), 919–932. https://doi.org/10.1016/j.cell.2008.10.012.
594. Tripathi, V.; Ellis, J. D.; Shen, Z.; Song, D. Y.; Pan, Q.; Watt, A. T.; Freier, S. M.; Bennett, C. F.; Sharma, A.; Bubulya, P. A.; Blencowe, B. J.; Prasanth, S. G.; Prasanth, K. V. The Nuclear-Retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Molecular Cell*. **2010**, *39*(6), 925–938. https://doi.org/10.1016/j.molcel.2010.
08.011.
595. Hutchinson, J. N.; Ensminger, A. W.; Clemson, C. M.; Lynch, C. R.; Lawrence, J. B.; Chess, A. A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. *BMC Genomics*. **2007**, *8*(1). https://doi.org/10.1186/1471-2164-8-39.
596. Wilusz, J. E.; JnBaptiste, C. K.; Lu, L. Y.; Kuhn, C.; Joshua-Tor, L.; Sharp, P. A. A triple helix stabilizes the 3′ ends of long noncoding RNAs that lack poly(A) tails. *Genes & Development*. **2012**, *26*(21), 2392–2407. https://doi.org/10.1101/gad.204438.112.
597. Clemson, C. M.; Hutchinson, J. N.; Sara, S. A.; Ensminger, A. W.; Fox, A. H.; Chess, A.; Lawrence, J. B. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Molecular Cell*. **2009**, *33*(6), 717–726. https://doi.org/10.1016/j.molcel.2009.01.026.
598. Brown, J. A.; Bulkley, D.; Wang, J.; Valenstein, M. L.; Yario, T. A.; Steitz, T. A.; Steitz, J. A. Structural insights into the stabilization of MALAT1 noncoding RNA by a bipartite triple helix. *Nature Structural & Molecular Biology*. **2014**, *21*(7), 633–640. https://doi.org/10.1038/nsmb.2844.
599. Arun, G.; Aggarwal, D.; Spector, D. L. MALAT1 Long Non-Coding RNA: functional implications. *Non-Coding RNA*. **2020**, *6*(2), 22. https://doi.org/10.3390/ncrna6020022.
600. Yang, L.; Lin, C.; Liu, W.; Zhang, J.; Ohgi, K. A.; Grinstein, J. D.; Dorrestein, P. C.; Rosenfeld, M. G. ncRNA- and Pc2 Methylation-Dependent Gene Relocation between Nuclear Structures Mediates Gene Activation Programs. *Cell*. **2011**, *147*(4), 773–788. https://doi.org/10.1016/j.cell.2011.08.054.
601. Tripathi, V.; Song, D. Y.; Zong, X.; Shevtsov, S. P.; Hearn, S.; Fu, X.; Dundr, M.; Prasanth, K. V. SRSF1 regulates the assembly of pre-mRNA processing factors in nuclear speckles. *Molecular Biology of the Cell*. **2012**, *23*(18), 3694–3706. https://doi.org/10.1091/mbc.e12-03-0206.
602. Engreitz, J. M.; Sirokman, K.; McDonel, P.; Shishkin, A. A.; Surka, C.; Russell, P.; Grossman, S. R.; Chow, A. Y.; Guttman, M.; Lander, E. S. RNA-RNA interactions enable specific targeting of noncoding RNAs to nascent Pre-MRNAs and chromatin sites. *Cell*. 2**014**, *159*(1), 188–199. https://doi.org/10.1016/j.cell.2014.08.018.
603. Fei, J.; Jadaliha, M.; Harmon, T. S.; Li, I. T. S.; Hua, B.; Hao, Q.; Holehouse, A. S.; Reyer, M.; Sun, Q.; Freier, S. M.; Pappu, R. V.; Prasanth, K. V.; Ha, T. Quantitative analysis of multilayer organization of proteins and RNA in nuclear speckles at super resolution. *Journal of Cell Science*. **2017**, *130*(24): 4180–4192. https://doi.org/10.1242/jcs.206854.
604. Lu, Z.; Zhang, Q. C.; Lee, B.; Flynn, R. A.; Smith, M. A.; Robinson, J. T.; Davidovich, C.; Gooding, A. R.; Goodrich, K. J.; Mattick, J. S.; Mesirov, J. P.; Cech, T. R.; Chang, H. Y. RNA duplex map in living cells reveals Higher-Order Transcriptome structure. *Cell*. **2016**, *165*(5), 1267–1279. https://doi.org/10.1016/j.cell.2016.04.028.

605. Cai, Z.; Cao, C.; Ji, L.; Ye, R.; Wang, D.; Xia, C.; Wang, S.; Du, Z.; Hu, N.; Yu, X.; Chen, J.; Wang, L.; Yang, X.; He, S.; Xue, Y. RIC-seq for global in situ profiling of RNA–RNA spatial interactions. *Nature.* **2020**, *582*(7812), 432–437. https://doi.org/10.1038/s41586-020-2249-1.
606. Ma, X.; Wang, J.; Wang, J.; X, C.; MA, Wang, X.; Liu, F. Malat1 as an evolutionarily conserved lncRNA, plays a positive role in regulating proliferation and maintaining undifferentiated status of early-stage hematopoietic cells. *BMC Genomics*. **2015**, *16*(1). https://doi.org/10.1186/s12864-015-1881-x.
607. Lelli, A.; Nolan, K. A.; Santambrogio, S.; Gonçalves, A. F.; Schönenberger, M. J.; Guinot, A.; Frew, I. J.; Marti, H. H.; Hoogewijs, D.; Wenger, R. H. Induction of long noncoding RNA MALAT1 in hypoxic mice. *Hypoxia*. **2015**, 45. https://doi.org/10.2147/hp.s90555.
608. Sallé-Lefort, S.; Miard, S.; Nolin, M.; Boivin, L.; Paré, M.; Debigaré, R.; Picard, F. Hypoxia upregulates Malat1 expression through a CaMKK/AMPK/HIF-1α axis. *International Journal of Oncology*. **2016**, *49*(4), 1731–1736. https://doi.org/10.3892/ijo.2016.3630.
609. Huang, Z.; Huang, L.; Shen, S.; Li, J.; Lu, H.; Mo, W.; Dang, Y.; Luo, D.,; Chen, G.; Feng, Z. Sp1 cooperates with Sp3 to upregulate MALAT1 expression in human hepatocellular carcinoma. *Oncology Reports*. **2015**, *34*(5), 2403–2412. https://doi.org/10.3892/or.2015.4259.
610. Zhao, Y.; Yang, Y.; Trovik, J.; Sun, K.; Zhou, L.; Jiang, P.; Lau, T.; Hoivik, E. A.; Salvesen, H. B.; Sun, H.; Wang, H. A novel WNT Regulatory Axis in endometrioid Endometrial Cancer. *Cancer Research*. **2014**, *74*(18), 5103–5117. https://doi.org/10.1158/0008-5472.can-14-0427.
611. Kuo, I.; Wu, C.; Chang, J.; Huang, Y.; Lin, C.; Yan, J.; Sheu, B.; Lu, P.; Chang, W.; Lai, W.; Wang, Y. Low SOX17 expression is a prognostic factor and drives transcriptional dysregulation and esophageal cancer progression. *International Journal of Cancer*. **2014**, *135*(3), 563–573. https://doi.org/10.1002/ijc.28695.
612. Fan, Y.; Shen, B.; Tan, M.; Mu, X.; Qin, Y.; Zhang, F.; Liu, Y. TGF-β–Induced Upregulation of malat1 Promotes Bladder Cancer Metastasis by Associating with suz12. *Clinical Cancer Research*. **2014**, *20*(6), 1531–1541. https://doi.org/10.1158/1078-0432.ccr-13-1455.
613. Sun, D.; Li, X.; He, Y.; Li, W.; Wang, Y.; Wang, H.; Jiang, S.; Xin, Y. YAP1 enhances cell proliferation, migration, and invasion of gastric cancer in vitro and in vivo. *Oncotarget*. **2016**, *7*(49), 81062–81076. https://doi.org/10.18632/oncotarget.13188.
614. Sun, H.; Lin, D.; Cao, Q.; Pang, B.; Gae, D. D.; Lee, V. K. M.; Lim, H. J.; Doan, N.; Said, J. W.; Gery, S.; Chow, M.; Mayakonda, A.; Forscher, C.; Tyner, J. W.; Koeffler, H. P. Identification of a novel SYK/C-MYC/MALAT1 signaling pathway and its potential therapeutic value in ewing sarcoma. *Clinical Cancer Research.* **2017**, *23*(15), 4376–4387. https://doi.org/10.1158/1078-0432.ccr-16-2185.
615. Wang, X.; Li, M.; Wang, Z.; Han, S.; Tang, X.; Ge, Y.; Zhou, L.; Zhou, C.; Yuan, Q.; Yang, M. Silencing of Long Noncoding RNA MALAT1 by miR-101 and miR-217 Inhibits Proliferation, Migration, and Invasion of Esophageal Squamous Cell Carcinoma Cells. *J. Biol. Chem.* **2014**, *290*, 3925–3935. https://doi.org/10.1074/jbc.M114.596866.
616. Radhakrishnan, S.K.; Lee, C.S.; Young, P.; Beskow, A.; Chan, J.Y.; Deshaies, R.J. Transcription factor Nrf1 mediates the proteasome recovery pathway after proteasome inhibition in mammalian cells. *Mol. Cell* **2010**, *38*, 17–28. https://doi.org/10.1016/j.molcel.2010.02.029.

617. Amodio, N.; Stamato, M.A.; Juli, G.; Morelli, E.; Fulciniti, M.; Manzoni, M.; Taiana, E.; Agnelli, L.; Cantafio, M.E.G.; Romeo, E.; et al. Drugging the lncRNA MALAT1 via LNA gapmeR ASO inhibits gene expression of proteasome subunits and triggers anti-multiple myeloma activity. *Leukemia* **2018**, *32*, 1948–1957. https://doi.org/10.1038/s41375-018-0067-3.
618. Li, C.; Chang, L.; Chen, Z.; Liu, Z.; Wang, Y.; Ye, Q. The role of lncRNA MALAT1 in the regulation of hepatocyte proliferation during liver regeneration. *Int. J. Mol. Med.* **2017**, *39*, 347. https://doi.org/10.3892/ijmm.2017.2854.
619. Leucci, E.; Patella, F.; Waage, J.; Holmstrøm, K.; Lindow, M.; Porse, B.; Kauppinen, S.; Lund, A.H. microRNA-9 targets the long non-coding RNA MALAT1 for degradation in the nucleus.m*Sci. Rep.* **2013**, *3*, 2535. https://doi.org/10.1038/srep02535.
620. Zhang, D.; Xue, J.; Peng, F. The regulatory activities of MALAT1 in the development of bone and cartilage diseases. *Front. Endocrinol.* **2022**, *13*, 1054827. https://doi.org/10.3389/fendo.2022.1054827.
621. Zhang, C.; Wang, J.; Guo, L.; Peng, M. Long non-coding RNA MALAT1 regulates cell proliferation, invasion and apoptosis by modulating the Wnt signaling pathway in squamous cell carcinoma. *Am. J. Transl. Res.* **2021**, *13*, 9233. PMID: 34540039; PMCID: PMC8430056.
622. Tripathi, V.; Shen, Z.; Chakraborty, A.; Giri, S.; Freier, S.M.; Wu, X.; Zhang, Y.; Gorospe, M.; Prasanth, S.G.; Lal, A.; et al. Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. *PLoS Genet.* **2013**, *9*, e1003368. https://doi.org/10.1371/journal.pgen.1003368.
623. Yang, F.; Yi, F.; Han, X.; Du, Q.; Liang, Z. MALAT-1 interacts with hnRNP C in cell cycle regulation. *FEBS Lett.* **2013**, *587*, 3175–3181. https://doi.org/10.1016/j.febslet.2013.07.048.
624. Dai, X.; Liu, L.; Liang, Z.; Guo, K.; Xu, S.; Wang, H. Silencing of lncRNA MALAT1 inhibits cell cycle progression via androgen receptor signaling in prostate cancer cells. *Pathol. Res. Pract.* **2019**, *215*, 712–721. https://doi.org/10.1016/j.prp.2019.01.011.
625. Hu, L.; Wu, Y.; Tan, D.; Meng, H.; Wang, K.; Bai, Y.; Yang, K. Up-regulation of long noncoding RNA MALAT1 contributes to proliferation and metastasis in esophageal squamous cell carcinoma. *J. Exp. Clin. Cancer Res.* **2015**, *34*, 7. https://doi.org/10.1186/s13046-015-0123-z.
626. Hajibabaei, S.; Nafissi, N.; Azimi, Y.; Mahdian, R.; Rahimi-Jamnani, F.; Valizadeh, V.; Rafiee, M.H.; Azizi, M. Targeting long non-coding RNA MALAT1 reverses cancerous phenotypes of breast cancer cells through microRNA-561-3p/TOP2A axis. *Sci. Rep.* **2023**, *13*, 8652. https://doi.org/10.1038/s41598-023-35639-x.
627. Hou, J.; Zhang, G.; Wang, X.; Wang, Y.; Wang, K. Functions and mechanisms of lncRNA MALAT1 in cancer chemotherapy resistance. *Biomark. Res.* **2023**, *11*, 23. https://doi.org/10.1186/s40364-023-00467-8.
628. Arun, G.; Diermeier, S.; Akerman, M.; Chang, K.; Wilkinson, J. E.; Hearn, S.; Kim, Y.; MacLeod, A. R.; Krainer, A. R.; Norton, L.; Brogi, E.; Egeblad, M.; Spector, D. L. Differentiation of mammary tumors and reduction in metastasis upon Malat1 lncRNA loss. *Genes & Development*. **2015**, *30*(1), 34–51. https://doi.org/10.1101/gad.270959.115.

629. Malakar, P.; Shilo, A.; Mogilevsky, A.; Stein, I.; Pikarsky, E.; Nevo, Y.; Benyamini, H.; Elgavish, S.; Zong, X.; Prasanth, K. V.; Karni, R. Long noncoding RNA MALAT1 promotes hepatocellular carcinoma development by SRSF1 upregulation and MTOR activation. *Cancer Research*. **2017**, *77*(5), 1155–1167. https://doi.org/10.1158/0008-5472.can-16-1508.
630. Spector, D.L.; Lamond, A.I. Nuclear Speckles. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, a000646. https://doi.org/10.1101/cshperspect.a000646.
631. Miao, H.; Wu, F.; Li, Y.; Qin, C.; Zhao, Y.; Xie, M.; Dai, H.; Yao, H.; Cai, H.; Wang, Q.; et al. MALAT1 modulates alternative splicing by cooperating with the splicing factors PTBP1 and PSF. *Sci. Adv.* **2022**, *8*, eabq7289. https://doi.org/10.1126/sciadv.abq7289.
632. Zhang, Z. C.; Tang, C.; Dong, Y.; Zhang, J.; Yuan, T.; Tao, S. C.; Li, X. L. Targeting the long noncoding RNA MALAT1 blocks the pro-angiogenic effects of osteosarcoma and suppresses tumour growth. *Int. J. Biol. Sci.* **2017**, *13*, 1398. https://doi.org/10.7150/ijbs.22249.
633. Wang, D.; Ding, L.; Wang, L.; Zhao, Y.; Sun, Z.; Karnes, R.J.; Zhang, J.; Huang, H. LncRNA MALAT1 enhances oncogenic activities of EZH2 in castration-resistant prostate cancer. *Oncotarget* **2015**, *6*, 41045. https://doi.org/10.18632/oncotarget.5728.
634. Jin, Y.; Feng, S. J.; Qiu, S.; Shao, N.; Zheng, J. H. LncRNA MALAT1 promotes proliferation and metastasis in epithelial ovarian cancer via the PI3K-AKT pathway. *Eur. Rev. Med. Pharmacol. Sci.* **2017**, *21*, 3176–3184. PMID: 28770968.
635. Zhang, J.; Gill, A. J. M.; Issacs, J. D.; Atmore, B.; Johns, A.; Delbridge, L. W.; Lai, R.; McMullen, T. P. W. The Wnt/β-catenin pathway drives increased cyclin D1 levels in lymph node metastasis in papillary thyroid cancer. *Hum. Pathol.* **2012**, *43*, 1044–1050. https://doi.org/10.1016/j.humpath.2011.08.013.
636. Peng, N.; He, J.; Li, J.; Huang, H.; Huang, W.; Liao, Y.; Zhu, S. Long noncoding RNA MALAT1 inhibits the apoptosis and autophagy of hepatocellular carcinoma cell by targeting the microRNA-146a/PI3K/Akt/mTOR axis. *Cancer Cell Int.* **2020**, *20*, 165. https://doi.org/10.1186/s12935-020-01231-w.
637. Zheng, X.; Ren, J.; Peng, B.; Ye, J.; Wu, X.; Zhao, W.; Li, Y.; Chen, R.; Gong, X.; Bai, C.; et al. MALAT1 overexpression promotes the growth of colon cancer by repressing β-catenin degradation. *Cell. Signal.* **2020**, *73*, 109676. https://doi.org/10.1016/j.cellsig.2020.109676.
638. Sun, Y.; Qin, B. Long noncoding RNA MALAT1 regulates HDAC4-mediated proliferation and apoptosis via decoying of miR-140-5p in osteosarcoma cells. *Cancer Med.* **2018**, *7*, 4584–4597. https://doi.org/10.1002/cam4.1677.
639. Kim, S.H.; Kim, S.H.; Yang, W.I.; Kim, S.J.; Yoon, S.O. Association of the long non-coding RNA MALAT1 with the polycomb repressive complex pathway in T and NK cell lymphoma. *Oncotarget* **2017**, *8*, 31305. https://doi.org/10.18632/oncotarget.15453.
640. Jin, C.; Yan, B.; Lu, Q.; Lin, Y.; Ma, L. Reciprocal regulation of Hsa-miR-1 and long noncoding RNA MALAT1 promotes triple-negative breast cancer development. *Tumor Biology*. **2015**, *37*(6), 7383–7394. https://doi.org/10.1007/s13277-015-4605-6.
641. Wang, J.; Su, L.; Chen, X.; Li, P.; Cai, Q.; Yu, B.; Liu, B.; Wu, W.; Zhu, Z. MALAT1 promotes cell proliferation in gastric cancer by recruiting SF2/ASF. *Biomed. Pharmacother.* **2014**, *68*, 557–564. https://doi.org/10.1016/j.biopha.2014.04.007.
642. Yang, M.H.; Hu, Z.Y.; Xu, C.; Xie, L.Y.; Wang, X.Y.; Chen, S.Y.; Li, Z.G. MALAT1 promotes colorectal cancer cell proliferation/migration/invasion via PRKA kinase anchor protein 9. *Biochim. Biophys. Acta* **2015**, *1852*, 166–174. https://doi.org/10.1016/j.bbadis.2014.11.013.

643. Qi, Y.; Ooi, H.S.; Wu, J.; Chen, J.; Zhang, X.; Tan, S.; Yu, Q.; Li, Y.Y.; Kang, Y.; Li, H.; et al. MALAT1 long ncRNA promotes gastric cancer metastasis by suppressing PCDH10. *Oncotarget* **2016**, *7*, 12693–12703. https://doi.org/10.18632/oncotarget.7281.
644. Shi, D.; Zhang, Y.; Lu, R.; Zhang, Y. The long non-coding RNA MALAT1 interacted with miR-218 modulates choriocarcinoma growth by targeting Fbxw8. *Biomed. Pharmacother.* **2018**, *97*, 543–550. https://doi.org/10.1016/j.biopha.2017.10.083.
645. Tan, X.; Huang, Z.; Li, X. Long Non-Coding RNA MALAT1 Interacts With miR-204 to Modulate Human Hilar Cholangiocarcinoma Proliferation, Migration, and Invasion by Targeting CXCR4. *J. Cell. Biochem.* **2017**, *118*, 3643–3653. https://doi.org/10.1002/jcb.25862.
646. Zhang, T. H.; Liang, L. Z.; Liu, X. L.; Wu, J. N.; Su, K.; Chen, J. Y.; Zheng, Q. Y.; Huang, H. Z.; Liao, G. Q. Long non-coding RNA MALAT1 interacts with miR-124 and modulates tongue cancer growth by targeting JAG1. *Oncol. Rep.* **2017**, *37*, 2087–2094. https://doi.org/10.3892/or.2017.5445.
647. Wang, Y.; Zhang, Y.; Hu, K.; Qiu, J.; Hu, Y.; Zhou, M.; Zhang, S. Elevated long noncoding RNA MALAT-1 expression is predictive of poor prognosis in patients with breast cancer: A meta-analysis. *Biosci. Rep.* **2020**, *40*, 20200215. https://doi.org/10.1042/BSR20200215.
648. Schmidt, L.H.; Spieker, T.; Koschmieder, S.; Humberg, J.; Jungen, D.; Bulk, E.; Hascher, A.; Wittmer, D.; Marra, A.; Hillejan, L.; et al. The Long Noncoding MALAT-1 RNA Indicates a Poor Prognosis in Non-small Cell Lung Cancer and Induces Migration and Tumor Growth. *J. Thorac. Oncol.* **2011**, *6*, 1984–1992. https://doi.org/10.1097/JTO.0b013e3182307eac.
649. Ma, K.X.; Wang, H.J.; Li, X.R.; Li, T.; Su, G.; Yang, P.; Wu, J.W. Long noncoding RNA MALAT1 associates with the malignant status and poor prognosis in glioma. *Tumor Biol.* **2015**, *36*, 3355–3359. https://doi.org/10.1007/s13277-014-2969-7.
650. Guffanti, A.; Iacono, M.; Pelucchi, P.; Kim, N.; Soldà, G.; Croft, L.J.; Taft, R.J.; Rizzi, E.; Askarian-Amiri, M.; Bonnal, R.J.; et al. A transcriptional sketch of a primary human breast cancer by 454 deep sequencing. *BMC Genom.* **2009**, *10*, 163. https://doi.org/10.1186/1471-2164-10-163.
651. Chen, Q.; Su, Y.; He, X.; Zhao, W.; Wu, C.; Zhang, W.; Si, X.; Dong, B.; Zhao, L.; Gao, Y.; et al. Plasma long non-coding RNA MALAT1 is associated with distant metastasis in patients with epithelial ovarian cancer. *Oncol. Lett.* **2016**, *12*, 1361–1366. https://doi.org/10.3892/ol.2016.4800.
652. Guo C.; Wang X.; Chen L.; Li M.; Li M.; Hu Y.; Ding W.; Wang X. Long non-coding RNA MALAT1 regulates ovarian cancer cell proliferation, migration and apoptosis through Wnt/β-catenin signaling pathway. *PubMed*. **2018**, *22*(12), 3703–3712. https://doi.org/10.26355/eurrev_201806_15249.
653. Pei C.; Gong X.; Zhang Y. LncRNA MALAT-1 promotes growth and metastasis of epithelial ovarian cancer via sponging microrna-22. Am J Transl Res. **2020**, *12*:6977. http://www.ajtr.org/index.html.
654. Wang, Y.; Wang, X.; Han, L.; Hu, D. LncRNA MALAT1 Regulates the Progression and Cisplatin Resistance of Ovarian Cancer Cells via Modulating miR-1271-5p/E2F5 Axis. *Cancer Management and Research*, *Volume*. **2020**, *12*, 9999–10010. https://doi.org/10.2147/cmar.s261979.
655. Li, Q.; Shi, J.; Xu, X. MicroRNA-1271-5p inhibits the tumorigenesis of ovarian cancer through targeting E2F5 and negatively regulates the mTOR signaling pathway. *Research Square (Research Square)*. **2020**. https://doi.org/10.21203/rs.3.rs-17976/v1.

656. Fellenberg, J.; Sähr, H.; Kunz, P.; Zhao, Z.; Liu, L.; Tichy, D.; Herr, I. Restoration of miR-127-3p and miR-376a-3p counteracts the neoplastic phenotype of giant cell tumor of bone derived stromal cells by targeting COA1, GLE1 and PDIA6. *Cancer Letters*. **2016**, *371*(1), 134–141. https://doi.org/10.1016/j.canlet.2015.10.039.
657. Lei, R.; Xue, M.; Zhang, L.; Lin, Z. Long noncoding RNA MALAT1-regulated microRNA 506 modulates ovarian cancer growth by targeting iASPP. *OncoTargets and Therapy*. **2016**, *Volume 10*, 35–46. https://doi.org/10.2147/ott.s112686.
658. Lin, Q.; Guan, W.; Ren, W.; Zhang, L.; Zhang, J.; Xu, G. MALAT1 affects ovarian cancer cell behavior and patient survival. *Oncology Reports*. **2018**, *39*, 2644-2652. https://doi.org/10.3892/or.2018.6384.
659. Gordon, M. A.; Babbs, B.; Cochrane, D. R.; Bitler, B. G.; Richer, J. K. The long non-coding RNA MALAT1 promotes ovarian cancer progression by regulating RBFOX2-mediated alternative splicing. *Molecular Carcinogenesis*. **2018**, *58*(2), 196–205. https://doi.org/10.1002/mc.22919.
660. Xu, W.; Yang, Z.; Lu, N. A new role for the PI3K/Akt signaling pathway in the epithelial-mesenchymal transition. *Cell Adhesion & Migration*. **2015**, *9*(4), 317–324. https://doi.org/10.1080/19336918.2015.1016686.
661. Jin, Y., Feng, S. J., Qiu, S., Shao, N., & Zheng, J. H. (2017). LncRNA MALAT1 promotes proliferation and metastasis in epithelial ovarian cancer via the PI3K-AKT pathway. *European review for medical and pharmacological sciences*, *21*(14), 3176–3184. https://www.europeanreview.org/article/13138.
662. Liu, X.; Lv, R.; Zhang, L.; Xu, G.; Bi, J.; Gao, F.; Zhang, J.; Xue, F.; Wang, F.; Wu, Y.; et al. Long noncoding RNA expression profile of infantile hemangioma identified by microarray analysis. *Tumour Biol.* **2016**, *37*, 15977–15987. https://doi.org/10.1007/s13277-016-5434-y.
663. Han, Y.; Liu, Y.; Nie, L.; Gui, Y.; Cai, Z. Inducing cell proliferation inhibition, apoptosis, and motility reduction by silencing long noncoding ribonucleic acid metastasis-associated lung adenocarcinoma transcript 1 in urothelial carcinoma of the bladder. *Urology* **2013**, *81*, 209.e1–209.e7. https://doi.org/10.1016/j.urology.2012.08.044.
664. Szpechcinski, A.; Dancewicz, M.; Kopinski, P.; Kowalewski, J.; Chorostowska-Wynimko, J. Real-time PCR quantification of plasma DNA in non-small cell lung cancer patients and healthy controls. *Eur. J. Med. Res.* **2009**, *14* (Suppl. S4), 237–240. https://doi.org/10.1186/2047-783X-14-S4-237.
665. Xie, L.Y.; Hu, Z.Y.; Wang, X.Y.; Li, Z.G. Expression and biological significance of long non-coding MALAT1 gene in human nasopharyngeal carcinoma cell lines. *J. Nanfang Med. Univ.* **2013**, *33*, 692. PMID: 23688988.
666. Kyurkchiyan, S.; Stancheva, G.; Petkova, V.; Hadzhiev, Y.; Dobriyanova, V.; Popova, D.; Kaneva, R.; Popov, T. Exploration of the association between HIF3α mRNA and lncRNA MALAT1 in laryngeal squamous cell carcinoma by correlation analysis. *Oncology Letters*. **2024**, *28*(1). https://doi.org/10.3892/ol.2024.14425.
667. Shih, C.; Chuang, L.; Tsai, M.; Chen, L.; Chuang, E. Y.; Lu, T.; Lai, L. Hypoxia-Induced MALAT1 promotes the proliferation and migration of breast cancer cells by sponging MIR-3064-5P. *Frontiers in Oncology*. **2021**, 11:658151–658151. https://doi.org/10.3389/fonc.2021.658151.
668. Zhao, C.; Ling, X.; Xia, Y.; Yan, B.; Guan, Q. The m6A methyltransferase METTL3 controls epithelial-mesenchymal transition, migration and invasion of breast cancer through the MALAT1/miR-26b/HMGA2 axis. *Cancer Cell International.* **2021**, **21**, 441. https://doi.org/10.1186/s12935-021-02113-5.

669. Zeng, C., Huang, W., Li, Y., & Weng, H. Roles of METTL3 in cancer: mechanisms and therapeutic targeting. *ournal of Hematology & Oncology*. **2020**, *13,* 117. https://doi.org/10.1186/s13045-020-00951-w.
670. Wang, N.; Cao, S.; Wang, X.; Zhang, L.; Yuan, H.; Ma, X. lncRNA MALAT1/miR-26a/26b/ST8SIA4 axis mediates cell invasion and migration in breast cancer cell lines. *Oncology Reports*. **2021**, *46*(2). https://doi.org/10.3892/or.2021.8132.
671. Feng, T.; Shao, F.; Wu, Q.; Zhang, X.; Xu, D.; Qian, K.; Xie, Y.; Wang, S.; Xu, N.; Wang, Y.; Qi, C. miR-124 downregulation leads to breast cancer progression via LncRNA-MALAT1 regulation and CDK4/E2F1 signal activation. *Oncotarget*. **2016**, *7*(13), 16205–16216. https://doi.org/10.18632/oncotarget.7578.
672. Zhou, R.M.; Wang, X.Q.; Yao, J.; Shen, Y.; Chen, S.N.; Yang, H.; Jiang, Q.; Yan, B. Identification and characterization of proliferative retinopathy-related long noncoding RNAs. *Biochem. Biophys. Res. Commun.* **2015**, *465*, 324–330. https://doi.org/10.1016/j.bbrc.2015.07.120.
673. Yan, C.; Chen, J.; Chen, N. Long noncoding RNA MALAT1 promotes hepatic steatosis and insulin resistance by increasing nuclear SREBP-1c protein stability. *Sci. Rep.* **2016**, *6*, 22640. https://doi.org/10.1038/srep22640.
674. Puthanveetil, P.; Chen, S.; Feng, B.; Gautam, A.; Chakrabarti, S. Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. *J. Cell. Mol. Med.* **2015**, *19*, 1418–1425. https://doi.org/10.1111/jcmm.12576.
675. Yan, B.; Tao, Z. F.; Li, X. M.; Zhang, H.; Yao, J.; Jiang, Q. Aberrant expression of long noncoding RNAs in early diabetic retinopathy. *Investig. Ophthalmol. Vis. Sci.* **2014**, *55*, 941–951. https://doi.org/10.1167/iovs.13-13221.
676. Piórkowska, K.; Zygmunt, K.; Hunter, W.; Wróblewska, K. MALAT1: A Long Non-Coding RNA with Multiple Functions and Its Role in Processes Associated with Fat Deposition. *Genes.* **2024**, *15*(4), 479. https://doi.org/10.3390/genes15040479.
677. Guo, F.; Li, Y.; Liu, Y.; Wang, J.; Li, G. Inhibition of metastasis-associated lung adenocarcinoma transcript 1 in caski human cervical cancer cells suppresses cell proliferation and invasion. *Acta Biochimica Et Biophysica Sinica*. **2010**, *42(3)*:224–9. https://doi.org/10.1093/abbs/gmq008.
678. Liu, S.; Song, L.; Zeng, S.; Zhang, L. MALAT1-miR-124-RBG2 axis is involved in growth and invasion of HR-HPV-positive cervical cancer cells. *Tumor Biology*. **2015**, *37*(1), 633–640. https://doi.org/10.1007/s13277-015-3732-4.
679. Tie, W.; Ge, F. MALAT1 inhibits proliferation of HPV16-Positive cervical cancer by sponging MIR-485-5P to promote expression of MAT2A. *DNA And Cell Biology*. **2021**, *40*(11), 1407–1417. https://doi.org/10.1089/dna.2020.6205.
680. Liang, T.; Wang, Y.; Jiao, Y.; Cong, S.; Jiang, X.; Dong, L.; Zhang, G.; Xiao, D. LNCRNA MALAT1 accelerates cervical carcinoma proliferation by suppressing MIR-124 expression in cervical tumor cells. *Journal of Oncology*. **2021**, *2021*, 1–11. https://doi.org/10.1155/2021/8836078.
681. Han, X.; Wang, Q.; Wang, Y.; Hu, B.; Dong, X.; Zhang, H.; Wang, W. Long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1/microRNA-202-3p/periostin axis modulates invasion and epithelial–mesenchymal transition in human cervical cancer. *Journal of Cellular Physiology*. **2019**, *234*(8):14170–14180. https://doi.org/10.1002/jcp.28113.
682. Lu, H.; He, Y.; Lin, L.; Qi, Z.; Ma, L.; Li, L.; Su, Y. Long non-coding RNA MALAT1 modulates radiosensitivity of HR-HPV+ cervical cancer via sponging miR-145. *Tumor Biol*. **2016**. **37**, 1683–1691. https://doi.org/10.1007/s13277-015-3946-5.

683. Yang L.; Bai H. S.; Deng Y.; Fan L. High malat1 expression predicts a poor prognosis of cervical cancer and promotes cancer cell growth and invasion. *Eur Rev Med Pharmacol Sci*. **2015**, *19*:3187–93. PMID: 26400521.
684. Xu, Y.; Zhang, Q.; Lin, F.; Zhu, L.; Huang, F.; Zhao, L.; Ou, R. Casiopeina II-gly acts on lncRNA MALAT1 by miR-17-5p to inhibit FZD2 expression via the Wnt signaling pathway during the treatment of cervical carcinoma. *Oncology Reports*. **2019**. https://doi.org/10.3892/or.2019.7268.
685. Wang N.; Hou M.; Zhan Y.; Shen X.; Xue H. MALAT1 promotes cisplatin resistance in cervical cancer by activating the PI3K/AKT pathway. *PubMed*. **2018**, *22*(22), 7653–7659. https://doi.org/10.26355/eurrev_201811_16382.
686. Liao, H.; Chen, Q.; Xiao, J. Reflections on the role of MALAT1 in gynecological cancer. *Cancer Management and Research*. **2020**, *Volume 12*, 13489–13500. https://doi.org/10.2147/cmar.s286804.
687. Farzaneh, M.; Najafi, S.; Dari, M. A. G.; Sheykhi-Sabzehpoush, M.; Dayer, D.; Cheraghzadeh, M.; Azizidoost, S. Functional roles of long noncoding RNA MALAT1 in gynecologic cancers. *Clinical & Translational Oncology*. **2022***, 25*(1), 48–65. https://doi.org/10.1007/s12094-022-02914-8.
688. Stephens, M.; Smith, N. J.; Donnelly, P. A New Statistical Method for Haplotype Reconstruction from Population Data. *The American Journal of Human Genetics.* **2001**, *68*(4), 978–989. https://doi.org/10.1086/319501.
689. Stephens, M.; Donnelly, P. A Comparison of Bayesian Methods for Haplotype Reconstruction from Population Genotype Data. *The American Journal of Human Genetics.* **2003**, *73*(5), 1162–1169. https://doi.org/10.1086/379378.
690. Gabriel, S. B.; Schaffner, S. F.; Nguyen, H.; Moore, J. M.; Roy, J.; Blumenstiel, B.; Higgins, J.; DeFelice, M.; Lochner, A.; Faggart, M.; Liu-Cordero, S. N.; Rotimi, C.; Adeyemo, A.; Cooper, R.; Ward, R.; Lander, E. S.; Daly, M. J.; Altshuler, D. The structure of haplotype blocks in the human genome. *Science.* **2002**, *296*(5576), 2225–2229. https://doi.org/10.1126/science.1069424.
691. Huang, H.; Wu, J.; Jin, G.; Zhang, H.; Ding, Y.; Hua, Z.; Zhou, Y.; Xue, Y.; Lu, Y.; Hu, Z.; et al. A 5-flanking region polymorphism in toll-like receptor 4 is associated with gastric cancer in a Chinese population. *J. Biomed. Res*. **2010**, 24, 100–106. https://doi.org/10.1016/s1674-8301(10)60017-6.
692. Tongtawee, T.; Simawaranon, T.; Wattanawongdon, W.; Dechsukhum, C.; Leeanansaksiri, W. Toll-like receptor 2 and 4 polymor phisms associated with Helicobacter pylori susceptibility and gastric cancer. *Turk. J. Gastroenterol*. **2018**, 30, 15–20. https://doi.org/10.5152/tjg.2018.17461.
693. Cheng, I.; Plummer, S.J.; Casey, G.; Witte, J.S. Toll-Like Receptor 4 Genetic Variation and Advanced Prostate Cancer Risk. Cancer Epidemiol. Biomark. Prev. 2007, 16, 352–355. https://doi.org/10.1158/1055-9965.epi-06-0429.
694. Luo, B.; Han, L.; Liu, S.; Wang, X.; Shi, Y.; Zhao, Z.; Zhao, C. Toll-like receptor gene polymorphisms and susceptibility to Epstein-Barr virus-associated and-negative gastric carcinoma in Northern China. *Saudi J. Gastroenterol*. **2015**, 21, 95. https://doi.org/10.4103/1319-3767.153832.
695. Suzuki, T.; Meguro, A.; Matsushima, M.; Masui, A.; Tsuda, S.; Nakamura, J.; Nishina, R.; Uchida, T.; Yuhara, H.; Igarashi, M.; et al. Investigation of the Association of TLR2 and TLR4 Polymorphisms with Susceptibility to Helicobac ter pylori-Related Gastrointestinal Diseases. *Open J. Intern. Med.* **2014**, 4, 52496. https://doi.org/10.4236/ojim.2014.44020.

696. Semlali, A.; Jalouli, M.; Parine, N. R.; Al Amri, A.; Arafah, M.; Al Naeem, A.; Abdullah, S.; Rouabhia, M.; Alanazi, M. Toll-like receptor-4 as a predictor of clinical outcomes of estrogen receptor-negative breast cancer in Saudi women. *OncoTargets Ther*. **2017**, 10, 1207–1216. https://doi.org/10.2147/ott.s112165.
697. Kim, J.; Cho, Y. A.; Choi, I. J.; Lee, Y. S.; Kim, S. Y.; Hwang, J. A.; Cho, S. J.; Kook, M. C.; Kim, C. G.; Kim, Y. W. Effects of Polymorphisms of Innate Immunity Genes and Environmental Factors on the Risk of Noncardia Gastric Cancer. *Cancer Res. Treat*. **2013**, 45, 313–324. https://doi.org/10.4143/crt.2013.45.4.313.
698. Proença, M. A. TLR2 and TLR4 polymorphisms influence mRNA and protein expression in colorectal cancer. *World J. Gastroenterol*. **2015**, 21, 7730. https://doi.org/10.3748/wjg.v21.i25.7730.
699. Shui, I. M.; Stark, J. R.; Penney, K. L.; Schumacher, F. R.; Epstein, M. M.; Pitt, M. J.; Stampfer, M. J.; Tamimi, R. M.; Lindstrom, S.; Sesso, H. D.; et al. Genetic variation in the toll-like receptor 4 and prostate cancer incidence and mortality. *Prostate*. **2011**, 72, 209–216. https://doi.org/10.1002/pros.21423.
700. Chen, Y. C.; Giovannucci, E.; Lazarus, R.; Kraft, P.; Ketkar, S.; Hunter, D. J. Sequence Variants of Toll-Like Receptor 4 and Susceptibility to Prostate Cancer. *Cancer Res.* **2005**, 65, 11771–11778. https://doi.org/10.1158/0008-5472.can-05-2078.
701. Slattery, M. L.; Herrick, J. S.; Bondurant, K. L.; Wolff, R. K. Toll-like receptor genes and their association with colon and rectal cancer development and prognosis. *Int. J. Cancer*. **2011**, 130, 2974–2980. https://doi.org/10.1002/ijc.26314.
702. Wang, M. H.; Helzlsouer, K.J.; Smith, M.W.; Hoffman-Bolton, J.A.; Clipp, S.L.; Grinberg, V.; De Marzo, A.M.; Isaacs, W.B.; Drake, C.G.; Shugart, Y.Y.; et al. Association of IL10 and Other immune response- and obesity-related genes with prostate cancer in CLUE II. *Prostate*. **2009**, 69, 874–885. https://doi.org/10.1002/pros.20933.
703. Lindström, S.; Hunter, D. J.; Grönberg, H.; Stattin, P.; Wiklund, F.; Xu, J.; Chanock, S. J.; Hayes, R.; Kraft, P. Sequence Variants in the TLR4 and TLR6-1-10 Genes and Prostate Cancer Risk. Results Based on Pooled Analysis from Three Independent Studies. *Cancer Epidemiol. Biomark. Prev*. **2010**, 19, 873–876. https://doi.org/10.1158/1055-9965.epi-09-0618.
704. Zidi, S.; Verdi, H.; Yilmaz-Yalcin, Y.; Yazici, A.; Gazouani, E.; Mezlini, A.; Atac, F. B.; Yacoubi-Loueslati, B. Involvement of Toll-like receptors in cervical cancer susceptibility among Tunisian women. *Bull. Du Cancer*. **2014**, 101, E31–E35. https://doi.org/10.1684/bdc.2014.2037.
705. Zidi, S.; Sghaier, I.; Gazouani, E.; Mezlini, A.; Yacoubi-Loueslati, B. Evaluation of Toll-Like Receptors 2/3/4/9 Gene Polymor phisms in Cervical Cancer Evolution. *Pathol. Oncol. Res*. **2015**, 22, 323–330. https://doi.org/10.1007/s12253-015-0009-6.
706. Pandey, S.; Mittal, R. D.; Srivastava, M.; Srivastava, K.; Singh, S.; Srivastava, S.; Mittal, B. Impact of Toll-like receptors [TLR] 2 (−196 to −174 del) and TLR4 (Asp299Gly, Thr399Ile) in cervical cancer susceptibility in North Indian women. *Gynecol. Oncol*. **2009**, 114, 501–505. https://doi.org/10.1016/j.ygyno.2009.05.032.
707. Jin, Y.; Qiu, S.; Shao, N.; Zheng, J. Association of toll-like receptor gene polymorphisms and its interaction with HPV infection in determining the susceptibility of cervical cancer in Chinese Han population. *Mamm. Genome*. **2017**, 28, 213–219. https://doi.org/10.1007/s00335-017-9691-x.

708. Ashton, K. A.; Proietto, A.; Otton, G.; Symonds, I.; McEvoy, M.; Attia, J.; Scott, R. J. Toll-Like Receptor (TLR) and Nucleosome binding Oligomerization Domain (NOD) gene polymorphisms and endometrial cancer risk. *BMC Cancer*. **2010**, 10, 382–387. https://doi.org/10.1186/1471-2407-10-382.
709. Wang, A. C.; Wu, F. X.; Gao, Y. S.; Sheng, X. G. Toll-like receptor 4 single-nucleotide polymorphisms Asp299Gly and Thr399Ile in ovarian cancers. *Oncol. Lett*. **2014**, 8, 438–440. https://doi.org/10.3892/ol.2014.2113.
710. Kania, K. D.; Haręża, D.; Wilczyński, J. R.; Wilczyński, M.; Jarych, D.; Malinowski, A.; Paradowska, E. The Toll-like Receptor 4 Polymorphism Asp299Gly Is Associated with an Increased Risk of Ovarian Cancer. *Cells*. **2022**, *11*, 3137. https://doi.org/10.3390/cells11193137.
711. Theodoropoulos, G. E.; Saridakis, V.; Karantanos, T.; Michalopoulos, N. V.; Zagouri, F.; Kontogianni, P.; Lymperi, M.; Gazouli, M.; Zografos, G. C. Toll-like receptors gene polymorphisms may confer increased susceptibility to breast cancer development. *Breast*. **2012**, *21*:534–538. https://doi.org/10.1016/j.breast.2012.04.001.
712. De Oliveira, J. G.; Rossi, A. F. T.; Nizato, D. M.; Miyasaki, K.; Silva, A. E. Profiles of Gene Polymorphisms in Cytokines and Toll-Like Receptors with Higher Risk for Gastric Cancer. *Digestive Diseases and Sciences*. **2012**, *58*: 978–988. https://doi.org/10.1007/s10620-012-2460-5.
713. Oliveira, J. G. D. Polymorphisms of the TLR2 and TLR4 genes are associated with risk of gastric cancer in a Brazilian population. *World J. Gastroenterol*. **2012**, 18, 1235. https://doi.org/10.3748/wjg.v18.i11.1235.
714. Garza-Gonzalez, E.; Bosques-Padilla, F.J.; Mendoza-Ibarra, S. I.; Flores-Gutierrez, J. P.; Maldonado-Garza, H. J.; Perez-Perez, G. I. Assessment of the toll-like receptor 4 Asp299Gly, Thr399Ile and interleukin-8-251 polymorphisms in the risk for the development of distal gastric cancer. *BMC Cancer*. **2007**, 7, 70. https://doi.org/10.1186/1471-2407-7-70.
715. Trejo-de la, O. A.; Torres, J.; Pérez-Rodríguez, M.; Camorlinga-Ponce, M.; Luna, L. F.; Abdo-Francis, J. M.; Lazcano, E.; Maldonado-Bernal, C. TLR4 single-nucleotide polymorphisms alter mucosal cytokine and chemokine patterns in Mexican patients with Helicobacter pylori-associated gastroduodenal diseases. *Clin. Immunol*. **2008**, 129, 333–340. https://doi.org/10.1016/j.clim.2008.07.009.
716. Santini, D.; Angeletti, S.; Ruzzo, A.; Dicuonzo, G.; Galluzzo, S.; Vincenzi, B.; Calvieri, A.; Pizzagalli, F.; Graziano, N.; Ferraro, E.; et al. Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms in gastric cancer of intestinal and diffuse histotypes. *Clin. Exp. Immunol*. **2008**, 154, 360–364. https://doi.org/10.1111/j.1365-2249.2008.03776.x.
717. Hold, G. L.; Rabkin, C. S.; Chow, W.; Smith, M. G.; Gammon, M. D.; Risch, H. A.; Vaughan, T. L.; McColl, K. E.; Lissowska, J.; Zatonski, W.; et al. A Functional Polymorphism of Toll-Like Receptor 4 Gene Increases Risk of Gastric Carcinoma and Its Precursors. *Gastroenterology.* **2007**, 132, 905–912. https://doi.org/10.1053/j.gastro.2006.12.026.
718. Companioni, O.; Bonet, C.; Muñoz, X.; Weiderpass, E.; Panico, S.; Tumino, R.; Palli, D.; Agnoli, C.; Vineis, P.; Boutron-Ruault, M. C. Polymorphisms of Helicobacter pylori signaling pathway genes and gastric cancer risk in the European prospective investigation into cancer-eurgast cohort. *Int. J. Cancer*. **2013**, 134, 92–101. https://doi.org/10.1002/ijc.28357.
719. Qadri, Q.; Rasool, R.; Afroze, D.; Naqash, S.; Gulzar, G. M.; Yousuf, A.; Siddiqi, M. A.; Shah, Z. A. Study of TLR4 and IL-8 Gene Polymorphisms in H.pylori-Induced

Inflammation in Gastric Cancer in an Ethnic Kashmiri Population. *Immunol. Investi*g. **2013**, 43, 324–336. https://doi.org/10.3109/08820139.2013.854378.
720. Zhou,Q.; Wang, C.; Wang, X.; Wu,X.; Zhu, Z.; Liu, B.; Su, L. Association between TLR4 (+896A/G and+1196C/T)Polymorphisms and Gastric Cancer Risk: An Updated Meta-Analysis. *PLoS ONE*. **2014**, 9, e109605. https://doi.org/10.1371/journal.pone.0109605.
721. Reilly, F.; Burke, J. P.; Lennon, G.; Kay, E. W.; McNamara, D. A.; Cullen, G.; Doherty, G.A.; Mulcahy, H.; Martin, S.; Winter, D.C.; et al. Acase–control study examining the association of smad7 and TLR single nucleotide polymorphisms on the risk of colorectal cancer in ulcerative colitis. *Color. Dis*. **2021**, 23, 1043–1048. https://doi.org/10.1111/codi.15550.
722. Asghari, A.; Nowras, T.; Tavakoli, T.; Fakharian, T.; Razavi, F. E.; Soltaninejad, E.; Naseri, M. Association between rs4986790 and rs4986791 Polymorphisms in TLR4 with Colorectal Cancer Risk in Iranian Population. *Russ. J. Genet*. **2021**, 57, 740–744. https://doi.org/10.1134/s1022795421060028.
723. Kopp, T. I.; Andersen, V.; Tjonneland, A.; Vogel, U. Polymorphisms in NFKB1 and TLR4 and Interaction with Dietary and Life Style Factors in Relation to Colorectal Cancer in a Danish Prospective Case-Cohort Study. *PLoS ONE*. **2015**, 10, e0116394. https://doi.org/10.1371/journal.pone.0116394.
724. Li, X. X.; Sun, G.P.; Meng, J.; Li, X.; Tang, Y. X.; Li, Z.; Wang, M. F.; Liang, G. F.; Lu, X. B. Role of Toll-Like Receptor 4 in Colorectal Carcinogenesis: A Meta-Analysis. *PLoS ONE*. **2014**, 9, e93904. https://doi.org/10.1371/journal.pone.0093904.
725. Moaaz, M.; Youssry, S.; Moaz, A.; Abdelrahman, M. Study of Toll-Like Receptor 4 Gene Polymorphisms in Colorectal Cancer: Correlation with Clinicopathological Features. *Immunol. Investig*. **2020**, 49, 571–584. https://doi.org/10.1080/08820139.2020.1716787.
726. Kutikhin, A. G.; Yuzhalin, A. E.; Volkov, A. N.; Zhivotovskiy, A. S.; Brusina, E. B. Correlation between genetic polymorphisms within IL-1B and TLR4 genes and cancer risk in a Russian population: A case-control study. *Tumor Biol*. **2014**, 35, 4821–4830. https://doi.org/10.1007/s13277-014-1633-6.
727. Weng, P. H.; Huang, Y. L.; Page, J. H.; Chen, J. H.; Xu, J.; Koutros, S.; Berndt, S.; Chanock, S.; Yeager, M.; Witte, J. S.; et al. Polymorphisms of an Innate Immune Gene, Toll-Like Receptor 4, and Aggressive Prostate Cancer Risk: A Systematic Review and Meta-Analysis. *PLoS ONE*. **2014**, 9, e110569. https://doi.org/10.1371/journal.pone.0110569.
728. Winchester, D. A.; Till, C.; Goodman, P. J.; Tangen, C. M.; Santella, R. M.; Johnson-Pais, T. L.; Leach, R. J.; Xu, J.; Zheng, S. L.; Thompson, I. M.; et al. Association between variants in genes involved in the immune response and prostate cancer risk in men randomized to the finasteride arm in the Prostate Cancer Prevention Trial. *Prostate*. **2017**, 77, 908–919. https://doi.org/10.1002/pros.23346.
729. Kurt, H.; Ozbayer, C.; Bayramoglu, A.; Gunes, H. V.; Degirmenci, R.; Oner, K. S.; Metintas, M. Determination of the Relationship Between rs4986790 and rs4986791 Variants of TLR4 Gene and Lung Cancer. *Inflammation*. **2015**, 39, 166–171. https://doi.org/10.1007/s10753-015-0235-9.
730. Bergmann, C.; Bachmann, H. S.; Bankfalvi, A.; Lotfi, R.; Pütter, C.; Wild, C. A.; Schuler, P. J.; Greve, J.; Hoffmann, T. K.; Lang, S.; et al. Toll-like receptor 4 single-nucleotide polymorphisms Asp299Gly and Thr399Ile in head and neck squamous cell carcinomas. *J. Transl. Med*. **2011**, 9, 139. https://doi.org/10.1186/1479-5876-9-139.

731. Srivastava, K.; Srivastava, A.; Kumar, A.; Mittal, B. Significant association between toll-like receptor gene polymorphisms and gallbladder cancer. *Liver Int*. **2010**, 30, 1067–1072. https://doi.org/10.1111/j.1478-3231.2010.02268.x.
732. Gast, A.; Bermejo, J. L.; Claus, R.; Brandt, A.; Weires, M.; Weber, A.; Plass, C.; Sucker, A.; Hemminki, K.; Schadendorf, D.; et al. Association of Inherited Variation in Toll-Like Receptor Genes with Malignant Melanoma Susceptibility and Survival. *PLoS ONE*. **2011**, 6, e24370. https://doi.org/10.1371/journal.pone.0024370.
733. AlKhulaifi, F. M.; Alkhuriji, A.; Mansour, L.; Al-jurayyan, A.; Al-Mulhim, N. M.; Tashkandy, Y. A.; Aldossari, G. S.; Alomar, S. Association between Toll-like receptor 4 polymorphism and Acute Lymphoblastic Leukemia susceptibility in Saudi Arabian patients. *J. King Saud Univ.–Sci*. **2022**, 34, 101985. https://doi.org/10.1016/j.jksus.2022.101985.
734. Zhu, L.; Yuan, H.; Jiang, T.; Wang, R.; Ma, H.; Zhang, S. Association of TLR2 and TLR4 Polymorphisms with Risk of Cancer: A Meta-Analysis. *PLoS ONE*. **2013**, 8, e82858. https://doi.org/10.1371/journal.pone.0082858.
735. Li, P.; He, C.Y.; Xu, Q.; Sun, L. P.; Ha, M. W.; Yuan, Y. Effect of the 2081G/A Polymorphism of the TLR4 Gene and Its Interaction with Helicobacter pylori Infection on the Risk of Gastric Cancer in Chinese Individuals. *Genet. Test. Mol. Biomark*. **2014**, 18, 610–615. https://doi.org/10.1089/gtmb.2014.0047.
736. Zhao, D.; Wu, Y. H.; Zhao, T. C.; Jia, Z. F.; Cao, D. H.; Yang, N.; Wang, Y. Q.; Cao, X.Y.; Jiang, J. Single-nucleotide polymorphisms in Toll-like receptor genes are associated with the prognosis of gastric cancer and are not associated with Helicobacter pylori infection. *Infect. Genet. Evol*. **2019**, 73, 384–389. https://doi.org/10.1016/j.meegid.2019.06.005.
737. Oetting, W. S.; Guan, W.; Schladt, D. P.; Leduc, R. E.; Jacobson, P. A.; Matas, A. J.; Chinnakotla, S.; Schröppel, B.; Murphy, B. T.; Israni, A. K. Donor polymorphisms of toll-like receptor 4 associated with graft failure in liver transplant recipients. *Liver Transplant*. **2012**, 18, 1399–1405. https://doi.org/10.1002/lt.23549.
738. Shen, Y.; Bu, M.; Zhang, A.; Liu, Y.; Fu, B. Toll-like receptor 4 gene polymorphism downregulates gene expression and involves in susceptibility to bladder cancer. *Tumor Biol*. **2014**, 36, 2779–2784. https://doi.org/10.1007/s13277-014-2902-0.
739. Song, J.; Kim, D. Y.; Kim, C. S.; Kim, H. J.; Lee, D. H.; Lee, H.; Ko, W.; Lee, G. The association between Toll-like receptor 4 (TLR4) polymorphisms and the risk of prostate cancer in Korean men. *Cancer Genet. Cytogenet*. **2009**, 190, 88–92. https://doi.org/10.1016/j.cancergencyto.2008.12.011.
740. Lu, Q.; Takata, Y.; Ou, X.; Cao, D.; Xie, T.; Chen, X. Potential lncRNA diagnostic biomarkers for early gastric cancer. *Mol. Med. Rep.* **2017**, 16, 9545–9552. https://doi.org/10.3892/mmr.2017.7770.
741. Chen, H.; Yuan, X.; Zhou, L.; Huang, J.; Tao, L.; Cheng, L.; Tian, J. Cisplatin and paclitaxel target significant long noncoding RNAs in laryngeal squamous cell carcinoma. *Med. Oncol*. **2014**, 31, 246. https://doi.org/10.1007/s12032-014-0246-7.
742. Saito, Y.; Kasamatsu, A.; Yamamoto, A.; Shimizu, T.; Yokoe, H.; Sakamoto, Y.; Ogawara, K.; Shiiba, M.; Tanzawa, H.; Uzawa, K. ALY as a potential contributor to metastasis in human oral squamous cell carcinoma. *J. Cancer Res. Clin. Oncol.* **2012**, 139, 585–594. https://doi.org/10.1007/s00432-012-1361-5.
743. Florentin, J.; Zhao, J.; Tai, Y.; Sun, W.; Ohayon, L.; O'Neil, S.; Arunkumar, A.; Zhang, X.; Zhu, J.; Aaraj, Y.A.; et al. Loss of Amphiregulin drives inflammation and endothelial apoptosis in pulmonary hypertension. *Life Sci. Alliance.* **2022**, 5, e202101264. https://doi.org/10.26508/lsa.202101264.

744. Li, H.; Yang, L. Identification of novel immune infiltration-related biomarkers of sepsis based on bioinformatics analysis. *Cell. Mol. Biol*. **2023**, 69, 205–209. https://doi.org/10.14715/cmb/2023.69.12.33.
745. Wain, L. V.; Vaez, A.; Jansen, R. C.; Joehanes, R.; Van Der Most, P. J.; Erzurumluoglu, A. M.; O'Reilly, P. F.; Cabrera, C.; Warren, H. R.; Rose, L. M.; et al. Novel blood pressure locus and gene discovery using Genome-Wide association study and expression data sets from blood and the kidney. *Hypertension*. **2017**, 70, e4–e19. https://doi.org/10.1161/hypertensionaha.117.09438.
746. Iniesta, R.; Campbell, D.; Venturini, C.; Faconti, L.; Singh, S.; Irvin, M. R.; Cooper-DeHoff, R. M.; Johnson, J. A.; Turner, S. T.; Arnett, D. K.; et al. Gene variants at LOCI related to blood pressure account for variation in response to antihypertensive drugs between black and white individuals. *Hypertension*. **2019**, 74, 614–622. https://doi.org/10.1161/hypertensionaha.118.12177.
747. Salemi, M.; Barone, C.; Romano, C.; Zolezzi, F.; Romano, C.; Scavuzzo, C.; Salluzzo, R.; Scillato, F.; Signorelli, M.; Kapetis, D.; et al. Gene expression profiling and qRT-PCR expression of RRP1B, PCNT, KIF21A and ADRB2 in leucocytes of Down's syn drome subjects. *J. Genet*. **2012**, 93 (Suppl. S1), 18–23. https://doi.org/10.1007/s12041-012-0132-z.
748. Hsieh, S.; Look, M. P.; Sieuwerts, A. M.; Foekens, J. A.; Hunter, K. W. Distinct inherited metastasis susceptibility exists for dif ferent breast cancer subtypes: A prognosis study. *Breast Cancer Res*. **2009**, 11. https://doi.org/10.1186/bcr2412.
749. Nanchari, S.R.; Cingeetham, A.; Meka, P.; Surekha, D.; Tipirisetti, N.; Padala, C.; Annamaneni, S.; Hanumanth, S.R.; Digu marthi, R.R.; Satti, V. Rrp1B gene polymorphism (1307T>C) in metastatic progression of breast cancer. *Tumor Biol*. **2014**, 36, 615–621. https://doi.org/10.1007/s13277-014-2613-6.
750. Ugenskienė, R.; Myrzaliyeva, D.; Jankauskaitė, R.; Gedminaitė, J.; Jančiauskienė, R.; Šepetauskienė, E.; Juozaitytė, E. The con tribution of SIPA1 and RRP1B germline polymorphisms to breast cancer phenotype, lymph node status and survival in a group of Lithuanian young breast cancer patients. *Biomarkers*. **2016**, 21, 363–370. https://doi.org/10.3109/1354750x.2016.1141989.
751. Al-Husseini R. M. A.; Hussain, R. A.; Abed, A. M. Assess the contribution of ribosomal RNA processing 1B gene polymorphisms in breast cancer in Iraqi patients. Ann. Biol. 2020, 36, 26–33.
752. Brooks, R.; Kizer, N.; Nguyen, L.; Jaishuen, A.; Wanat, K.; Nugent, E. K.; Grigsby, P.; Allsworth, J. E.; Rader, J. S. Polymorphisms in MMP9 and SIPA1 are associated with increased risk of nodal metastases in early-stage cervical cancer. *Gynecologic Oncology.* **2010**, *116*(3), 539–543. https://doi.org/10.1016/j.ygyno.2009.09.037.
753. Roberts, Hong, C.; Edge, S.; Yao, S.; Nesline, M.; Ambrosone, C. P1-09-06: Single nucleotide polymorphisms in the BRMS1 and SIPA1 metastasis suppressor genes as prognostic markers in breast cancer patients. *Cancer Research.* **2011**, *71*(24_Supplement), P1-06. https://doi.org/10.1158/0008-5472.sabcs11-p1-09-06.
754. Qu, S.; Cai, H.; Long, J.; Cai, Q.; Courtney, R.; Shu, X.; Gao, Y.; Zheng, W. Abstract 891: Association of genetic polymorphisms of SIPA1 with breast cancer risk and survival among Chinese women. *Cancer Research*. **2010**, *70*(8_Supplement), 891. https://doi.org/10.1158/1538-7445.am10-891.

755. Gaudet, M. M.; Hunter, K.; Pharoah, P.; Dunning, A. M.; Driver, K.; Lissowska, J.; Sherman, M.; Peplonska, B.; Brinton, L. A.; Chanock, S.; Garcia-Closas, M. Genetic variation in SIPA1 in relation to breast cancer risk and survival after breast cancer diagnosis. *International Journal of Cancer.* **2009**, *124*(7), 1716–1720. https://doi.org/10.1002/ijc.23919.
756. Roberts, M. R.; Sucheston-Campbell, L. E.; Zirpoli, G. R.; Higgins, M.; Freudenheim, J. L.; Bandera, E. V.; Ambrosone, C. B.; Yao, S. Single nucleotide variants in metastasis-related genes are associated with breast cancer risk, by lymph node involvement and estrogen receptor status, in women with European and African ancestry. *Molecular Carcinogenesis.* **2016**, *56*(3), 1000–1009. https://doi.org/10.1002/mc.22565.
757. Hsieh, S.; Smith, R. A.; Lintell, N. A.; Hunter, K. W.; Griffiths, L. R. Polymorphisms of the SIPA1 gene and sporadic breast cancer susceptibility. *BMC Cancer.* **2009**, *9*(1). https://doi.org/10.1186/1471-2407-9-331.
758. Mackawy, A. M.; Megahed, O. SIPA1 Gene Polymorphisms and the Risk of Breast Carcinoma among the Egyptian Females: A Pilot Study. *Journal of Integrative Oncology.* **2018**, *07*(01). https://doi.org/10.4172/2329-6771.1000202.
759. Nguyen, H. T.; Yen, L. N.; Ngoc, T. B. The Relationship between the Mutation rs3741378 of SIPA1 Gene and Breast Cancer in Vietnamese Women. *Int J Hum Genet.* **2014**, *14*(3,4): 161-168. https://doi.org/10.31901/24566330.2014/14.03-4.07.
760. Gdowicz-Kłosok, A.; Giglok, M.; Drosik, A.; Suwiński, R.; Butkiewicz, D. The SIPA1 -313A>G polymorphism is associated with prognosis in inoperable non-small cell lung cancer. *Tumor Biology.* **2014**, *36*(2), 1273–1278. https://doi.org/10.1007/s13277-014-2753-8.
761. Tian, J.; Liu, Y.; Zhu, B.; Tian, Y.; Zhong, R.; Chen, W.; Lu, X.; Zou, L.; Shen, N.; Qian, J.; Li, H.; Miao, X.; Wang, L. SF3A1 and pancreatic cancer: new evidence for the association of the spliceosome and cancer. *Oncotarget*. **2015**, 6(35), 37750–37757. https://doi.org/10.18632/oncotarget.5647.
762. Yang, Y.; Lu, W.; Ning, M.; Zhou, X.; Wan, X.; Mi, Q.; Yang, X.; Zhang, D.; Zhang, Y.; Jiang, B.; He, L.; Liu, J.; Zou, Y. A functional SNP rs895819 on pre-miR-27a is associated with bipolar disorder by targeting NCAM1. *Communications Biology.* **2022**, *5*(1). https://doi.org/10.1038/s42003-022-03263-6.
763. Guo, W.; Dong, Z.; Bai, Y.; Guo, Y.; Shen, S.; Kuang, G.; Xu, J. Associations between polymorphisms of HOTAIR and risk of gastric cardia adenocarcinoma in a population of north China. *Tumor Biology*. **2014**, 36(4), 2845–2854. https://doi.org/10.1007/s13277-014-2912-y.
764. Aglan, S.; Elsammak, M.; Elsammak, O.; El-Bakoury, E.; Elsheredy, H.; Ahmed, Y.; Sultan, M.; Awad, A. Evaluation of serum Nestin and HOTAIR rs12826786 C>T polymorphism as screening tools for breast cancer in Egyptian women. *Journal of Medical Biochemistry*. **2021**, 40(1), 17–25. https://doi.org/10.5937/jomb0-25295.
765. Bayram, S.; Sümbül, A. T.; Dadaş, E. A functional HOTAIR rs12826786 C>T polymorphism is associated with breast cancer susceptibility and poor clinicopathological characteristics in a Turkish population: a hospital-based case–control study. *Tumor Biology*. **2015**, 37(4), 5577–5584. https://doi.org/10.1007/s13277-015-4430-y.
766. Oliveira, A. I.; Xavier-Magalhães, A.; Moreira-Barbosa, C.; Magalhães, H.; Henrique, R.; Jerónimo, C.; Costa, B. M. Influence of HOTAIR rs920778 and rs12826786 genetic variants on prostate cancer risk and progression-free survival. *Biomarkers in Medicine*. **2018**, 12(3), 257–264. https://doi.org/10.2217/bmm-2017-0258.

767. Li, J.; Cui, Z.; Li, H.; Lv, X.; Gao, M.; Yang, Z.; Bi, Y.; Zhou, B.; Yin, Z. Long non-coding RNA HOTAIR polymorphism and susceptibility to cancer: an updated meta-analysis. *Environmental Health and Preventive Medicine*. **2018**, 23(1). https://doi.org/10.1186/s12199-018-0697-0.
768. Liu, X.; Zhao, Y.; Li, Y.; Lin, F.; Zhang, J. Association between HOTAIR genetic polymorphisms and cancer susceptibility: A meta-analysis involving 122,832 subjects. *Genomics*. **2020**, *112*(5), 3036–3055. https://doi.org/10.1016/j.ygeno.2020.05.018.
769. Martins, E. P.; De Castro, J. V.; Fontes, R.; Monteiro-Reis, S.; Henrique, R.; Jerónimo, C.; Costa, B. M. Relevance of HOTAIR rs920778 and rs12826786 Genetic Variants in Bladder Cancer Risk and Survival. *Cancers*. **2024**, *16*(2), 434. https://doi.org/10.3390/cancers16020434.
770. Xavier-Magalhães, A.; Oliveira, A. I.; De Castro, J. V.; Pojo, M.; Gonçalves, C. S.; Lourenço, T.; Viana-Pereira, M.; Costa, S.; Linhares, P.; Vaz, R.; Nabiço, R.; Amorim, J.; Pinto, A. A.; Reis, R. M.; Costa, B. M. Effects of the functional HOTAIR rs920778 and rs12826786 genetic variants in glioma susceptibility and patient prognosis. *Journal of Neuro-Oncology*. **2017**, *132*(1), 27–34. https://doi.org/10.1007/s11060-016-2345-0.
771. Wang, B.; Yuan, F.; Zhang, F.; Miao, Z.; Jiang, D. A systematic review and meta-analysis of the association between HOTAIR polymorphisms and susceptibility to breast cancer. *Archives of Medical Science*. **2019**, *19*(1):128-137. https://doi.org/10.5114/aoms.2019.87537.
772. Khorshidi, H. R.; Taheri, M.; Noroozi, R.; Soudyab, M.; Sayad, A.; Ghafouri-Fard, S. Investigation of the association of HOTAIR single nucleotide polymorphisms and risk of breast cancer in an Iranian population. *International Journal of Cancer Management*. **2017**, *10*(5). https://doi.org/10.5812/ijcm.7498.
773. Ülger, Y.; Dadaş, E.; Kaya, B. Y.; Sümbül, A. T.; Genç, A.; Bayram, S. The analysis of lncRNA HOTAIR rs12826786 C>T polymorphism and gastric cancer susceptibility in a Turkish population: lack of any association in a hospital-based case–control study. *Irish Journal of Medical Science*. **2017**, *186*(4), 859–865. https://doi.org/10.1007/s11845-017-1596-x.
774. Dadaş, E.; Aydın, M. Effect of HOTAIR rs12826786 and rs1899663 polymorphisms on lung cancer susceptibility and clinicopathological characteristics in a turkish population: a hospital-based case-control study. *Cellular and Molecular Biology*. **2018**, *64*(7), 97–102. https://doi.org/10.14715/cmb/2018.64.7.17.
775. Xu, T.; Zhou, Y.; Zhang, Y.; Yang, C.; Yang, H.; Zhu, S. Association between HOTAIR polymorphisms and cancer risk：a meta-analysis based on twenty-one case-control studies. *PubMed*. **2019**, *24*(1), 354–367. https://pubmed.ncbi.nlm.nih.gov/30941992.
776. Ke, C.; Feng, X.; Li, J.; Chen, S.; Hu, X. Association between long non-coding RNA HOTAIR polymorphism and lung cancer risk: A systematic review and meta-analysis. *Experimental and Therapeutic Medicine*. **2022**, *24*(2). https://doi.org/10.3892/etm.2022.11477.
777. Kashani, S.; Sasan, H. A.; Bahari, G.; Mollashahi, B.; Hashemi, S. M.; Taheri, M. Association between HOTAIR Polymorphisms and Lymphoma. *Asian Pacific Journal of Cancer Prevention*. **2021**, *22*(9), 2831–2835. https://doi.org/10.31557/apjcp.2021.22.9.2831.
778. Jin, H.; Lu, X.; Ni, J.; Sun, J.; Gu, B.; Ding, B.; Zhu, H.; Ma, C.; Cui, M.; Xu, Y.; Zhang, Z.; Lercher, M.; Chen, J.; Gao, N.; Wang, S. HOTAIR rs7958904 polymorphism is associated with increased cervical cancer risk in a Chinese population. *Scientific Reports*. **2017**, *7*(3144). https://doi.org/10.1038/s41598-017-03174-1.

779. Kim, J. O.; Jun, H. H.; Kim, E. J.; Lee, J. Y.; Park, H. S.; Ryu, C. S.; Kim, S.; Oh, D.; Kim, J. W.; Kim, N. K. Genetic Variants of HOTAIR Associated With Colorectal Cancer Susceptibility and Mortality. *Frontiers in Oncology*. **2020**, *10*: 72. https://doi.org/10.3389/fonc.2020.00072.
780. Lin, Y.; Guo, W.; Li, N.; Fu, F.; Lin, S.; Wang, C. Polymorphisms of long non-coding RNA HOTAIR with breast cancer susceptibility and clinical outcomes for a southeast Chinese Han population. *Oncotarget*. **2017**, *9*(3), 3677–3689. https://doi.org/10.18632/oncotarget.23343.
781. Wu, B.; Liu, J.; Wang, B.; Liao, X.; Cui, Z.; Ding, N. Association on polymorphisms in LncRNA HOTAIR and susceptibility to HNSCC in Chinese population. *PubMed*. **2018**, *22*(3), 702–706. https://doi.org/10.26355/eurrev_201802_14296.
782. Zhou, Q.; Chen, F.; Fei, Z.; Zhao, J.; Liang, Y.; Pan, W.; Liu, X.; Zheng, D. Genetic variants of lncRNA HOTAIR contribute to the risk of osteosarcoma. *Oncotarget*. **2016**, *7*(15), 19928–19934. https://doi.org/10.18632/oncotarget.7957.
783. Xue, Y.; Gu, D.; Ma, G.; Zhu, L.; Hua, Q.; Chu, H.; Tong, N.; Chen, J.; Zhang, Z.; Wang, M. Genetic variants in lncRNA HOTAIR are associated with risk of colorectal cancer. *Mutagenesis*. **2014**, *30*(2), 303–310. https://doi.org/10.1093/mutage/geu076],
784. Wu, H.; Shang, X.; Shi, Y.; Yang, Z.; Zhao, J.; Yang, M.; Li, Y.; Xu, S. Genetic variants of lncRNA HOTAIR and risk of epithelial ovarian cancer among Chinese women. *Oncotarget*. **2016**, *7*(27), 41047–41052. https://doi.org/10.18632/oncotarget.8535.
785. Ge, Y.; Jiang, R.; Zhang, M.; Wang, H.; Zhang, L.; Tang, J.; Liang, C. Analyzing 37,900 Samples Shows Significant Association between Hotair Polymorphisms and Cancer Susceptibility: A Meta-Analysis. *The International Journal of Biological Markers*. **2017**, *32*(2), 231–242. https://doi.org/10.5301/jbm.5000235.
786. Zhang, Z.; Tong, X.; Zhang, W.; Fu, W. Association between the HOTAIR polymorphisms and cancer risk: an updated meta-analysis. *Oncotarget*. **2016**, *8*(3), 4460–4470. https://doi.org/10.18632/oncotarget.13880.
787. Zhang, J.; Liu, X.; You, L.; Zhou, R. Significant association between long non-coding RNA HOTAIR polymorphisms and cancer susceptibility: a meta-analysis. *OncoTargets and Therapy*. **2016**, 3335. https://doi.org/10.2147/ott.s107190.
788. Cho, S. H.; Kim, J. H.; Park, H. W.; Park, H. S.; An, H. J.; Kim, Y. R.; Ahn, E. H.; Lee, W. S.; Kim, N. K. Associations between HOTAIR polymorphisms rs4759314, rs920778, rs1899663, and rs7958904 and risk of primary ovarian insufficiency in Korean women. *Maturitas*. **2021**, *144*, 74–80. https://doi.org/10.1016/j.maturitas.2020.10.023.
789. Guo, L.; Lu, X.; Zheng, L.; Liu, X.; Hu, M. Association of Long Non-Coding RNA HOTAIR Polymorphisms with Cervical Cancer Risk in a Chinese Population. *PLoS ONE*. **2016**, *11*(7), e0160039. https://doi.org/10.1371/journal.pone.0160039.
790. Liu, Y.; Zhang, Q.; Ni, R. Association between genetic variants (rs920778, rs4759314, and rs217727) in LncRNAs and cervical cancer susceptibility in Chinese population: A systematic review and meta-analysis. *Frontiers in Genetics*. **2022**, *13*. https://doi.org/10.3389/fgene.2022.988207.
791. Weng, S.; Wu, W.; Hsiao, Y.; Yang, S.; Hsu, C.; Wang, P. Significant association of long non-coding RNAs HOTAIR genetic polymorphisms with cancer recurrence and patient survival in patients with uterine cervical cancer. *International Journal of Medical Sciences*. **2018**, *15*(12), 1312–1319. https://doi.org/10.7150/ijms.27505.

792. Zhang, X.; Zhou, L.; Fu, G.; Sun, F.; Shi, J.; Wei, J.; Lu, C.; Zhou, C.; Yuan, Q.; Yang, M. The identification of an ESCC susceptibility SNP rs920778 that regulates the expression of lncRNAHOTAIRvia a novel intronic enhancer. *Carcinogenesis*. **2014**, *35*(9), 2062–2067. https://doi.org/10.1093/carcin/bgu103.
793. Wang, C.; Li, Y.; Li, Y.; Zhang, H.; Gong, H.; Yuan, Y.; Li, W.; Liu, H.; Chen, J. HOTAIR lncRNA SNPs rs920778 and rs1899663 are associated with smoking, male gender, and squamous cell carcinoma in a Chinese lung cancer population. *Acta Pharmacologica Sinica*. **2018**, *39*(11), 1797–1803. https://doi.org/10.1038/s41401-018-0083-x.
794. Li, H.; Tang, X.; Liu, Y.; Li, W.; Chen, Q.; Pan, Y. Association of Functional Genetic Variants of HOTAIR with Hepatocellular Carcinoma (HCC) Susceptibility in a Chinese Population. *Cellular Physiology and Biochemistry*. **2017**, *44*(2), 447–454. https://doi.org/10.1159/000485011.
795. Lv, Z.; Kou, C.; Chen, N.; Jia, L.; Sun, X.; Gao, Y.; Bai, R.; Yang, M.; Cui, J. Single nucleotide polymorphisms in HOTAIR are related to breast cancer risk and prognosis in the northeastern Chinese population. *Frontiers in Oncology*. **2021**, *11*. https://doi.org/10.3389/fonc.2021.706428.
796. Yan, R.; Cao, J.; Song, C.; Chen, Y.; Wu, Z.; Wang, K.; Dai, L. Polymorphisms in lncRNA HOTAIR and susceptibility to breast cancer in a Chinese population. *Cancer Epidemiology*. **2015**, *39*(6), 978–985. https://doi.org/10.1016/j.canep.2015.10.025.
797. Bayram, S.; Sümbül, A. T.; Batmacı, C. Y.; Genç, A. Effect of HOTAIR rs920778 polymorphism on breast cancer susceptibility and clinicopathologic features in a Turkish population. *Tumor Biology*. **2015**, *36*(5), 3863–3870. https://doi.org/10.1007/s13277-014-3028-0.
798. Rajagopal, T.; Seshachalam, A.; Akshaya, R.; Rathnam, K. K.; Talluri, S.; Jothi, A.; Dunna, N. R. Association of HOTAIR (rs920778 and rs1899663) and NME1 (rs16949649 and rs2302254) gene polymorphisms with breast cancer risk in India. *Gene*. **2020**, *762*, 145033. https://doi.org/10.1016/j.gene.2020.145033.
799. Hassanzarei, S.; Hashemi, M.; Sattarifard, H.; Hashemi, S. M.; Bahari, G.; Ghavami, S. Genetic polymorphisms of HOTAIR gene are associated with the risk of breast cancer in a sample of southeast Iranian population. *Tumor Biology*. **2017**, *39*(10), 101042831772753. https://doi.org/10.1177/1010428317727539.
800. De Silva, S.; Hewag, A. S.; Shafeeu, F. A.; Manoharan, V.; De Silva, K. A pilot study of detecting RS920778 and RS1899663 genetic variants of HOTAIR in a cohort of sporadic breast cancer patients in Sri Lanka. *Archives of Breast Cancer*. **2024**, *11*(2), 204–207. https://doi.org/10.32768/abc.2024112204-207.
801. Qiu, H.; Wang, X.; Guo, R.; Liu, Q.; Wang, Y.; Yuan, Z.; Li, J.; Shi, H. HOTAIR rs920778 polymorphism is associated with ovarian cancer susceptibility and poor prognosis in a Chinese population. *Future Oncology*. **2017**, *13*(4), 347–355. https://doi.org/10.2217/fon-2016-0290.
802. Su, S.; Hsieh, M.; Lin, C.; Chuang, C.; Liu, Y.; Yeh, C.; Yang, S. Impact of HOTAIR gene polymorphism and environmental risk on oral cancer. *Journal of Dental Research*. **2018**, *97*(6), 717–724. https://doi.org/10.1177/0022034517749451.
803. Anber, N.; Tarabay, M. M.; Elmougy, R.; Abdel-Dayem, M. A.; Elbendary, E. Y. Association of HOTAIR gene rs920778 (C > T) and rs4759314 (A > G) polymorphism with breast cancer in Egyptian women. *Molecular Biology Reports*. **2023**, *50*(11), 9153–9163. https://doi.org/10.1007/s11033-023-08725-6.

804. Minn, A. K. K.; Sato, N.; Mieno, M. N.; Arai, T.; Muramatsu, M. Association study of long non-coding RNA HOTAIR rs920778 polymorphism with the risk of cancer in an elderly Japanese population. *Gene*. **2020**, *729*, 144263. https://doi.org/10.1016/j.gene.2019.144263.
805. Beiramzadeh, A.; Heidari, Z.; Norozi, M.; Saravani, M. Association Between HOTAIR rs920778 and H19 rs3741219 Polymorphisms with Hashimoto's Thyroiditis (HT) and Graves' Disease (GD). *Reports of Biochemistry and Molecular Biology*. **2022**, *11*(3), 377–385. https://doi.org/10.52547/rbmb.11.3.377.
806. Namarvar, M. K.; Pouladi, N.; Ghaffarin, S. Association of HOTAIR (rs920778 C>T) Polymorphism with Thyroid Cancer Susceptibility in the Northwest of Iran. *Scientific Journal of Kurdistan University of Medical Sciences*. **2022**, *27*(4), 1–12. https://doi.org/10.52547/sjku.27.4.1.
807. Sargazi, S.; Abghari, A. Z.; Mirinejad, S.; Nia, M. H.; Majidpour, M.; Danesh, H.; Saravani, R.; Sheervalilou, R.; Shakiba, M.; Abghari, F. Z. Long noncoding RNA HOTAIR polymorphisms and susceptibility to bipolar disorder: a preliminary case–control study. *Nucleosides Nucleotides & Nucleic Acids*. **2022**, *41*(7), 684–701. https://doi.org/10.1080/15257770.2022.2065017.
808. Majidpour, M.; Saravani, R.; Sargazi, S.; Sargazi, S.; Harati-Sadegh, M.; Khorrami, S.; Sarhadi, M.; Alidadi, A. A study on associations of long noncoding RNA HOTAIR polymorphisms with genetic susceptibility to chronic kidney disease. *Journal of Clinical Laboratory Analysis*. **2024**. https://doi.org/10.1002/jcla.25086.
809. Safari, M.; Noroozi, R.; Taheri, M.; Ghafouri-Fard, S. The rs12826786 in HOTAIR lncRNA Is Associated with Risk of Autism Spectrum Disorder. *Journal of Molecular Neuroscience*. **2019**, *70*(2), 175–179. https://doi.org/10.1007/s12031-019-01421-w.
810. Sadeghi, Z.; Mokhtari, M. J. Association of rs12826786 and rs1899663, Long Non-coding RNA HOTAIR Polymorphic Variants, with Susceptibility to Rheumatoid Arthritis Disease. *Indian Journal of Clinical Biochemistry*. **2024**. https://doi.org/10.1007/s12291-024-01238-4.
811. Shaker, O. G.; Abdelaleem, O. O.; Mahmoud, R. H.; Abdelghaffar, N. K.; Ahmed, T. I.; Said, O. M.; Zaki, O. M. Diagnostic and prognostic role of serum miR-20b, miR-17-3p, HOTAIR, and MALAT1 in diabetic retinopathy. *IUBMB Life*. **2018**, *71*(3), 310–320. https://doi.org/10.1002/iub.1970.
812. Biswas, S.; Feng, B.; Chen, S.; Liu, J.; Aref-Eshghi, E.; Gonder, J.; Ngo, V.; Sadikovic, B.; Chakrabarti, S. The Long Non-Coding RNA HOTAIR is a critical epigenetic mediator of angiogenesis in diabetic retinopathy. *Investigative Ophthalmology & Visual Science*. **2021**, *62*(3), 20. https://doi.org/10.1167/iovs.62.3.20.
813. Sun, Y.; Chou, Y.; Tsai, H.; Hsiao, Y.; Lee, C.; Yang, S.; Ting, K.; Wang, P. Impact of genetic variants of long noncoding RNA Metastasis-Associated lung adenocarcinoma Transcript 1 on uterine cervical cancer. *Journal of Cancer.* **2022**, *13*(7), 2150–2158. https://doi.org/10.7150/jca.70730.
814. Safan, M.; Elhelbawy, N.; Elhelbawy, M.; Mostafa, B.; Bayomy, N. Metastasis-associated lung adenocarcinoma transcript 1 gene polymorphism in Egyptian patients with hepatocellular carcinoma. *Menoufia Medical Journal.* **2022**, *35*(4), 1625. https://doi.org/10.4103/mmj.mmj_200_22.
815. Ji, X.; Zhang, J.; Liu, L.; Lin, Z.; Pi, L.; Lin, Z.; Tian, N.; Lin, X.; Chen, S.; Yu, X.; Gao, Y. Association of tagSNPs at lncRNA MALAT-1 with HCC Susceptibility in a Southern Chinese Population. *Scientific Reports.* **2019**, *9*(1). https://doi.org/10.1038/s41598-019-47165-w.

816. Tong, G.; Tong, W.; He, R.; Cui, Z.; Li, S.; Zhou, B.; Yin, Z. MALAT1 polymorphisms and lung cancer susceptibility in a Chinese Northeast Han population. *International Journal of Medical Sciences.* **2022**, *19*(8), 1300–1306. https://doi.org/10.7150/ijms.73026.
817. Lin, S.; Lu, J.; Hsieh, W.; Chou, Y.; Su, T.; Tsai, T.; Tsai, Y.; Yang, P.; Yang, S. Evaluation of the clinical significance of long non-coding RNA MALAT1 genetic variants in human lung adenocarcinoma. *Aging.* **2024**, *16*(6), 5740–5750. https://doi.org/10.18632/aging.205675.
818. Kelishadi, Z. R.; Mojbafan, M.; Aminimoghaddam, S.; Akouchekian, M.; Talebi, S. Examination of the Expression Level HOTAIR, MALAT1and H19 Genes and Single Nucleotide Polymorphisms rs920778, rs619586 and rs2839698 Respectively in the Same Genes, in Response to Treatment of Endometrial Cancer Patients. *SSRN Electronic Journal.* **2022**. https://doi.org/10.2139/ssrn.4246444.
819. Gao, X. R.; Wang, X. Y.; Li, X. Y.; Sun, Y. Q.; Zhang, S. L. Clinical Significance and Function of MALAT1 Gene Expression and the rs619586 Polymorphism in Colorectal Cancer. *PubMed.* **2022**, *35*(8), 768–771. https://doi.org/10.3967/bes2022.101.
820. Wang, M.; Liu, J. Retracted: MALAT1 rs619586 polymorphism functions as a prognostic biomarker in the management of differentiated thyroid carcinoma. *Journal of Cellular Physiology.* **2019**, *235*(2), 1700–1710. https://doi.org/10.1002/jcp.29089.
821. Ding, Y.; Wen, Y.; Chuang, C.; Lin, C.; Yang, Y.; Liu, Y.; Chang, W.; Chang, L.; Yang, S.; Chien, M. Combined impacts of genetic variants of Long Non-Coding RNA MALAT1 and the environmental carcinogen on the susceptibility to and progression of oral squamous cell carcinoma. *Frontiers in Oncology.* **2021**, *11*. https://doi.org/10.3389/fonc.2021.684941.
822. Gong, W. J.; Yin, J. Y.; Li, X. P.; Fang, C.; Xiao, D.; Zhang, W.; Zhou, H.; Li, X.; Liu, Z. Association of well-characterized lung cancer lncRNA polymorphisms with lung cancer susceptibility and platinum-based chemotherapy response. *Tumor Biol.* **2016**, 37, 8349–8358. https://doi.org/10.1007/s13277-015-4497-5.
823. Hu, J.; Wang, S.; Chou, Y.; Chiu, K.; Li, J.; Chen, C.; Hung, S.; Yang, C.; Ou, Y.; Cheng, C.; Lin, C.; Yang, S. Associations between LncRNA MALAT1 Polymorphisms and Lymph Node Metastasis in Prostate Cancer. *Diagnostics.* **2021**, *11*(9), 1692. https://doi.org/10.3390/diagnostics11091692.
824. Cao, L.; Yan, G.; Yu, S.; Li, F.; Su, Z.; Hou, X.; Xiao, J.; Tian, T. Associations of MALAT1 and its functional single nucleotide polymorphisms with cancer. *Pathology – Research and Practice.* **2022**, *236*, 153988. https://doi.org/10.1016/j.prp.2022.153988.
825. Chen, M.; Cai, D.; Gu, H.; Yang, J.; Fan, L. MALAT1 rs619586 A/G polymorphisms are associated with decreased risk of lung cancer. *Medicine.* **2021**, *100*(12), e23716. https://doi.org/10.1097/md.0000000000023716.
826. Zhao, K.; Jin, S.; Wei, B.; Cao, S.; Xiong, Z. Association study of genetic variation of lncRNA MALAT1 with carcinogenesis of colorectal cancer. *Cancer Management and Research.* **2018**, *Volume 10*, 6257–6261. https://doi.org/10.2147/cmar.s177244.
827. Wen, J.; Chen, L.; Tian, H.; Li, J.; Zhang, M.; Cao, Q.; Zhang, W.; Chen, S.; Shi, L. Effect of MALAT1 polymorphisms on papillary thyroid cancer in a Chinese population. *Journal of Cancer.* **2019**, *10*(23), 5714–5721. https://doi.org/10.7150/jca.28887.
828. Yuan, N.; Chang, N.; Lee, N.; Yang, N.; Su, N.; Lin, N.; Yang, N.; Chien, N. Genetic Variants of lncRNA MALAT1 Exert Diverse Impacts on the Risk and Clinicopathologic Characteristics of Patients with Hepatocellular Carcinoma. *Journal of Clinical Medicine.* **2019**, *8*(9), 1406. https://doi.org/10.3390/jcm8091406.

829. Ni, W.; Wang, X.; Sun, Y.; Gao, X. Meta-analysis of the association between MALAT1 rs619586 A>G polymorphism and cancer risk. *Journal of International Medical Research.* **2020**, *48*(7), 030006052094196. https://doi.org/10.1177/0300060520941969.
830. Zheng, L.; Rong, L.; Cheng, Z. Association Between LncRNA MALAT1 Polymorphisms and Cancer Risk: A Meta-Analysis Based on7007 Cases and 8791 Controls. *Research Square (Research Square).* **2020**. https://doi.org/10.21203/rs.3.rs-42022/v1.
831. Qu, Y.; Shao, N.; Yang, W.; Wang, J.; Cheng, Y. Association of polymorphisms in MALAT1 with the risk of esophageal squamous cell carcinoma in a Chinese population. *OncoTargets and Therapy.* **2019**, *Volume 12*, 2495–2503. https://doi.org/10.2147/ott.s191155.
832. Chen, G.; Zhang, M.; Liang, Z.; Chen, S.; Chen, F.; Zhu, J.; Zhao, M.; He, J.; Hua, W.; Duan, P. Association of polymorphisms in MALAT1 with the risk of endometrial cancer in Southern Chinese women. *Journal of Clinical Laboratory Analysis.* **2019**, *34*(4). https://doi.org/10.1002/jcla.23146.
833. Liu, T.; Ma, J.; Hou, D.; Wang, W.; Cao, H. Haplotype-GGGT in long non-coding RNA MALAT1 inhibits brain metastatic lung cancer and lymph nodes of lung cancer via the MALAT1/miR-328/KATNB1. *Aging*. **2023**, *15*(6), 1918–1930. https://doi.org/10.18632/aging.204563.
834. Zheng, J.; Pang, C.; Du, W.; Wang, L.; Sun, L.; Xing, Z. An allele of rs619586 polymorphism in MALAT1 alters the invasiveness of meningioma via modulating the expression of collagen type V alpha (COL5A1). *Journal of Cellular and Molecular Medicine.* **2020**, *24*(17), 10223–10232. https://doi.org/10.1111/jcmm.15637.
835. Che, D.; Yang, Y.; Xu, Y.; Fang, Z.; Pi, L.; Fu, L.; Zhou, H.; Tan, Y.; Lu, Z.; Li, L.; Liang, Q.; Xuan, Q.; Gu, X. The lncRNA MALAT1 rs619586 G Variant Confers Decreased Susceptibility to Recurrent Miscarriage. *Frontiers in Physiology.* **2019**, *10*. https://doi.org/10.3389/fphys.2019.00385.
836. Li, Q.; Zhu, W.; Zhang, B.; Wu, Y.; Yan, S.; Yuan, Y.; Zhang, H.; Li, J.; Sun, K.,; Wang, H.; Yu, T. The MALAT1 gene polymorphism and its relationship with the onset of congenital heart disease in Chinese. *Bioscience Reports.* **2018**, *38*(3). https://doi.org/10.1042/bsr20171381.
837. Hu, W.; Ding, H.; Ouyang, A.; Zhang, X.; Xu, Q.; Han, Y.; Zhang, X.; Jin, Y. LncRNA MALAT1 gene polymorphisms in coronary artery disease: a case–control study in a Chinese population. *Bioscience Reports.* **2019**, *39*(3). https://doi.org/10.1042/bsr20182213.
838. Wang, G.; Li, Y.; Peng, Y.; Tang, J.; Li, H.. Association of polymorphisms in *MALAT1* with risk of coronary atherosclerotic heart disease in a Chinese population. *Lipids Health Dis.* **2018**, *17*, 75. https://doi.org/10.1186/s12944-018-0728-2.
839. Fathy, N.; Kortam, M. A.; Shaker, O. G.; Sayed, N. H. Long noncoding RNAs MALAT1 and ANRIL gene variants and the risk of cerebral ischemic Stroke: an association study. *ACS Chemical Neuroscience.* **2021**, *12*(8), 1351–1362. https://doi.org/10.1021/acschemneuro.0c00822.
840. Samadi-Khouzani, A.; Parizi, P. K.; Ghafari, F.; Esmaeili, S.; Peymani, M.; Momtazi-Borojeni, A. A. Association between rs619586 (A/G) polymorphism in the gene encoding lncRNA-MALAT1 with type 2 diabetes susceptibility among the Isfahan population in Iran. *Int J Diabetes Dev Ctries.* **2022**, 42, 77–81. https://doi.org/10.1007/s13410-021-00949-1.

841. Chang, W.; Zhang, L.; Wen, L.; Huang, Q.; Tong, X.; Tao, Y.; Chen, G. Association of tag single nucleotide polymorphisms (SNPs) at lncRNA MALAT1 with type 2 diabetes mellitus susceptibility in the Chinese Han population: A case-control study. *Gene.* **2023**, *851*, 147008. https://doi.org/10.1016/j.gene.2022.147008.
842. Ghafouri-Fard, S.; Gholipour, M.; Abak, A.; Hussen, B. M.; Oskooei, V. K.; Taheri, M.; Rakhshan, A. Association analysis of MALAT1 polymorphisms and risk of psoriasis among Iranian patients. *International Journal of Immunogenetics.* **2021**, *49*(2), 83–87. https://doi.org/10.1111/iji.12562.
843. Yue, J.; Zheng, S. Analysis of association between MALAT1 haplotype and the severity of Normal Tension Glaucoma (NTG). *Research Square (Research Square).* **2020**. https://doi.org/10.21203/rs.3.rs-21631/v1.
844. Wu, S.; Sun, H.; Wang, Y.; Yang, X.; Meng, Q.; Yang, H.; Zhu, H.; Tang, W.; Li, X.; Aschner, M.; Chen, R. MALAT1 rs664589 Polymorphism Inhibits Binding to miR-194-5p, Contributing to Colorectal Cancer Risk, Growth, and Metastasis. *Cancer Research.* **2019**, *79*(20), 5432–5441. https://doi.org/10.1158/0008-5472.can-19-0773.
845. Yu, N. H.; Tu, N. S.; Shen, N. C.; Bai, N. X.; Sun, N. J.; Shi, N. M.; Li, N. C.; Yuan, N. H. Effects of the lncRNA MALAT1 gene region rs664589 site mutation on acute myocardial infarction in Chinese Han. *Cellular and Molecular Biology*. **2024**, *70*(1), 119–127. https://doi.org/10.14715/cmb/2024.70.1.16.
846. Mou, X.; Wang, J.; Wang, L.; Wang, S. Correlation Between Single Nucleotide Polymorphisms of the rs664589 Locus in the Long-Chain Noncoding RNA Lung Adenocarcinoma Metastasis-Associated Gene 1, Hypertension, and Its Mechanism. *Genetic Testing and Molecular Biomarkers.* **2020**, *24*(3), 120–130. https://doi.org/10.1089/gtmb.2019.0193.
847. Yao, Y.; Liang, Y.; Dong, X.; Liu, S.; Zhang, S.; Liu, W.; Li, Y.; Shi, L.; Yan, Z.; Yao, Y. Association of Long Non-Coding RNAs (lncRNAs) ANRIL and MALAT1 Polymorphism with Cervical Cancer. *Pharmacogenomics and Personalized Medicine.* **2022**, *Volume 15*, 359–375. https://doi.org/10.2147/pgpm.s358453.
848. Hong, J. H.; Jin, E.; Chang, I. A.; Kang, H.; Lee, S.; Sung, J. K. Association of long noncoding RNA MALAT1 polymorphisms with gastric cancer risk in Korean individuals. *Molecular Genetics & Genomic Medicine.* **2020**, *8*(12). https://doi.org/10.1002/mgg3.1541.
849. Wang, W.; Xiong, W.; Zheng, J.; Jin, Y.; Dong, L.; Feng, X.; Ban, Y.; Chen, B. The contribution of MALAT1 gene rs3200401 and MEG3 gene rs7158663 to the risk of lung, colorectal, gastric and liver cancer. *Pathology – Research and Practice.* **2022**, *240*, 154212. https://doi.org/10.1016/j.prp.2022.154212.
850. Li, K.; Han, Z.; Wu, J.; Ye, H.; Sun, G.; Shi, J.; Zhang, J.; Wang, P. The Relationship between MALAT1 Polymorphism rs3200401 C > T and the Risk of Overall Cancer: A Meta-Analysis. *Medicina.* **2022**, *58*(2), 176. https://doi.org/10.3390/medicina58020176.
851. Wang, J.; Xiang, J.; Wu, L.; Bai, Y.; Chen, Z.; Yin, X.; Wang, Q.; Guo, W.; Peng, Y.; Guo, H.; Xu, P. A genetic variant in long non-coding RNA MALAT1 associated with survival outcome among patients with advanced lung adenocarcinoma: a survival cohort analysis. *BMC Cancer.* **2017**, *17*(1). https://doi.org/10.1186/s12885-017-3151-6.
852. Volkogon, A.; Kolnoguz, O.; Harbuzova, V.; Ataman, A. Long Non-Coding RNA MALAT1 Gene Polymorphism is Associated with Disease-Free Survival in Bladder Cancer Patients. *Galician Medical Journal.* **2020**, *27*(2), E202025. https://doi.org/10.21802/gmj.2020.2.5.

853. Andrii, V. Analysis of association between RS3200401 Long NonCoding RNA MALAT1 gene polymorphism and prostate adenocarcinoma development in Ukrainian population. *Journal of Urology & Nephrology Studies.* **2019**, *1*(5). https://doi.org/10.32474/juns.2019.01.000122.
854. Fawzy, M. S.; Ibrahiem, A. T.; Osman, D. M.; Almars, A. I.; Alshammari, M. S.; Almazyad, L. T.; Almatrafi, N. D. A.; Almazyad, R. T.; Toraih, E. A. Angio-Long Noncoding RNA MALAT1 (rs3200401) and MIAT (rs1061540) Gene Variants in Ovarian Cancer. *Epigenomes.* **2024**, *8*(1), 5. https://doi.org/10.3390/epigenomes8010005.
855. Li, Y.; Zhang, Y.; Xu, X.; Bi, L.; Zhang, M. Association of lncRNA Polymorphisms with Myocardial Infarction: A Case-Control Study and a Meta-*Analysis. J Clin Chem Lab Med*. **2018** 1: 113.
856. Zhang, T.; Luo, J., Liu; F., Zhang, X.; Luo, F., Yang, Y.; Li, X. Long noncoding RNA MALAT1 polymorphism predicts MACCEs in patients with myocardial infarction. *BMC Cardiovascular Disorders.* **2022**, *22*(1). https://doi.org/10.1186/s12872-022-02590-0.
857. Elsayed, Y. H. M.; Eldeen, L. A. T.; Saleh, O. M.; Mahmoud, M. A. E. W. MALAT1 Gene Rs3200401 polymorphism in Patients with arterial Hypertension. *International Journal of Research Publication and Reviews.* **2023**, *4*(7), 1781–1787. https://doi.org/10.55248/gengpi.4.723.48907.
858. Ismail, N. M.; Toraih, E. A.; Almars, A. I.; Ageeli, E. A.; Fawzy, M. S.; Maher, S. A. Genotype Triad for HOTAIR rs10783618, LINC-ROR rs1942347, and MALAT1 rs3200401 as Molecular Markers in Systemic Lupus Erythematous. *Diagnostics.* **2022**, *12*(5), 1197. https://doi.org/10.3390/diagnostics12051197.
859. Shkurat, T. P.; Ammar, M.; Bocharova, O.; Teplyakova, E.; Aleksandrova, A.; Ali, R.; Lipovich, L. The role of genetic variants in the long Non-Coding RNA genes MALAT1 and H19 in the pathogenesis of childhood obesity. *Non-Coding RNA.* **2023**, *9*(2), 22. https://doi.org/10.3390/ncrna9020022.
860. El-Sayed, E. H.; Fathy, A.; Younes, S. E. A.; Al-Shahaly, M. H.; Omar, H. H. Long Non-coding RNA Genes Polymorphisms H19 (rs2251375) and MALAT1 (rs3200401) Association with Rheumatoid Arthritis and Their Correlation with Disease Activity in a Cohort of Egyptian Patients: A Pilot Study. *Biochemical Genetics.* **2023**, *61*(6), 2443–2456. https://doi.org/10.1007/s10528-023-10383-z.

LIST OF SCIENTIFIC PUBLICATIONS

Publications Related to the Results of the Dissertation:

1. **Žilienė, E.**; Inčiūra, A.; Ugenskienė, R.; Juozaitytė, E. Pathomorphological manifestations and the course of the cervical cancer disease determined by variations in the TLR4 gene // Diagnostics. Basel, Switzerland: MDPI. ISSN: 2075-4418, 2023-06, Vol.13 (12), p.1999. doi.org/10.3390/diagnostics13121999 [Citav. rodiklis: 3, bendr. cit. rod.: 3.324, kvartilis: Q1 (2023. InCites JCR SCIE)].
2. **Balčiūnienė, E.**, Inčiūra, A., Juozaitytė, E., & Ugenskienė, R. (2024). Impact of RRP1B variants on the phenotype, progression, and metastasis of cervical cancer // Cancers. Basel, Switzerland: MDPI. ISSN: 2072-6694, 2024-04, 16(7), 1250. doi.org/10.3390/cancers16071250 [Citav. rodiklis: 4.5, bendr. cit. rod.: 4.973, kvartilis: Q1 (2023. InCites JCR SCIE)].

Other publications:

1. Bekampytė, Justina; Savukaitytė, Aistė; Bartnykaitė, Agnė; Ugenskienė, Rasa; **Žilienė, Eglė**; Inčiūra, Arturas; Juozaitytė, Elona. TIRAP rs8177376, rs611953, rs3802814, and rs8177374 polymorphisms and their association with cervical cancer phenotype and prognosis // Genes. Basel : MDPI, 2022, vol. 13, no. 8, p. 1-11, ISSN 2073-4425. doi:10.3390/genes13081365 [Citav. rodiklis: 3.5, bendr. cit. rod.: 4.2, kvartilis: Q2 (2022. InCites JCR SCIE)].
2. Golubickaitė, Ieva; Ugenskienė, Rasa; **Žilienė, Eglė**; Beniušytė, Jurgita; Inčiūra, Arturas; Poškienė, Lina; Juozaitytė, Elona. POLG gene variants in cervical cancer patients and their associations with clinical and pathomorphological tumor characteristics // Journal of Clinical Medicine. Basel : MDPI, 2021, vol. 10, no. 9, ISSN 2077-0383. doi:10.3390/jcm10091838 [Citav. rodiklis: 4.964, bendr. cit. rod.: 8.28, kvartilis: Q2 (2021. InCites JCR SCIE)].
3. Golubickaitė, Ieva; Ugenskienė, Rasa; Čepaitė, Justė; **Žilienė, Eglė**; Inčiūra, Arturas; Poškienė, Lina; Juozaitytė, Elona. Mitochondria-related TFAM gene variants and their effects on patients with cervical cancer // Biomedical reports. Athens : Spandidos publications, 2021, vol. 15, iss. 6, p. 1-7, ISSN 2049-9434, 2049-9442. doi:10.3892/br.2021.1482 Emerging Sources Citation Index (Web of Science); PubMed; Scopus; Index Copernicus. [S1] [M.kr.: M001].

CONFERENCE PRESENTATIONS

1. **Balčiūnienė, Eglė**; Ugenskienė, Rasa; Juozaitytė, Elona; Inčiūra, Arturas. Examination of polymorphism rs3741378 in the SIPA1 gene and its correlation with pathomorphological features in cervical cancer and disease progression // International Health Sciences Conference for All (IHSC for All) "Precision Medicine" : Abstract book 2024 : [March 25-26, 2024, Kaunas] / Edited by Ignas Lapeikis, Livija Petrokaitė, p. 443–445, ISSN 3030-0711.
2. **Balčiūnienė, Eglė**; Ugenskienė, Rasa; Juozaitytė, Elona; Inčiūra, Arturas. Investigation of Polymorphism rs8819 in the SRSF1 Gene: Implications for Pathomorphological Characteristics in Cervical Cancer and Disease Progression // 9th Kaunas / Lithuania International Hematology / Oncology Colloquium : 24 May 2024 : Online Poster Abstract Book / Editor Elona Juozaitytė, p. 9 - 10, ISBN 978-609-8343-00-7.
3. **Žilienė, Eglė**; Inčiūra, Arturas; Ugenskienė, Rasa; Juozaitytė, Elona. Analysis of SIPA1 and RRP1B Gene Variants and Their Association with the Course of Cervical Cancer // 6th Kaunas / Lithuania International Hematology / Oncology Colloquium : 28 May 2021, Kaunas / Lithuania : Online Poster Abstract Book / Editor Elona Juozaitytė; Abstracts' Reviewers Arturas Inčiūra, Viktoras Rudžianskas, Milda Rudžianskienė, Aistė Savukaitytė ; Kaunas Region Society of Oncologists, Hematologists and Transfusiologists. Kaunas : Eventas, 2021. ISBN 9786099616728, p. 20-20, ISBN 9786099616728.
4. **Žilienė, Eglė**; Dubinskiy, Yury; Ugenskienė, Rasa; Inčiūra, Arturas; Juozaitytė, Elona; Bartnykaitė, Agnė; Brazaitytė, Rūta. MyD88 Gene Polymorphisms and Their Associations with Tumour Pathomorphological Characteristics and Progression of Disease in Patients with Gynaecological Malignancies // 5th International Hematology / Oncology Colloquium : 26 June 2020, Kaunas / Lithuania : Online Poster Abstract Book / Editor Elona Juozaitytė; Abstracts' Reviewers Arturas Inčiūra, Rolandas Gerbutavičius, Sigita Liutkauskienė; Kaunas Region Society of Oncologists, Hematologists and Transfusiologists. Kaunas : Eventas, 2020. ISBN 9786099616704, p. 16-16, no. 16, ISBN 9786099616704.
5. **Žilienė, Eglė**; Inčiūra, Arturas; Ugenskienė, Rasa; Bartnykaitė, Agnė; Brazaitytė, Rūta; Juozaitytė, Elona. The Analysis of Polymorphisms in the TLR4 Gene and Their Associations with the Pathomorphological Characteristics of Cervical Cancer and the Course of the Disease // 5th

International Hematology / Oncology Colloquium : 26 June 2020, Kaunas / Lithuania : Online Poster Abstract Book / Editor Elona Juozaitytė ; Abstracts' Reviewers Arturas Inčiūra, Rolandas Gerbutavičius, Sigita Liutkauskienė ; Kaunas Region Society of Oncologists, Hematologists and Transfusiologists. Kaunas : Eventas, 2020. ISBN 9786099616704, p. 14-15, no. 15, ISBN 9786099616704.

6. Brazytė, Laura; Bekampytė, Justina; Ugenskienė, Rasa; **Žilienė, Eglė**; Inčiūra, Arturas; Juozaitytė, Elona. The Role of SULT1A1 and UGT1A1 Polymorphisms in Patients with Cervical Cancer // 8th Kaunas / Lithuania International Hematology / Oncology Colloquium : 12 May 2023 : online poster abstract book / Editor Elona Juozaitytė ; Abstracts' reviewers: Rolandas Gerbutavičius, Arturas Inčiūra, Dietger Niederwieser, Domas Vaitiekus. Kaunas : Eventas, 2023. ISBN 9786099616773, p. 6-7, ISBN 9786099616773, 9786099616773.
7. Karumazondo, Nyasha; Bekampytė, Justina; Ugenskienė, Rasa; **Žilienė, Eglė**; Inčiūra, Arturas; Juozaitytė, Elona. FAS and FASL Gene Variant Analysis and their Effect on Cervical Cancer Prognosis Assessment // 8th Kaunas / Lithuania International Hematology / Oncology Colloquium : 12 May 2023 : online poster abstract book / Editor Elona Juozaitytė ; Abstracts' reviewers: Rolandas Gerbutavičius, Arturas Inčiūra, Dietger Niederwieser, Domas Vaitiekus. Kaunas : Eventas, 2023. ISBN 9786099616773, p. 12-14, ISBN 9786099616773, 9786099616773.
8. Bekampytė, Justina; Ugenskienė, Rasa; **Žilienė, Eglė**; Inčiūra, Arturas; Juozaitytė, Elona. Association of TIRAP rs8177376 and rs8177374 Polymorphisms with Cervical Cancer Phenotype and Prognosis // 7th Kaunas / Lithuania International Hematology / Oncology Colloquium : 26 May 2022, Kaunas, Lithuania : Online Poster Abstract Book / Editor Elona Juozaitytė ; Abstracts' Reviewers Rolandas Gerbutavičius, Arturas Inčiūra, Dietger Niederwieser, Domas Vaitiekus ; Kaunas Region Society of Oncologists, Hematologists and Transfusiologists. Kaunas : Eventas, 2022. ISBN 9786099616759, p. 4-5, ISBN 9786099616759.
4. Golubickaitė, Ieva; Ugenskienė, Rasa; **Žilienė, Eglė**; Beniušytė, Jurgita; Inčiūra, Arturas; Juozeitytė, Elona. The Effect of germline POLG gene variants on cervical cancer pathomorfological characteristics and disease outcome // European Journal of Human Genetics : EJHG : Abstracts from 54th European Society of Human Genetics (ESHG) Conference: e-Posters : Virtual conference : August 28-31, 2021 / European Society for Human Genetics (ESHG). London : Nature Publishing Group, 2022, vol. 30, suppl. 1, p. 381-381, ISSN 1018-4813, 1476-5438. doi:10.1038/s41431-021-01026-1. [Citav. rodiklis: 5.2, bendr. cit. rod.: 5.05, kvartilis: Q1 (2022. InCites JCR SCIE)].

9. Čepaitė, Justė; Ugenskienė, Rasa; **Žilienė, Eglė**; Inčiūra, Arturas; Juozaitytė, Elona. Influence of IL-6 and IL-1β Gene Polymorphisms on Clinical and Morphological Characteristics of Cervical Tumors and Patient Survival // 6th Kaunas / Lithuania International Hematology / Oncology Colloquium : 28 May 2021, Kaunas / Lithuania : Online Poster Abstract Book / Editor Elona Juozaitytė; Abstracts' Reviewers Arturas Inčiūra, Viktoras Rudžianskas, Milda Rudžianskienė, Aistė Savukaitytė; Kaunas Region Society of Oncologists, Hematologists and Transfusiologists. Kaunas : Eventas, 2021. ISBN 9786099616728, p. 6-6, ISBN 9786099616728.
10. Matlašaitytė, Kamilė; Bekampytė, Justina; Ugenskienė, Rasa; **Žilienė, Eglė**; Inčiūra, Arturas; Juozaitytė, Elona. The Investigation of Associations between Glutathione-S-Transferase Gene Polymorphisms and Cervical Cancer Prognosis // 6th Kaunas / Lithuania International Hematology / Oncology Colloquium : 28 May 2021, Kaunas / Lithuania: Online Poster Abstract Book / Editor Elona Juozaitytė ; Abstracts' Reviewers Arturas Inčiūra, Viktoras Rudžianskas, Milda Rudžianskienė, Aistė Savukaitytė ; Kaunas Region Society of Oncologists, Hematologists and Transfusiologists. Kaunas : Eventas, 2021. ISBN 9786099616728, p. 11-11, ISBN 9786099616728.

diagnostics

MDPI

Article

Pathomorphological Manifestations and the Course of the Cervical Cancer Disease Determined by Variations in the *TLR4* Gene

Eglė Žilienė [1,*], Arturas Inčiūra [1], Rasa Ugenskienė [1,2] and Elona Juozaitytė [1]

[1] Institute of Oncology, Lithuanian University of Health Sciences, LT-50161 Kaunas, Lithuania; arturas.inciura@lsmu.lt (A.I.); rasa.ugenskiene@lsmu.lt (R.U.); elona.juozaityte@lsmu.lt (E.J.)
[2] Department of Genetics and Molecular Medicine, Lithuanian University of Health Sciences, LT-50161 Kaunas, Lithuania
* Correspondence: egle.ziliene@lsmu.lt; Tel.: +370-68213898

Abstract: Cervical cancer (CC) is often associated with human papillomavirus (HPV). Chronic inflammation has been described as one of the triggers of cancer. The immune system fights diseases, including cancer. The genetic polymorphism of pathogen recognition receptors potentially influences the infectious process, development, and disease progression. Many candidate genes SNPs have been contradictory demonstrated to be associated with cervical cancer by association studies, GWAS. *TLR4* gene activation can promote antitumor immunity. It can also result in immunosuppression and tumor growth. Our study aimed to investigate eight selected polymorphisms of the *TLR4* gene (rs10759932, rs1927906, rs11536898, rs11536865, rs10983755, rs4986790, rs4986791, rs11536897) and to determine the impact of polymorphisms in genotypes and alleles on the pathomorphological characteristics and progression in a group of 172 cervical cancer subjects with stage I–IV. Genotyping was performed by RT-PCR assay. We detected that the CA genotype and A allele of rs11536898 were significantly more frequent in patients with metastases ($p = 0.026$; $p = 0.008$). The multivariate logistic regression analysis confirmed this link to be significant. The effect of rs10759932 and rs11536898 on progression-free survival (PFS) and overall survival (OS) has been identified as important. In univariate and multivariate Cox analyses, AA genotype of rs11536898 was a negative prognostic factor for PFS ($p = 0.024$; $p = 0.057$, respectively) and OS ($p = 0.008$; $p = 0.042$, respectively). Rs11536898 C allele predisposed for longer PFS (univariate and multivariate: $p = 0.025$; $p = 0.048$, respectively) and for better OS (univariate and multivariate: $p = 0.010$; $p = 0.043$). The worse prognostic factor of rs10759932 in a univariate and multivariate Cox analysis for survival was CC genotype: shorter PFS ($p = 0.032$) and increased risk of death ($p = 0.048$; $p = 0.015$, respectively). The T allele of rs10759932 increased longer PFS (univariate and multivariate: $p = 0.048$; $p = 0.019$, respectively) and longer OS (univariate and multivariate: $p = 0.037$; $p = 0.009$, respectively). Our study suggests that SNPs rs10759932 and rs11536898 may have the potential to be markers contributing to the assessment of the cervical cancer prognosis. Further studies, preferably with larger groups of different ethnic backgrounds, are needed to confirm the results of the current study.

Keywords: cervical cancer; *TLR4*; polymorphisms; genotype; metastases; survival

Citation: Žilienė, E.; Inčiūra, A.; Ugenskienė, R.; Juozaitytė, E. Pathomorphological Manifestations and the Course of the Cervical Cancer Disease Determined by Variations in the *TLR4* Gene. *Diagnostics* **2023**, *13*, 1999. https://doi.org/10.3390/diagnostics13121999

Academic Editor: Mara Carsote

Received: 8 March 2023
Revised: 31 May 2023
Accepted: 3 June 2023
Published: 7 June 2023

1. Introduction

In today's oncology, the genetic features of the host that determine the pathophysiology of cancer and the course of the disease are intensively studied. The genetic influence on cancer is multifunctional. The risk of cancer is increased by additional factors that activate the immune system and cause inflammation. Inflammatory mediators can contribute to neoplasia by inducing mutations, adaptive responses, resistance to apoptosis, and environmental changes such as stimulation of angiogenesis [1–3].

Scientific studies suggest that membrane-associated innate Toll-like receptors (TLRs) as pattern recognition receptors (PRRs) play a main role in activating the immune response

associated with autoimmune diseases, inflammation, and tumor-associated diseases. The human TLRs family consists of 10 members (TLR1–TLR10). They are expressed in human immune cells and many tumors. Each of their expressions elicits a different response. These are transmembrane proteins that can recognize pathogen-associated molecular patterns (PAMPs) or host damage-associated molecular patterns (DAMPs) to activate innate and adaptive immune responses by triggering activation of NF-κB, AP1, CREB, c/EBP, and IRF transcription factors. TLRs mediate changes in the expression of chemokines and pro-inflammatory cytokines and activate the response of cytotoxic lymphocytes, thereby eliminating pathogens and host debris [4–7]. The signaling pathway of TLRs begins in the cytoplasmic TIR domain, which contains adaptors such as MyD88, TIRAP, and TRIF that modulate TLR signaling pathways, helping to recognize antigenic molecules (lipopolysaccharides, nucleic acids). This activates the protein complex, such as NF-kB, IRFs, MAP kinases, via the MyD88-dependent way on the recruitment of members of the IRAK family, TRIF-dependent way or MyD88-independent pathway. It regulates the production of cytokines, chemokines, type I interferon, thus eliminating antigens. Negative regulation of the signal path helps protect the host from inflammatory damage [8–11]. Studies have shown that TLRs can produce the desired antitumor effects by inducing apoptosis, autophagy, and necrosis in tumor cells [12–14]. TLR expression correlates with cancer prognosis [15,16]. Activation of TLRs becomes a target for cancer immunotherapy [17–22].

TLR4 gene, which consists of three exons and is localized on chromosome 9q33.1, is one of the most studied TLRs. Mutations in the *TLR4* gene have been shown to induce resistance of pathogens to lipopolysaccharides in mice [23]. *TLR4* mutations are associated with endotoxin hyporesponsiveness in humans [24]. The TLR4 receptor is likely to be associated with several diseases because of the range of ligands (both pathogen-related and endogenous) identified as agonists of *TLR4* [25]. *TLR4* is linked to a range of diseases with potential treatments targeting the *TLR4* pathway [26–67]. TLR4 activation can not only cause antitumor immunity but also, conversely, promote immunosuppression and influence tumor growth [68]. Changes in *TLR4* gene expression are involved in carcinogenesis. Activated *TLR4* increases inflammatory cytokines and cell proliferation, migration, invasion, and survival. Overexpression of *TLR4* in malignant cells promotes tumor growth and metastases [69]. High expression of *TLR4* is likely associated with the poor survival outcome of patients with solid cancers [70]. Cervical cancer, which starts in the cervix, is a widespread health problem and one of the most common oncological diseases. According to the World Health Organization, it is the fourth most commonly diagnosed cancer in women. It also ranks fourth in the world for cancer-related deaths among women. There were an estimated 604,000 new cases of cervical cancer and 342,000 deaths from the disease worldwide in 2020. *TLR4* promotes HPV-positive cervical tumor growth and facilitates the formation of a local immunosuppressive microenvironment. These conditions may lead to CC development [71]. *TLR4* expression was reported in accordance with histopathological grade in human papillomavirus (HPV)-infected cervical cells: the level was higher in invasive cervical cancers (ICC) than in cervical intraepithelial neoplasia (CIN) and low in normal cervical tissues. Moreover, higher *TLR4* expression in HPV-positive cervical cancer cell lines SiHa and HeLa, compared with the HPV-negative cell line C33A, was observed, indicating a role for HPV infection in *TLR4* regulation [72]. A link between increased *TLR4* expression and the severity of cervical lesions was found and was closely associated with FIGO stage, lymph node metastases, and tumor size in CC. In the advanced stages of FIGO, larger tumor sizes, and higher *TLR4* expression levels were observed [73]. Various factors influencing the development of CC have been identified. Cervical tumorigenesis is often initiated by high-risk HPV [74,75].

Single nucleotide polymorphism (SNP) is likely to affect cancer susceptibility. The influence of polymorphisms of the *TLR4* gene on various cancerous diseases was investigated [76–79]. To comprehensively analyze the impact of germinal polymorphisms on the course of the disease, the main components influencing the spread of cancer are investigated. A review of the global literature focused on the influence of *TLR4* gene polymorphisms and

expression on the course of various cancers (tumor proliferation, differentiation, metastases, prognosis, and patient survival). An association between *TLR4* polymorphisms and a risk of hypersensitivity to HPV16/18 infection in women and increased risk of cervicitis, the precancerous lesion, has been identified [80–84]. However, there are very few studies on the impact of *TLR4* gene polymorphisms on the pathomorphological features or course of cervical cancer.

The *TLR4* gene was chosen for the analyses as it encodes the protein that interferes with HPV, leading to dysregulation of the local immune microenvironment and tumorigenesis. With limited reports on the role of *TLR4* polymorphisms in cervical cancer, we performed a study to investigate eight selected polymorphisms of the *TLR4* gene (rs10759932, rs1927906, rs11536898, rs11536865, rs10983755, rs4986790, rs4986791, rs11536897), whose links to other cancers from other research have been published. We analyzed the distribution of polymorphisms in genotypes and alleles in a group of patients with cervical cancer, and we determined the correlations between SNPs and tumor pathomorphological parameters and the course of the disease.

2. Materials and Methods

2.1. Study Subjects

The study of interest in cervical cancer was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-10 and P1-BE-2-10/2014). One hundred seventy-two adult patients treated at the Hospital of Lithuanian University of Health Sciences Kauno Clinics with stage I–IV of cervical cancer, who agreed to participate in the retrospective study, were enrolled consecutively. Patient exclusion criteria were other malignancies and incomplete medical documentation. A written informed consent was obtained from all the participants. Subjects were recruited from October 2014 to August 2020. The follow-up period was until November 2020. All patients were treated according to standard protocols. The vast majority underwent chemoradiation therapy (69.2%), while others underwent surgery followed by radiotherapy or systemic treatment.

2.2. Methods

Clinical data on participants, tumor morphological characteristics, and the course of the disease were collected from the medical records. The cervical cancer diagnosis was made by a gynecologist performing a gynecological and radiological examination, and based on the histopathology of cervical biopsies. All carcinoma cases were staged following the recommendations of the International Federation of Gynecology and Obstetrics (FIGO). The tumor grading system was based on architectural and cytologic (nuclear) criteria. The age at the time of diagnosis, tumor size (T), lymph node involvement (N), the spread of metastases (M), stage, differentiation degree (G), presence of disease progression, and death of patients were considered as clinicopathological features in this analysis.

2.3. SNP Selection

Genotype data were identified using online databases—The International HapMap Project (http://www.HapMap.org, accessed on 1 October 2020) and the 1000 Genomes Project (http://www.1000genomes.org, accessed on 1 October 2020). The selection criteria of *TLR4* SNPs were as follows: SNPs were detected in other populations, related to the outcomes of different diseases reported in other studies, SNPs have not been widely analyzed before among patients with CC, a minor allele frequency (MAF) of SNP was $\geq$5% in the European population, and SNPs might be a functional site mapped.

2.4. DNA Extraction and Genotyping

DNA was extracted from leucocytes of peripheral venous blood samples collected in vacuum tubes with ethylenediaminetetraacetate (EDTA) and stored in a laboratory biobank at -20 °C. DNA genotyping was performed at the Institute of Oncology (Lithuanian University of Health Sciences). Genomic DNA extraction was performed using a genomic

DNA purification Kit (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). SNPs in the *TLR4* gene were determined using the TaqMan® probe SNP Genotyping Assay (Thermo Fisher Scientific, Baltics, Vilnius, Lithuania). Molecular genetic studies were performed using the real-time polymerase chain reaction (RT-PCR) method. PCR was used to amplify a particular segment of DNA based on the protocol. The candidate SNPs, location, region, MAF in the European population, and the primers used for RT-PCR are listed in Table 1.

Table 1. SNPs genomic region, MAF.

SNP	Genomic Position in chr9	Region/Location	MAF/Highest Population MAF
rs10983755 G > A	117702392	Promoter, 5′-UTR, intergenic variant −2081	0.07/0.31 (A)
rs10759932 T > C	117702866	Promoter, 5′-UTR, −1607	0.18/0.35 (C)
rs11536865 G > C	117703745	Promoter, 5′-UTR, regulatory region variant −729	0.04/0.24 (C)
rs4986790 A > G Asp299Gly	117713024	Exon, 3′-UTR, missense variant 896	0.06/0.14 (G)s
rs4986791 C > T Thr399Ile	117713324	Exon, 3′-UTR, missense variant 1196	0.04/0.17 (T)
rs11536897 G > A	117717732	3′-UTR 3084	0.04/0.11 (A)
rs1927906 T > C	117717837	3′-UTR 3189	0.21/0.49 (C)
rs11536898 C > A	117717932	3′-UTR 3284	0.13/0.27 (A)

3. Results

3.1. Tumour Characteristics and SNP Frequencies

In our study, 90.1% of the participants were of Lithuanian nationality, while the remaining nationalities were Polish, Estonian, Ukrainian, and German. The majority of patients were aged ≥50 years old (71.5%) and had the squamous-type histopathology variant of cervical cancer (92.3%). Other rare histopathology variants, including adenocarcinoma, adenosquamous cell carcinoma, mucinous adenocarcinoma, accounted for 8.7% of the subjects. The vast majority of tumors (65.7%) were moderately differentiated (G2). The most commonly diagnosed stage was stage IIB cancer (32.0%). Slightly less than half of the subjects (44.8%) had pathological regional lymph nodes. Nine patients had pathologic paraaortic lymph nodes, which were categorized as stage IIIC2 cancer. Distant metastases were diagnosed in ten patients. The detailed distribution of clinicopathological features can be found in the Table 2.

We performed Hardy–Weinberg equilibrium testing for each SNP. Three of the SNPs did not follow Hardy–Weinberg equilibrium (HWE). In the cases of SNP rs11536865, all the cases had the GG genotype. For SNP rs10983755, the distribution of genotype was as follows: GG—92.4%, GA—7.6%, AA—0%. For SNP rs11536897, the distribution of genotype was as follows: GG—96.0%, AG—4.0%, AA—0%. Others SNPs distribution of genotypes was as follows: Rs10759932 TT—70.4%, TC—27.3%, CC—4%; rs1927906 TT—77.3%, TC—22.1%, CC—0.6%; rs11536898 CC—75.0%, CA—22.1%, AA—2.9%; Rs4986790 AA—86.0%, AG—13.4%, GG—0.6%; Rs4986791 CC—85.5%, CT—13.9%, TT—0.6%; The total count and frequencies of TLR4 genotypes and alleles are presented in Table 3.

Table 2. General clinicopathological characteristics and frequencies of 172 study participants.

Variables	Subgroups	Frequencies (Count/%)
Age (years) (mean ± SD: 55.4 ± 13.5)	≥50	123/71.5%
	<50	49/28.5%
Histology	Squamous	157/92.3%
	Non-squamous	15/8.7%
Tumor size (T)	T1A	1/0.6%
	T1B	25/14.6%
	T2A	4/2.3%
	T2B	80/46.5%
	T3A	13/7.6%
	T3B	38/22.1%
	T3C	4/2.3%
	T4A	4/2.3%
	T4B	3/1.7%
Pathological regional lymph nodes status	N0	95/55.2%
	N1	77/44.8%
Distant metastases	M0	162/94.2%
	M1	10/5.8%
Stage	IA	1/0.6%
	IB	15/8.7%
	IIA	5/2.9%
	IIB	12/7.0%
	IIIA	9/5.2%
	IIIB	12/7.0%
	IIIC1	53/31.0%
	IIIC2	9/5.2%
	IVA	3/1.7%
	IVB	10/5.8%
Grade	1	13/7.6%
	2	113/65.7%
	3	46/26.7%

3.2. *Association Analysis*

In our study, we analyzed the potential associations between SNPs and tumor clinicopathological features. However, no statistically significant correlations between rs10759932, rs1927906, rs11536865, rs10983755, rs4986790, rs4986791, rs11536897, and tumor size, nodes status, metastases, tumor cells differentiation, stage were found performing logistic regression. All the analyzed polymorphisms were not related to the patients' age at the time of diagnosis ($p > 0.05$). Nevertheless, we detected a significant link between SNP rs11536898 and metastases (M). Carrying the A allele statistically significantly increased the chance of having metastases (OR = 5.068, 95% CI: 1.357–18.918, $p = 0.008$). This finding was partially confirmed by the genotype model, as patients with the CA genotype had a 4.735 higher risk for distal metastases than patients with the CC genotype (95% CI: 1.204–18.626, $p = 0.026$). This may be due to the fact that only five patients with the AA genotype were determined in our study, which may have affected *p*-value in this comparison. All the results are presented in Tables 4 and 5. Furthermore, the multivariate logistic regression analysis confirmed the significant link between rs11536898 and metastases. In the multivariate analysis (Model No.1), the CA genotype significantly increased the risk to having metastases (OR = 4.609, 95% CI: 1.166–18.212, $p = 0.029$), and the A allele increased the risk for metastases (OR = 5.044, 95% CI: 1.346–18.899, $p = 0.016$) when adjusting for the age group at the diagnosis. The relationship remains statistically significant when adjusting for the age at the diagnosis and tumor differentiation (G) (Model No.2): the CA genotype significantly increased the risk of having metastases (OR = 4.419, 95% CI: 1.111–17.576, $p = 0.035$). Additionally, the A allele increased the risk for metastases (OR = 4.884, 95% CI: 1.297–18.392, $p = 0.019$). We did not include tumor size and nodules in multivariate models because we observed complete data separation (Table 6).

Table 3. The distribution of *TLR4* genotypes and alleles.

SNP		Genotypes Frequencies		Alleles Frequencies	
Rs10759932 T > C	TT	121	0.704	T	0.841
	TC	47	0.273	C	0.159
	CC	4	0.023		
Rs1927906 T > C	TT	133	0.773	T	0.883
	TC	38	0.221	C	0.117
	CC	1	0.006		
Rs11536898 C > A	CC	129	0.750	C	0.861
	CA	38	0.221	A	0.139
	AA	5	0.029		
Rs11536865 G > C	GG	172	1.000	G	1.000
	GC	0	0		
	CC	0	0		
Rs10983755 G > A	GG	159	0.924	G	0.962
	GA	13	0.076	A	0.038
	AA	0	0		
Rs4986790 A > G	AA	148	0.860	A	0.927
	AG	23	0.134	G	0.073
	GG	1	0.006		
Rs4986791 C > T	CC	147	0.854	C	0.924
	CT	24	0.140	T	0.076
	TT	1	0.006		
Rs11536897 G > A	GG	165	0.959	G	0.979
	GA	7	0.041	A	0.021
	AA	0	0		

3.3. Survival Analysis

In our study group, disease progression was observed in 30.2% of patients during the follow-up period. The localization of progression were as follows: local progression was found in 13.36% of all subjects, positive lymph nodes (N+) were detected in 3.66%, local progression and positive nodes were found in 4.08%, and in other cases (9.3%), progression included local progression, positive lymph nodes, and distant metastases. There were 40 instances of death (23.3%) during the follow-up period. In all cases, the cause of death was cancer progression.

The effect of the SNPs on survival (PFS and OS) was analyzed in the genotype and allelic models. The PFS ranged from 1 to 201 months (median 13). The OS also went from 1 to 201 months (median 16.5). No significant link between rs1927906, rs11536865, rs10983755, rs4986790, rs4986791, rs11536897 genotypes or alleles and survival was detected. However, the effect of two SNPs (rs10759932 and rs11536898) on PFS and OS has been identified as important. The Kaplan—Meier method showed a link between rs10759932 CC genotype and OS (p = 0.049 for Log Rank, p = 0.018 for Breslow, and p = 0.028 for Tarone–Ware). Cox's regression analysis demonstrated the influence of the CC genotype on shorter PFS and OS compared to patients with the TT genotype (OR = 2.918, 95% CI: 0.894–9.530, p = 0.049; OR = 3.340, 95% CI: 1.006–11.095, p = 0.048, respectively). Our multivariate Cox's regression model included tumor T, N, G, and the age of patients. In the adjusted analysis, the CC genotype increased the risk of progression by almost four times compared to the TT genotype (OR = 3.674, 95% CI: 1.115–12.108, p = 0.032) and increased the risk of faster mortality (OR = 4.608, 95% CI: 1.344–15.801, p = 0.015). The T allele was significant for PFS (Log Rank p = 0.049, Breslow p = 0.042, Tarone–Ware p = 0.042) and OS (Log Rank p = 0.031, Breslow p = 0.018, Tarone–Ware p = 0.023) in the allelic model. Carrying the T allele increased the possibility of longer PFS (OR = 0.331, 95% CI: 0.103–1.067, p = 0.048) and longer OS (OR = 0.284, 95% CI: 0.087–0.928, p = 0.037). The holders of T allele had an increased chance of longer PFS (OR = 0.244, 95% CI: 0.075–0.795, p = 0.019) and a decreased risk of shorter OS (OR = 0.200, 95% CI: 0.059–0.674, p = 0.009) when the adjustment for tumor T, N, G and the age of patients (Tables 7 and 8).

Table 4. Univariate logistic regression analysis between SNP's and tumor characteristics.

		Possitive T3–T4 Versus T1–2			Possitive N1 Versus N0			Possitive M1 Versus M0			Possitive G3 Versus G1 + G2		
SNP	**Genotype, alleles**	**OR**	**95% CI**	***p*-Value**	**OR**	**95% CI**	***p*-Value**	**OR**	**95% CI**	***p*-Value**	**OR**	**95% CI**	***p*-Value**
Rs10759932	TC vs. TT	1.276	0.639–2.551	0.490	1.272	0.647–2.499	0.486	2.158	0.554–8.413	0.268	1.110	0.520–2.370	0.787
	CC vs. TT	1.881	0.256–13.834	0.535	1.327	0.181–9.734	0.781	7.733	0.678–88.176	0.099	2.903	0.392–21.495	0.297
	T allele + vs. T -	0.570	0.078–4.150	0.574	0.806	0.111–5.861	0.831	0.170	0.016–1.800	0.097	0.355	0.049–2.596	0.290
	C allele + vs. C -	1.317	0.6673–2.577	0.421	1.276	0.662–2.460	0.467	2.522	0.697–9.122	0.147	1.210	0.584–2.504	0.608
Rs1927906	TC vs. TT	1.033	0.489–2.183	0.932	0.668	0.318–1.403	0.567	1.543	0.379–6.278	0.545	1.919	0.887–4.154	0.098
	CC vs. TT	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	T allele + vs. T -	2.758	2.261–3.364	0.187	2.250	1.903–2.660	0.265	1.062	1.023–1.103	0.803	3.800	2.957–4.883	0.097
	C allele + vs. C -	1.107	0.530–2.310	0.787	0.716	0.345–1.484	0.368	1.500	0.369–6.097	0.325	2.056	0.962–4.398	0.060
Rs11536898	CA vs. CC	1.405	0.671–2.944	0.368	1.543	0.746–3.191	0.242	4.735	1.204–18.626	**0.026**	1.576	0.722–3.439	0.253
	AA vs. CC	2.898	0.469–17.989	0.253	2.083	0.337–12.898	0.430	7.812	0.704–86.710	0.094	0.758	0.082–7.030	0.807
	C allele + vs. C -	0.374	0.061–2.300	0.271	0.530	0.086–3.258	0.487	0.228	0.023–2.254	0.169	1.475	0.161–13.555	0.730
	A allele + vs. A -	1.529	0.757–3.090	0.235	1.597	0.798–3.197	0.184	5.068	1.357–18.918	**0.008**	1.463	0.689–3.106	0.320
Rs10983755	AG vs. GG	1.088	0.340–3.482	0.887	1.483	0.477–4.613	0.496	1.389	0.162–11.900	0.764	2.550	0.809–8.035	0.110
	AA vs. GG	*	*	*	*	*	*	*	*	*	*	*	*
	G allele + vs. G -	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele + vs. A -	1.088	0.340–3.482	0.887	1.483	0.477–4.613	0.494	1.389	0.162–11.900	0.763	2.550	0.809–8.035	0.110
Rs4986790	AG vs. AA	1.152	0.467–2.841	0.758	0.501	0.195–1.289	0.152	1.667	0.331–8.388	0.536	1.600	0.628–4.077	0.325
	GG vs. AA	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele + vs. A -	2.758	2.261–3.364	0.187	2.250	1.903–2.660	0.265	1.062	1.023–1.103	0.803	3.800	2.957–4.883	0.267
	G allele + vs. G -	1.280	0.532–3.082	0.581	0.572	0.231–1.419	0.225	1.591	0.317–7.986	0.570	1.800	0.727–4.455	0.199
Rs4986791	TC vs. CC	1.305	0.542–3.143	0.553	0.581	0.234–1.441	0.241	1.580	0.315–7.929	0.580	1.486	0.588–3.756	0.402
	TT vs. CC	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	C allele + vs. C -	2.758	2.261–3.364	0.187	2.250	1.903–2.660	0.265	1.062	1.023–1.103	0.803	3.800	2.957–4.883	0.267
	T allele + vs. T -	1.435	0.608–3.389	0.408	0.653	0.271–1.573	0.340	1.511	0.302–7.567	0.613	1.672	0.682–4.103	0.258
Rs11536897	AG vs. GG	0.682	0.128–3.623	0.653	0.922	0.200–4.251	0.917	0.000	0.000	1.000	1.100	0.206–5.877	0.911
	AA vs. GG	*	*	*	*	*	*	*	*	*	*	*	*
	G allele + vs. G -	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele + vs. A -	0.682	0.128–3.623	0.651	0.922	0.200–4.251	0.917	0.939	0.904–0.977	0.502	1.100	0.206–5.877	0.911

T3–T4, T1–2—tumor size, N1—pathological regional lymph nodes, N0—no pathological regional lymph nodes, M1—distant metastases, M0—no distant metastases, G1 + G2, G3—tumor grade. * OR could not be estimated because of zero value within a cell.

Table 5. Univariate logistic regression analysis: the odds ratio for associations between SNPs and the age of patients, stage groups, and expected prognosis of the disease.

		Possitive Stage III–IV Versus Stage I–II			Possitive Worse Prognosis: T3–T4 + G3 Versus T1–T2 + G1–G2			Age (Years): ≤50 vs. >50		
SNP	**Genotype, Alleles**	**OR**	**95% CI**	***p*-Value**	**OR**	**95% CI**	***p*-Value**	**OR**	**95% CI**	***p*-Value**
Rs10759932	TC vs. TT	1.088	0.551–2.148	0.808	1.143	0.396–3.298	0.805	1.575	0.793–3.134	0.195
	CC vs. TT	0.806	0.110–5.911	0.832	4.000	0.237–67.473	0.336	5.854	0.590–58.052	0.131
	T allele + vs. T -	1.270	0.175–9.232	0.813	0.259	0.016–4.304	0.346	0.195	0.020–1.915	0.120
	C allele + vs. C -	1.062	0.549–2.055	0.857	1.273	0.463–3.500	0.640	1.734	0.891–3.377	0.104
Rs1927906	TC vs. TT	0.655	0.317–1.350	0.251	2.159	0.785–5.940	0.136	0.896	0.393–1.178	0.642
	CC vs. TT	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	T allele + vs. T -	1.800	1.574–2.058	0.372	0.000	0.000	1.000	2.672	2.201–3.243	0.378
	C allele + vs. C -	0.691	0.338–1.414	0.310	2.429	0.907–6.506	0.078	0.900	0.429–1.891	0.852
Rs11536898	CA vs. CC	.1.123	0.541–2.334	0.755	1.725	0.579–5.140	0.328	1.227	0.588–2.563	0.586
	AA vs. CC	1.225	0.198–7.582	0.827	2.156	0.184–25.271	0.541	0.422	0.046–3.885	0.446
	C allele + vs. C -	0.838	0.136–5.146	0.848	0.524	0.045–6.045	0.604	2.485	0.272–22.734	0.651
	A allele + vs. A -	1.135	0.564	2.281	1.776	0.631–4.997	0.277	1.103	0.544–2.240	0.856
Rs10983755	AG vs. GG	1.291	0.405–4.119	0.666	2.430	0.535–11.030	0.250	1.453	0.466–4.528	0.520
	AA vs. GG	*	*	*	*	*	*	*	*	*
	G allele + vs. G -	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele + vs. A -	1.291	0.405–4.119	0.666	2.430	0.535–11.030	0.250	1.453	0.366–4.528	0.520
Rs4986790	AG vs. AA	0.570	0.235–1.383	0.214	2.005	0.616–6.533	0.248	0.876	0.349–2.199	0.778
	GG vs. AA	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele + vs. A -	1.800	1.574–2.058	0.372	0.000	0.000	1.000	0.000	0.000	1.000
	G allele + vs. G -	0.627	0.264–1.492	0.289	2.406	0.781–7.416	0.126	0.986	0.405–2.402	0.975
Rs4986791	TC vs. CC	0.635	0.267–1.510	0.304	2.005	0.616–6.533	0.248	0.812	0.327–2.022	0.655
	TT vs. CC	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	C allele + vs. C -	1.800	1.574–2.058	0.372	0.000	0.000	1.000	0.000	0.000	1.000
	T allele + vs. T -	0.692	0.296–1.620	0.395	2.406	0.781–7.416	0.126	0.914	0.378–2.208	0.842
Rs11536897	AG vs. GG	0.581	0.126–2.677	0.486	0.000	0.000	1.000	0.000	0.000	1.000
	AA vs. GG	*	*	*	*	*	*	*	*	*
	G allele + vs. G -	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele + vs. A -	0.581	0.126–2.677	0.481	*	*	*	*	*	*

* OR could not be estimated because of zero value within a cell.

Table 6. Multivariate logistic regression analyses for metastases adjusted for Rs11536898 genotype, age at the diagnosis and tumor differentiation (G).

				Model No.1			Model No.2	
Dependent	**SNP**	**Covariates**	**Odds**	**95%CI**	***p***	**Odds**	**95%CI**	***p***
Positive M	Rs11536898	CA vs. CC	4.609	1.166–18.212	**0.029**	4.419	1.111–17.576	**0.035**
		AA vs. CC	9.452	0.803–111.217	0.074	9.871	0.827–117.76	0.070
		Age group	0.977	0.928–1.028	0.370	0.977	0.928–1.029	0.376
		Possitive G3 vs. G1+G2				1.729	0.445–6.716	0.429
				Model No.1			**Model No.2**	
Dependent	**SNP**	**Covariates**	**Odds**	**95%CI**	***p***	**Odds**	**95%CI**	***p***
Positive M	Rs11536898	A allele + vs. A -	5.044	1.346–18.899	**0.016**	4.884	1.297–18.392	**0.019**
		Age group	0.979	0.931–1.030	0.415	0.980	0.932–1.030	0.426
		Possitive G3 vs. G1+G2				1.670	0.433–6.439	0.456

Table 7. Cox's univariate model for PFS and OS.

			Progression-Free Survivol			**Overall Survival**	
SNP	**Genotype/Allele**	**HR**	**95% CI**	***p*-Value**	**HR**	**95% CI**	***p*-Value**
Rs10759932	TC vs. TT	0.884	0.472–1.653	0.699	0.818	0.382–1.752	0.606
	CC vs. TT	2.918	0.894–9.530	**0.049**	3.340	1.006–11.095	**0.048**
	T allele + vs. T -	0.331	0.103–1.067	**0.048**	0.284	0.087–0.928	**0.037**
	C allele + vs. C -	1.012	0.564–1.816	0.967	1.012	0.509–2.010	0.973
Rs1927906	TC vs. TT	0.975	0.498–1.910	0.975	0.695	0.306–1.576	0.383
	CC vs. TT	2.584	0.352–18.949	0.350	3.081	0.417–22.761	0.383
	T allele + vs. T -	0.385	0.053–2.807	0.346	0.301	0.041–2.216	0.239
	C allele + vs. C -	1.028	0.537–1.971	0.933	0.770	0.354–1.673	0.509
Rs11536898	CA vs. CC	1.103	0.586–2.073	0.762	1.294	0.636–2.633	0.476
	AA vs. CC	3.926	1.201–12.837	**0.024**	5.057	1.522–16.802	**0.008**
	C allele + vs. C -	0.261	0.081–0.844	**0.025**	0.212	0.065–0.691	**0.010**
	A allele + vs. A -	1.274	0.707–2.295	0.420	1.545	0.803–2.971	0.193
Rs10983755	AG vs. GG	0.508	0.123–2.097	0.349	0.341	0.043–2.290	0.253
	AA vs. GG	*	*	*	*	*	*
	G allele + vs. G -	0.000	0.000	1.000	0.000	0.000	1.000
	A allele + vs. A -	0.508	0.123–2.097	0.349	0.341	0.043–2.290	0.253
Rs4986790	AG vs. AA	1.482	0.716–3.069	0.290	1.062	0.444–2.542	0.892
	GG vs. AA	2.767	0.378–20.275	0.316	3.346	0.453–24.696	0.236
	A allele + vs. A -	0.385	0.053–2.807	0.346	0.301	0.041–2.216	0.239
	G allele + vs. G -	1.554	0.774–3.123	0.215	1.178	0.520–2.669	0.695
Rs4986791	TC vs. CC	1.426	0.689–2.952	0.339	1.029	0.430–2.461	0.950
	TT vs. CC	2.752	0.376–20.165	0.319	3.331	0.451–24.582	0.238
	C allele + vs. C -	1.499	0.746–3.010	0.256	0.301	0.041–2.216	0.239
	T allele + vs. T -	0.385	0.053–2.807	0.346	1.142	0.504–2.587	0.750
Rs11536897	AG vs. GG	0.425	0.058–3.084	0.397	1.314	0.316–5.454	0.707
	AA vs. GG	*	*	*	*	*	*
	G allele + vs. G -	0.000	0.000	1.000	0.000	0.000	1.000
	A allele + vs. A -	0.425	0.058–3.084	0.397	1.314	0.316–5.454	0.707

* OR could not be estimated because of zero value within a cell.

The rs11536898 AA genotype subgroup, compared to the CC genotype, was also significantly associated with PFS (Log Rank, $p = 0.014$, Breslow $p = 0.001$, Tarone–Ware $p = 0.003$) and OS (Log Rank, $p = 0.003$, Breslow $p < 0.001$, Tarone–Ware $p < 0.001$). The rs11536898 AA genotype compared to patients with the CC genotype decreased the likelihood for longer PFS (OR = 3.926, 95% CI: 1.201–12.837, $p = 0.024$) and shortened OS (OR = 5.057, 95% CI: 1.522–16.802, $p = 0.008$). In the multivariate Cox's regression analysis, the AA genotype remained a factor that shortened OS (OR = 3.735, 95% CI: 1.051–13.278, $p = 0.042$) and showed a borderline effect on PFS (OR = 3.306, 95% CI: 0.967–11.299, $p = 0.057$), when

the adjustment for tumor T, N, G and the age of patients. The rs11536898 C allele was significantly associated with PFS (Log Rank, $p = 0.015$, Breslow $p = 0.003$, Tarone–Ware $p = 0.005$) and OS (Log Rank, $p = 0.004$, Breslow $p < 0.001$, Tarone–Ware $p = 0.001$). No significant effect of the CA genotype on PFS was determined. This is in line with the allelic model, which demonstrated that the carriers of the C allele were less likely to have shorter PFS compared to non-carriers. The rs11536898 C allele predisposed to longer PFS (OR = 0.261, 95% CI: 0.081–0.844, $p = 0.025$) and longer OS (OR = 0.212, 95% CI: 0.065–0.691, $p = 0.010$). When adjusting for tumor T, N, G, and age of patients, the tendency remains statistically significant for PFS (OR = 0.291, 95% CI: 0.086–0.987, $p = 0.048$) and for OS (OR = 0.274, 95% CI: 0.078–0.959, $p = 0.043$) (Tables 7 and 8).

Table 8. Cox's multivariate model for PFS and OS. The adjusted ratio for associations between SNPs rs10759932 and rs11536898 and age at the time of diagnosis, tumor characteristics.

	Variables	Progression-Free Survivol			Overall Survival		
		HR	**95% CI**	***p*-Value**	**HR**	**95% CI**	***p*-Value**
Rs10759932	TC vs. TT	0.658	0.338–1.280	0.217	0.747	0.351–1.590	0.449
	CC vs. TT	3.674	1.115–12.108	**0.032**	4.608	1.344–15.801	**0.015**
	Age at diagnosis	0.993	0.971–1.016	0.566	1.017	0.991–1.043	0.199
	T3-T4 vs. T1-T2	5.540	2.870–10.694	<0.001	8.178	3.489–19.167	<0.001
	N1 vs. N0	1.340	0.709–2.534	0.368	1.775	0.854–3.689	0.124
	G3 vs. G1-2	0.913	0.490–1.704	0.776	0.773	0.384–1.556	0.471
Rs10759932	T allele + vs. T -	0.244	0.075–0.795	**0.019**	0.200	0.059–0.674	**0.009**
	Age at diagnosis	0.996	0.973–1.018	0.697	1.018	0.993–1.044	0.163
	T3-T4 vs. T1-T2	5.298	2.750–10.206	<0.001	8.045	3.430–18.871	<0.001
	N1 vs. N0	1.291	0.684–2.439	0.431	1.735	0.835–3.604	0.140
	G3 vs. G1-2	0.962	0.520–1.779	0.902	0.797	0.399–1.593	0.521
Rs11536898	CA vs. CC	0.858	0.440–1.675	0.654	1.090	0.522–2.277	0.819
	AA vs. CC	3.306	0.967–11.299	**0.057**	3.735	1.051–13.278	**0.042**
	Age at diagnosis	0.993	0.971–1.017	0.578	1.018	0.992–1.045	0.171
	T3-T4 vs. T1-T2	5.158	2.675–9.947	<0.001	7.658	3.280–17.876	<0.001
	N1 vs. N0	1.241	0.653–2.360	0.510	1.686	0.805–3.530	0.166
	G3 vs. G1-2	1.009	0.538–1.894	0.977	0.819	0.405–1.654	0.577
Rs11536898	C allele + vs. C -	0.291	0.086–0.987	**0.048**	0.274	0.078–0.959	**0.043**
	Age at diagnosis	0.994	0.971–1.017	0.612	1.018	0.992–1.044	0.176
	T3-T4 vs. T1-T2	5.077	2.645–9.747	0.000	7.694	3.298–17.951	0.000
	N1 vs. N0	1.232	0.648–2.342	0.525	1.694	0.810–3.540	0.161
	G3 vs. G1-2	1.018	0.543–1.907	0.955	0.817	0.405–1.651	0.574

Kaplan—Meier analysis was performed to generate survival curves for genotypes and alleles for both PFS and OS (Figure 1).

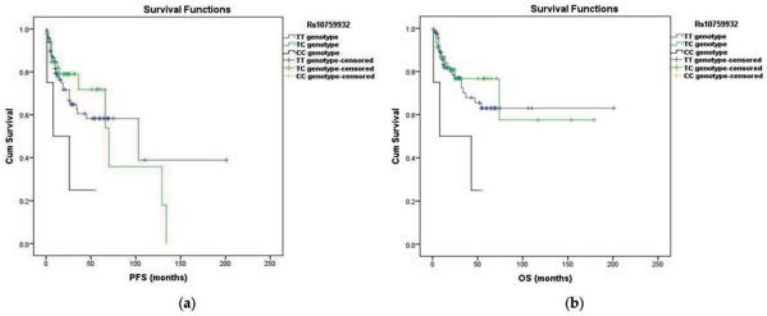

Figure 1. *Cont.*

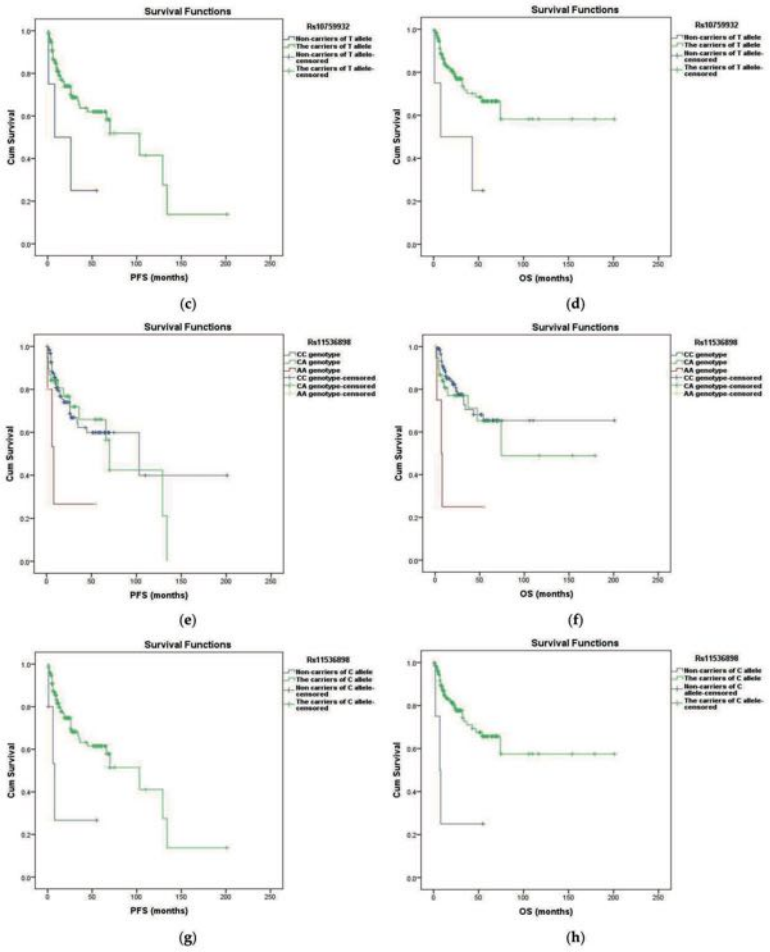

Figure 1. Kaplan—Meier survival curves for PFS and OS in patients with cervical cancer according rs10759932 and rs11536898 polymorphisms (n = 172). Kaplan—Meier survival curves for rs10759932 and rs11536898 polymorphisms in the genotype and allelic models demonstrating PFS and OS differences. The *X*-axis displays the number of months from cervical cancer, confirming the event date (PFS or OS), and the *Y*-axis indicates the survival probability. (**a**,**b**) Rs10759932 CC genotype increased the risk for shorter PFS and OS compared to patients with the TT genotype (95% CI: 0.894–9.530, $p = 0.049$; 95% CI: 1.006–11.095, $p = 0.048$, respectively); (**c**,**d**) Carrying the T allele increased the possibility of longer PFS (95% CI: 0.103–1.067, $p = 0.048$) and longer OS (95% CI: 0.087–0.928, $p = 0.037$); (**e**,**f**) Rs11536898 AA genotype compared to patients with the CC genotype shortened PFS (95% CI: 1.201–12.837, $p = 0.024$) and OS (95% CI: 1.522–16.802, $p = 0.008$); (**g**,**h**) Rs11536898 C allele predisposed to longer PFS (95% CI: 0.081–0.844, $p = 0.025$) and to longer OS (95% CI: 0.065–0.691, $p = 0.010$).

4. Discussion

The active investigation of the correlation between *TLR4* SNPs and CC is intriguing. The current study is the first to investigate analyzed SNPs in assessing the clinicopathological features as well as the course of CC. It establishes the relationship between SNPs in *TLR4* and CC, suggesting their potential as biomarkers that could be used for prognosticating the development of the disease. In the future, SNP detection in *TLR4* may be used to stratify patients, predict clinical manifestations of CC, assess risks of progression or relapse, and evaluate treatment efficacy. The study has many advantages: the dataset contains genetic data, tumor phenotype data, and survival information. However, there are limitations in our study. We cannot compare our results with others because we did not find any studies analyzing associations between these polymorphisms and clinicopathological characteristics of CC. In addition, our results may have been affected by the limited sample size. We hope to expand the study group in the future. Another weakness of this study is the lack of a control group to assess CC risk. Genotyping errors are expected to be minor. Thus, the resulting biases will likely to be small.

Two of the SNPs (rs10759932 and rs11536898) were significant in our analysis. Rs10759932 is located in the promoter region of the *TLR4* gene and may regulate the *TLR4* expression level by influencing the binding affinity of transcription factors [85]. We found that the rare homozygous rs10759932 CC genotype causes shorter PFS and OS. The allelic model did not contradict the survival results. The T allele showed the link to better survival, although the effect of the C allele on worse survival prognosis was not statistically significant. However, previously published studies provide contradictory data for rs10759932 correlation with cancers. Some researchers' findings could contribute to our study's results, indicating that the CC genotype is an indicator of a worse outcome. T. Tongtawee et al. investigated 400 patients with gastric lesions, including chronic gastritis, gastric atrophy, internal metaplasia, and gastric cancer. They found that the rs10759932 CC homozygous genotype significantly increased the risk of premalignant and malignant gastric lesion development [86]. The Cleveland case-control study in Caucasians and African Americans supported the influence of rs10759932 on prostate cancer risk. Men carrying the CC genotype for rs10759932 had a statistically significant increased risk of prostate cancer (p = 0.006) compared to men carrying the TT genotype [87]. On the other hand, other research provides opposite results. The study conducted in the Shandong Province of Northern China demonstrates that the rs10759932 polymorphism was associated with susceptibility to gastric cancer (GC) in both genotype and allelic frequency. However, genotype CC was the protective factor for GC. They believe that the genetic variant of *TLR4* rs10759932 might play an essential protective role in the development of GC [88]. Huang et al. found that the rs10759932 TC heterozygote and combined genotypes TC/CC were associated with a significantly reduced risk of gastric cancer in the high-risk population [85]. Similar results were obtained from a Japanese study where the rs10759932 TC/CC genotypes decreased the risk of gastric cancer. However, this did not reach statistical significance (p = 0.059) [89]. Several studies have not shown any correlations between rs10759932 polymorphisms and cancer. These include studies on breast cancer in the Saudi population [90], the risk of noncardia gastric cancer in Goyang [91], and the risk of colorectal cancer in Brazil [92]. A large nested case-control study of prostate cancer in the Physicians' Health Study (1982–2004), including 1267 controls and 1286 random prostate cancer cases, showed that genetic variation across this polymorphism is not strongly associated with prostate cancer risk or mortality [93]. The dissociation of research results possibly be due to sample size limitations, different ethnic groups, and the multicausal backgrounds promoting cancer development, including genetic factors, race, environment, and lifestyle.

In our study, rs11536898 was also associated with CC clinical outcomes. The rare AA genotype causes shorter PFS and OS compared to the CC genotype. The C allele was inversely associated with shorter PFS and OS. In addition, the AC genotype and A allele were associated with an increased risk of metastases. On the contrary, a significant association was revealed with the risk of prostate cancer. The Health Professionals Follow-

up Study (HPFS) found that men under the age of age 65 carrying two copies of the minor alleles of rs11536898 had a statistically significantly lower risk of prostate cancer compared to noncarriers (CC and CA versus AA: OR 0.59; 95% CI 0.41–0.86) [94]. However, many of the SNPs in this study were in high linkage disequilibrium with one another. The Physicians' Health Study found no statistically significant association between this SNP and the overall prevalence of prostate cancer. In addition, there were no significant associations between the SNP and cases of advanced/fatal or severe cancer, and there was also no evidence of associations between *TLR4* SNPs and prostate cancer-specific mortality or bone metastases [93]. Observational results from another population-based case-control study showed that rs11536898 was associated with colon cancer, where the AA vs. CA/CC genotype decreased colon cancer risk (OR 0.50, 95% CI 0.29, 0.87) [95]. However, other studies (the Washington County Cancer Registry, the Maryland Cancer Registry, Sweden, the Physicians' Health Study) found no significant interfaces between rs11536898 and cancer [93,96,97]. Although the data are unclear, we believe that the A allele may be associated with a worse prognosis.

Unfortunately, in our study, we did not find statistically significant associations with pathomorphological features or outcomes of cervical cancer for SNPs rs4986790 and rs4986791, which have been widely studied worldwide and are potentially associated with other cancers, influencing the risk or prognosis. Rs4986790 is a common polymorphism that causes an amino acid exchange (aspartate to glycine). In a study involving 122 Tunisian women with cervical cancer compared with 260 healthy control, the *TLR4* polymorphism Asp299Gly (rs4986790) was found to be associated with a higher risk of cervical cancer. The common homozygote Asp/Asp genotype and the Asp allele were associated with a higher risk of developing cervical cancer (OR 4.95, CI: 1.97–13.22) and (OR 5.17, CI: 2.11–13.50), respectively [98]. Another Tunisian case-control study with 130 cervical cancer patients and 260 controls showed that the rs4986790 dominant genotype Asp/Asp was significantly more frequent among cervical cancer cases with early stage (I + II) and advanced stage (III + IV) than controls. The major allele Asp was a risk factor for the I + II stage tumors [99]. Opposite results were reported in an Indian cervical cancer study involving 110 untreated cervical cancer patients and 141 healthy controls, where the minor allele G of rs4986790 was associated with an increased risk of cervical cancer, although a genotypic association was not found [83]. Pandey et al., in a study of North Indian women, did not observe an association between rs4986790 and rs4986791 with cervical cancer risk at the genotype, allele, and haplotype level. However, this study with 150 cervical cancer patients and 150 healthy female controls provided data showing that the Thr399Ile (rs4986791) polymorphism Thr/Ile genotype was significantly associated ($p = 0.044$) with Stage II cervical cancer and conferred a 2.51 fold risk of developing cervical cancer at an early stage [100]. A Chinese Han population study with 1262 participants, including 420 cervical cancer patients and 842 controls, did not find any significant association of rs4986791 with cervical cancer risk [101]. In the evaluation of other female-related cancers, the allele and genotype frequencies for the polymorphism rs4986790 were compared between 191 endometrial cancer cases and 291 controls in a study at the Hunter Centre, Australia, but no associations were observed for endometrial cancer risk [102]. The TLR4 Asp299Gly and Thr399Ile alleles were not detected in the 105 ovarian cancer patients in a study conducted in northern China. These results indicate that the *TLR4* 299Gly and 399Ile alleles were exhibited at a lower frequency in northern Chinese ovarian cancer patients compared to other studies [103]. Among the 70 women with ovarian cancer enrolled in the Poland study, the heterozygous variant and the recessive G allele of rs4986790 were more frequently found than in the 130 healthy individuals, indicating an increased risk of OC for its carriers. No difference in the distribution of rs4986791 between the cases and controls was observed [104]. A study conducted at the "Hippocratic" General Hospital of Athens, Greece, which included 261 breast cancer patients and 480 healthy individuals, showed that Gly carriers of rs4986790 (Asp/Gly & Gly/Gly genotype) and the Gly allele were more common among the breast cancer cases ($p = 0.0031$ and $p = 0.0061$, respectively) [105]. It was found to have a significant

association with breast cancer malignancy in the ER-patient groups for the rs4986790 in the Saudi Arabian population. In the ER-group, the AA genotype presented a significantly higher frequency in the patients compared to the controls. Similarly, the genotype AG was considerably less frequent in the cases compared to the controls [90].

TLR4 polymorphisms rs4986790 and rs4986791 may be associated with a significantly increased gastric cancer risk. Two publications by Juliana Garcia de Oliveira mention the significant influence of these SNPs on gastric cancer in the Brazilian population [106,107]. However, a study by Garza-Gonzalez et al. reported no correlation between *TLR4* polymorphisms and gastric cancer in the Mexican population [108]. A study by Trejo-de la et al. in a Mexican population found that the D299G (rs4986790) polymorphism was significantly associated with duodenal ulcer and showed a trend for association with gastric cancer [109]. In an Italian population case-control study by Santini et al. the Thr399Ile polymorphism was linked to increased susceptibility to gastric cancer [110]. Caucasian population-based case-control study data suggest that the *TLR4* + 896A > G polymorphism is a risk factor for non-cardia gastric carcinoma and its precursors [111]. The frequency of risk alleles of rs4986790 and rs4986791 in a nested case-control gastric cancer study in the European Prospective Study Cancer Group was low, and they could only estimate the association in the codominant model, which did not show significance [112]. In an Ethnic Kashmiri Population, no significance was observed in the appearance of gastric cancer, but odds Ratio analysis showed that carriers of the Asp299Gly G allele were significantly associated with the tumors in the distal part of the stomach. In contrast, carriers of the Thr399Ile T allele were associated with well-differentiated gastric adenocarcinoma [113]. The overall results from a meta-analysis of gastric cancer risk suggest that *TLR4* polymorphisms (+896A/G and +1196C/T) may be associated with a significantly increased risk of gastric cancer in Caucasians [114]. However, no SNPs were found at the sites Asp299Gly and Thr399Ile to be associated with susceptibility to GC in the Shangdong Province of Northern China [88].

Several studies have analyzed the association of rs4986790 and rs4986791 with colorectal cancer (CRC). However, studies conducted in Brazilian, Irish, Danish, and Iranian populations did not find a significant effect on CRC risk [92,115–117]. A meta-analysis supported the association of TLR4 genetic polymorphisms with an increased risk of CRC among Asians but not among Caucasians and Africans stratified by ethnic group [118]. An Egyptian study revealed that rs4986790 G allele carriers were more frequent in the CRC group compared to controls, and the T allele of rs4986791 was associated with an increased risk for CRC. Additionally, the rs4986791 CT/TT genotype was observed to be significantly linked to CRC. The G allele of rs4986790 predisposed to CRC progression, including high cancer stage IV, high grade III, positive lymph nodes (N2), and metastases [119]. The study conducted on Russian individuals with various solid tumors suggested that the A/G genotype for the rs4986790 SNP correlated with an 80% increased colorectal cancer. Rs4986790 polymorphisms were more evident in patients with rectal cancer separately [120].

Large case-control studies, meta-analysis have shown no significant association between rs4986790, rs4986791, and prostate cancer risk or clinical features [92,97,121,122].

Only a few studies have analyzed the associations of these SNPs with other cancers. A Turkish case-control study on lung cancer (NSCLC, SCLC) indicated that rs4986790 was not associated with lung cancer. However, the 3.857-fold risk was evaluated for the rs4986791 CT genotype compared to CC in lung cancer ($p = 0.041$) [123]. A study on patients with head and neck squamous cell carcinomas from Germany reported the results of the investigated SNPs rs4986790 and rs4986791. The Asp299Gly genotype, compared to Asp299Asp, was associated with poorer DFS ($p = 0.04$) and worse OS ($p = 0.04$). Patients with the rs4986790 wild-type genotype (*TLR4* Asp299Asp vs. *TLR4* Asp299Gly) had significantly longer DFS with adjuvant systemic treatment ($p = 0.004$). For another SNP rs4986791, a similar pattern was observed in DFS [124]. In a case-control study from the Indian population, rs4986791 was associated with a significantly elevated risk of gallbladder cancer [125]. Between melanoma cases and controls in Germany, patients carrying the minor allele for the rs4986790 polymorphism were associated with prolonged overall survival ($p = 0.01$)

and survival following metastases (p = 0.02) [126]. SNPs rs4986790 and rs1927906 were genotyped in a study of Saudi Arabian patients with acute lymphoblastic leukemia (ALL) and healthy controls. Only the AG showed a significant association with a protective effect against ALL (p = 0.002) [127].

Several meta-analyses have attempted to generalize these data. In a meta-analysis by Zhu L et al. based on 34 publications, *TLR4* rs4986790 and rs4986791 were found to increase overall cancer risk. The effect of rs4986790 on cancer risk was more evident in female-specific cancers and digestive cancers, especially for gastric cancer. The risks effect of rs4986791 was also prominent in gastric cancer. However, no significant association was observed between rs4986790 and prostate cancer risk. The association between rs4986791 and cancer risk was significant in both South Asians and East Asians, but not in Caucasians [128]. Ding et al. represented the results of a meta-analysis based on 55 publications. They found that Rs4986790 was not strongly associated with cancer risk. Meanwhile, the rs4986791 polymorphism has always been associated with reduced cancer risk in the general population. Moreover, they found that Caucasian female-specific cancers were significantly associated with rs4986790 polymorphisms in subgroup analysis by cancer type and race, while Asian digestive cancers were significantly influenced by the rs4986791 polymorphism [77].

Accumulated evidence has implicated *TLR4* polymorphism in modulating the risk and development of various types of cancers. However, we still need to replicate those findings. In our study, the results of these SNPs did not serve as indicators of possible disease progression biomarker.

We found no association of rs10983755, rs11536897, rs11536865, rs1927906 SNPs with clinical features and outcomes of CC. The rs10983755 polymorphism affects the risk of gastric carcinogenesis and can provide some protection against H. pylori infection [91,129]. However, the results showed no significant association with H. pylori infection, and there was no significant association of any examined genotype with the overall survival of GC in another Chinese population study. Patients with lymph node metastases undergoing postoperative chemotherapy and carrying the rs10983755 AA genotype had an HR of 0.328, compared to those carrying the GG + AG genotype [130]. The *TLR4* rs11536897 (−3084), rs1927906 (3189), and rs11536865 (−729G/C) polymorphisms are rare SNPs, and their functions remain unclear. In our study, all cases of rs11536865 had GG genotypes. There is a suggestion of an interaction between polymorphisms within *TLR4* and the HCV status [131]. The −729GC polymorphism was associated with an increased risk of bladder cancer. Moreover, the −729GC genotype significantly affected lower TLR4 mRNA and protein levels, suggesting that the polymorphism may lead to dysregulation of TLR4 expression, interfering with TLR4 promoter activity [132]. In a Korean men's case-control prostate cancer study, all 300 cases revealed the GG genotype at rs11536897 [133]. No link between SNPs rs11536897, rs1927906, and prostate cancer was found in the pooled Sweden case-control study and meta-analysis by Weng et al. [97,121].

The rs1927906 heterozygous CT was associated with a decreased cancer risk in Saudi Arabian patients with an ALL [127].

5. Conclusions

Our study suggests that SNPs rs10759932 and Rs11536898 may have the potential to be markers contributing to the assessment of cervical cancer survival prognosis. The SNP rs11536898 prompts us to consider its impact on cancer metastases and further research in this area. However, due to the limited sample size, a larger group of patients with cervical cancer is required to confirm the obtained results. Our results provide insight for future studies on cervical cancer and other infection-related cancer types, which can evaluate these polymorphisms to determine their functionality. Although evidence is accumulating for the importance of genetic variation in the etiology and development of cervical cancer, research investigating the role of immune-related gene variants in cervical cancer is still in its early stages. Further studies, preferably with larger groups of individuals from different ethnic

backgrounds, are needed to confirm the results of the current study. Therefore, identifying the variants responsible for maintaining the tumor immune response may provide more specific targets to combat cervical cancer development and disease progression. In the future, SNP detection in TLR4 may be used to predict the clinical manifestations, risk, and prognosis of CC.

Author Contributions: Conceptualization, E.Ž., A.I., R.U. and E.J.; Methodology, R.U. and A.I.; Formal analysis, E.Ž., A.I., R.U. and E.J.; Investigation, E.Ž. and R.U.; Resources, E.Ž. and A.I.; Data Curation, E.Ž; Writing—Original draft preparation, E.Ž.; Writing—Review and Editing, A.I., R.U. and E.J.; Visualization, E.Ž.; Supervision, A.I., R.U. and E.J.; Project Administration, Supervision, A.I., R.U. and E.J.; Funding Acquisition, E.Ž. and R.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Lithuanian University of Health Sciences.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors are grateful to the patients, nurses and colleagues who worked with the real-time PCR technique in the laboratory for this study.

Conflicts of Interest: The authors report no declaration of interest.

References

1. Coussens, L.M.; Werb, Z. Inflammation and cancer. *Nature* **2002**, *420*, 860–867. [CrossRef] [PubMed]
2. Emily, S.; Sigmund, A.W. Chronic inflammation and cancer. *Oncology* **2002**, *16*, 217–222. Available online: https://pubmed.ncbi.nlm.nih.gov/11866137/ (accessed on 1 January 2022).
3. Nitin, S.; Deepak, B.; Jagadish, P.R.; Pankaj, B.P.; Savita, S.T.; Veena, B.P. Inflammation and cancer Nitin Singh. *Ann. Afr. Med.* **2019**, *18*, 121–126.
4. Goldszmid, R.S.; Trinchieri, G. The price of immunity. *Nat. Immunol.* **2012**, *13*, 932–938. [CrossRef]
5. Ospelt, C.; Gay, S. TLRs and chronic inflammation. *Int. J. Biochem. Cell Biol.* **2010**, *42*, 495–505. [CrossRef]
6. Newton, K.; Dixit, V.M. Signaling in Innate Immunity and Inflammation. *Cold Spring Harb. Erspectives Biol.* **2012**, *4*, a006049. [CrossRef]
7. Pradere, J.P.; Dapito, D.H.; Schwabe, R.F. The Yin and Yang of Toll-like receptors in cancer. *Oncogene* **2013**, *33*, 3485–3495. [CrossRef]
8. Kawai, T.; Akira, S. TLR signaling. *Semin. Immunol.* **2007**, *19*, 24–32. [CrossRef]
9. Kawasaki, T.; Kawai, T. Toll-Like Receptor Signaling Pathways. *Front. Immunol.* **2014**, *5*, 461. [CrossRef]
10. Takeda, K.; Akira, S. TLR signaling pathways. *Semin. Immunol.* **2004**, *16*, 3–9. [CrossRef]
11. Lu, Y.C.; Yeh, W.C.; Ohashi, P.S. *LPS/TLR4* signal transduction pathway. *Cytokine* **2008**, *42*, 145–151. [CrossRef] [PubMed]
12. Cen, X.; Liu, S.; Cheng, K. The Role of Toll-Like Receptor in Inflammation and Tumor Immunity. *Front. Pharmacol.* **2018**, *9*, 878. [CrossRef] [PubMed]
13. Ou, L.; Lin, S.; Song, B.; Liu, J.; Lai, R.; Shao, L. The mechanisms of graphene-based materials-induced programmed cell death: A review of apoptosis, autophagy, and programmed necrosis. *Int. J. Nanomed.* **2017**, *12*, 6633–6646. [CrossRef] [PubMed]
14. Zhao, X.L.; Lin, Y.; Jiang, J.; Tang, Z.; Yang, S.; Lu, L.; Liang, Y.; Liu, X.; Tan, J.; Hu, X.G.; et al. High-mobility group box 1 released by autophagic cancer-associated fibroblasts maintains the stemness of luminal breast cancer cells. *J. Pathol.* **2017**, *243*, 376–389. [CrossRef]
15. Urban-Wojciuk, Z.; Khan, M.M.; Oyler, B.L.; Fåhraeus, R.; Marek-Trzonkowska, N.; Nita-Lazar, A.; Hupp, T.R.; Goodlett, D.R. The Role of TLRs in Anti-cancer Immunity and Tumor Rejection. *Front. Immunol.* **2019**, *10*, 2388. [CrossRef] [PubMed]
16. Keshavarz, A.; Pourbagheri-Sigaroodi, A.; Zafari, P.; Bagheri, N.; Ghaffari, S.H.; Bashash, D. Toll-like receptors (TLRs) in cancer; with an extensive focus on TLR agonists and antagonists. *IUBMB Life* **2020**, *73*, 10–25. [CrossRef]
17. Shi, M.; Chen, X.; Ye, K.; Yao, Y.; Li, Y. Application potential of toll-like receptors in cancer immunotherapy. *Medicine* **2016**, *95*, e3951. [CrossRef]
18. Huang, L.; Xu, H.; Peng, G. TLR-mediated metabolic reprogramming in the tumor microenvironment: Potential novel strategies for cancer immunotherapy. *Cell. Mol. Immunol.* **2018**, *15*, 428–437. [CrossRef]
19. Cheng, B.; Yuan, W.E.; Su, J.; Liu, Y.; Chen, J. Recent advances in small molecule based cancer immunotherapy. *Eur. J. Med. Chem.* **2018**, *157*, 582–598. [CrossRef]

20. Braunstein, M.J.; Kucharczyk, J.; Adams, S. Targeting Toll-Like Receptors for Cancer Therapy. *Target. Oncol.* **2018**, *13*, 583–598. [CrossRef]
21. Farooq, M.; Batool, M.; Kim, M.S.; Choi, S. Toll-Like Receptors as a Therapeutic Target in the Era of Immunotherapies. *Front. Cell Dev. Biol.* **2021**, *9*, 756315. [CrossRef] [PubMed]
22. Pahlavanneshan, S.; Sayadmanesh, A.; Ebrahimiyan, H.; Basiri, M. Toll-Like Receptor-Based Strategies for Cancer Immunotherapy. *J. Immunol. Res.* **2021**, *2021*, 9912188. [CrossRef] [PubMed]
23. Qureshi, S.T.; Larivière, L.; Leveque, G.; Clermont, S.; Moore, K.J.; Gros, P.; Malo, D. Endotoxin-tolerant Mice Have Mutations in Toll-like Receptor 4 (*Tlr4*). *J. Exp. Med.* **1999**, *189*, 615–625. [CrossRef]
24. Arbour, N.C.; Lorenz, E.; Schutte, B.C.; Zabner, J.; Kline, J.N.; Jones, M.; Frees, K.; Watt, J.L.; Schwartz, D.A. *TLR4* mutations are associated with endotoxin hyporesponsiveness in humans. *Nat. Genet.* **2000**, *25*, 187–191. [CrossRef]
25. O'Neill, L.A.J.; Bryant, C.E.; Doyle, S.L. Therapeutic Targeting of Toll-Like Receptors for Infectious and Inflammatory Diseases and Cancer. *Pharmacol. Rev.* **2009**, *61*, 177–197. [CrossRef] [PubMed]
26. Miller, S.I.; Ernst, R.K.; Bader, M.W. *LPS*, *TLR4* and infectious disease diversity. *Nat. Rev. Microbiol.* **2005**, *3*, 36–46. [CrossRef] [PubMed]
27. Olejnik, J.; Hume, A.J.; Mühlberger, E. Toll-like receptor 4 in acute viral infection: Too much of a good thing. *PLoS Pathog.* **2018**, *14*, e1007390. [CrossRef]
28. Shirey, K.A.; Blanco, J.C.G.; Vogel, S.N. Targeting *TLR4* Signaling to Blunt Viral-Mediated Acute Lung Injury. *Front. Immunol.* **2021**, *12*, 705080. [CrossRef]
29. Aguirre-García, M.; Rojas-Bernabé, A.; Gómez-García, A.R.; Escalona-Montaño, A. TLR-Mediated Host Immune Response to Parasitic Infectious Diseases. In *Toll-Like Receptors*; Rezaeli, N., Ed.; IntechOpen: London, UK, 2020; pp. 37–61. [CrossRef]
30. Li, H.; Sun, B. Toll-like receptor 4 in atherosclerosis. *J. Cell. Mol. Med.* **2007**, *11*, 88–95. [CrossRef]
31. Roshan, M.H.K.; Tambo, A.; Pace, N.P. The Role of *TLR2*, *TLR4*, and *TLR9* in the Pathogenesis of Atherosclerosis. *Int. J. Inflamm.* **2016**, *2016*, 1532832. [CrossRef]
32. Zeng, X.; Guo, R.; Dong, M.; Zheng, J.; Lin, H.; Lu, H. Contribution of *TLR4* signaling in intermittent hypoxia-mediated atherosclerosis progression. *J. Transl. Med.* **2018**, *16*, 106. [CrossRef]
33. Li, H.; Jiao, Y.; Xie, M. Paeoniflorin Ameliorates Atherosclerosis by Suppressing *TLR4*-Mediated NF-κB Activation. *Inflammation* **2017**, *40*, 2042–2051. [CrossRef] [PubMed]
34. Zakeri, A.; Russo, M. Dual Role of Toll-like Receptors in Human and Experimental Asthma Models. *Front. Immunol.* **2018**, *9*, 1027. [CrossRef] [PubMed]
35. Crespo-Lessmann, A.; Mateus, E.; Vidal, S.; Ramos-Barbón, D.; Torrejón, M.; Giner, J.; Soto, L.; Juárez, C.; Plaza, V. Expression of toll-like receptors 2 and 4 in subjects with asthma by total serum IgE level. *Respir. Res.* **2016**, *17*, 41. [CrossRef] [PubMed]
36. Zhao, J.; Shang, H.; Cao, X.; Huang, Y.; Fang, X.; Zhang, S.; Xie, M.; Xie, J.; Liu, X. Association of polymorphisms in *TLR2* and *TLR4* with asthma risk. *Medicine* **2017**, *96*, e7909. [CrossRef]
37. Li, Z.; Mao, X.; Liu, Q.; Song, H.; He, B.; Shi, P.; Zhang, Q.; Li, X.; Wang, J. Functional variations of the *TLR4* gene in association with chronic obstructive pulmonary disease and pulmonary tuberculosis. *BMC Pulm. Med.* **2019**, *19*, 184. [CrossRef] [PubMed]
38. Liu, M.; Xie, J.; Sun, Y. *TLR4/MyD88*/NF-κB-Mediated Inflammation Contributes to Cardiac Dysfunction in Rats of PTSD. *Cell. Mol. Neurobiol.* **2020**, *40*, 1029–1035. [CrossRef]
39. Jia, S.J.; Niu, P.P.; Cong, J.Z.; Zhang, B.K.; Zhao, M. *TLR4* signaling: A potential therapeutic target in ischemic coronary artery disease. *Int. Immunopharmacol.* **2014**, *23*, 54–59. [CrossRef]
40. Yu, L.; Feng, Z. The Role of Toll-Like Receptor Signaling in the Progression of Heart Failure. *Mediat. Inflamm.* **2018**, *2018*, 9874109. [CrossRef]
41. De Vicente, L.G.; Pinto, A.P.; da Rocha, A.L.; Pauli, J.R.; de Moura, L.P.; Cintra, D.E.; Ropelle, E.R.; da Silva, A.S. Role of *TLR4* in physical exercise and cardiovascular diseases. *Cytokine* **2020**, *136*, 155273. [CrossRef]
42. Cheng, Y.; Zhu, Y.; Huang, X.; Zhang, W.; Han, Z.; Liu, S. Association between *TLR2* and *TLR4* Gene Polymorphisms and the Susceptibility to Inflammatory Bowel Disease: A Meta-Analysis. *PLoS ONE* **2015**, *10*, e0126803. [CrossRef] [PubMed]
43. Dejban, P.; Nikravangolsefid, N.; Chamanara, M.; Dehpour, A.; Rashidian, A. The role of medicinal products in the treatment of inflammatory bowel diseases (IBD) through inhibition of *TLR4*/NF-kappaB pathway. *Phytother. Res.* **2020**, *35*, 835–845. [CrossRef] [PubMed]
44. Lu, Y.; Li, X.; Liu, S.; Zhang, Y.; Zhang, D. Toll-like Receptors and Inflammatory Bowel Disease. *Front. Immunol.* **2018**, *9*, 72. [CrossRef] [PubMed]
45. Sghaier, I.; Zidi, S.; Mouelhi, L.; Ghazoueni, E.; Brochot, E.; Almawi, W.; Loueslati, B. *TLR3* and *TLR4* SNP variants in the liver disease resulting from hepatitis B virus and hepatitis C virus infection. *Br. J. Biomed. Sci.* **2018**, *76*, 35–41. [CrossRef] [PubMed]
46. Kapil, S.; Duseja, A.; Sharma, B.K.; Singla, B.; Chakraborti, A.; Das, A.; Ray, P.; Dhiman, R.K.; Chawla, Y. Genetic polymorphism in *CD14* gene, a co-receptor of *TLR4* associated with non-alcoholic fatty liver disease. *World J. Gastroenterol.* **2016**, *22*, 9346. [CrossRef] [PubMed]
47. Weber, S.N.; Bohner, A.; Dapito, D.H.; Schwabe, R.F.; Lammert, F. *TLR4* Deficiency Protects against Hepatic Fibrosis and Diethylnitrosamine-Induced Pre-Carcinogenic Liver Injury in Fibrotic Liver. *PLoS ONE* **2016**, *11*, e0158819. [CrossRef]

48. Hritz, I.; Mandrekar, P.; Velayudham, A.; Catalano, D.; Dolganiuc, A.; Kodys, K.; Kurt-Jones, E.; Szabo, G. The critical role of toll-like receptor (TLR) 4 in alcoholic liver disease is independent of the common TLR adapter MyD88. *Hepatology* **2008**, *48*, 1224–1231. [CrossRef]
49. Santos-Martins, M.; Sameiro-Faria, M.; Ribeiro, S.; Rocha-Pereira, P.; Nascimento, H.; Reis, F.; Miranda, V.; Quintanilha, A.; Belo, L.; Beirão, I.; et al. *TLR4* and *TLR9* Polymorphisms Effect on Inflammatory Response in End-Stage Renal Disease Patients. *Eur. J. Inflamm.* **2014**, *12*, 521–529. [CrossRef]
50. Dessing, M.C.; Kers, J.; Damman, J.; Leuvenink, H.G.D.; van Goor, H.; Hillebrands, J.L.; Hepkema, B.G.; Snieder, H.; van den Born, J.; de Borst, M.H.; et al. Toll-Like Receptor Family Polymorphisms Are Associated with Primary Renal Diseases but Not with Renal Outcomes Following Kidney Transplantation. *PLoS ONE* **2015**, *10*, e0139769. [CrossRef]
51. Taha, I.M.; Abdu Allah, A.M.; Abd El Gayed, E.M. Expression of toll-like receptor 4 and its connection with type 2 diabetes mellitus. *Cell. Mol. Biol.* **2018**, *64*, 15–20. [CrossRef]
52. Wang, L.; Wang, J.; Fang, J.; Zhou, H.; Liu, X.; Su, S.B. High glucose induces and activates Toll-like receptor 4 in endothelial cells of diabetic retinopathy. *Diabetol. Metab. Syndr.* **2015**, *7*, 89. [CrossRef] [PubMed]
53. Wang, Z.; Ni, X.; Zhang, L.; Sun, L.; Zhu, X.; Zhou, Q.; Yang, Z.; Yuan, H. Toll-Like Receptor 4 and Inflammatory Micro-Environment of Pancreatic Islets in Type-2 Diabetes Mellitus: A Therapeutic Perspective. *Diabetes Metab. Syndr. Obes. Targets Ther.* **2020**, *13*, 4261–4272. [CrossRef] [PubMed]
54. Huang, Q.Q.; Pope, R.M. The role of Toll-like receptors in rheumatoid arthritis. *Curr. Rheumatol. Rep.* **2009**, *11*, 357–364. [CrossRef] [PubMed]
55. Dai, Q.; Li, Y.; Wang, M.; Li, Y.; Li, J. TlR2 and TlR4 are involved in the treatment of rheumatoid arthritis synovial fibroblasts with a medicated serum of asarinin through inhibition of Th1/Th17 cytokines. *Exp. Ther. Med.* **2020**, *19*, 3009–3016. [CrossRef] [PubMed]
56. Huang, N.Q.; Jin, H.; Zhou, S.Y.; Shi, J.S.; Jin, F. *TLR4* is a link between diabetes and Alzheimer's disease. *Behav. Brain Res.* **2017**, *316*, 234–244. [CrossRef]
57. Yang, J.; Wise, L.; Fukuchi, K.I. *TLR4* Cross-Talk With NLRP3 Inflammasome and Complement Signaling Pathways in Alzheimer's Disease. *Front. Immunol.* **2020**, *11*, 724. [CrossRef]
58. Miron, J.; Picard, C.; Lafaille-Magnan, M.; Savard, M.; Labonté, A.; Breitner, J.; Rosa-Neto, P.; Auld, D.; Poirier, J. Association of TLR4 with Alzheimer's disease risk and presymptomatic biomarkers of inflammation. *Alzheimers Dement.* **2019**, *15*, 951–960. [CrossRef]
59. Paudel, Y.N.; Angelopoulou, E.; Piperi, C.; Othman, I.; Aamir, K.; Shaikh, M.F. Impact of HMGB1, RAGE, and TLR4 in Alzheimer's Disease (AD): From Risk Factors to Therapeutic Targeting. *Cells* **2020**, *9*, 383. [CrossRef]
60. Perez-Pardo, P.; Dodiya, H.B.; Engen, P.A.; Forsyth, C.B.; Huschens, A.M.; Shaikh, M.; Voigt, R.M.; Naqib, A.; Green, S.J.; Kordower, J.H.; et al. Role of TLR4 in the gut-brain axis in Parkinson's disease: A translational study from men to mice. *Gut* **2018**, *68*, 829–843. [CrossRef]
61. Zhao, J.; Han, X.; Xue, L.; Zhu, K.; Liu, H.; Xie, A. Association of *TLR4* gene polymorphisms with sporadic Parkinson's disease in a Han Chinese population. *Neurol. Sci.* **2015**, *36*, 1659–1665. [CrossRef]
62. Kouli, A.; Horne, C.; Williams-Gray, C. Toll-like receptors and their therapeutic potential in Parkinson's disease and α-synucleinopathies. *Brain Behav. Immun.* **2019**, *81*, 41–51. [CrossRef] [PubMed]
63. Campolo, M.; Paterniti, I.; Siracusa, R.; Filippone, A.; Esposito, E.; Cuzzocrea, S. TLR4 absence reduces neuroinflammation and inflammasome activation in Parkinson's diseases in vivo model. *Brain Behav. Immun.* **2019**, *76*, 236–247. [CrossRef] [PubMed]
64. Obadia, N.; Andrade, G.; Leardini-Tristão, M.; Albuquerque, L.; Garcia, C.; Lima, F.; Daleprane, J.; Castro-Faria-Neto, H.C.; Tibiriçá, E.; Estato, V. *TLR4* mutation protects neurovascular function and cognitive decline in high-fat diet-fed mice. *J. Neuroinflamm.* **2022**, *19*, 104. [CrossRef] [PubMed]
65. Asadzadeh Manjili, F.; Yousefi-Ahmadipour, A.; Kazemi Arababadi, M. The roles played by *TLR4* in the pathogenesis of multiple sclerosis; A systematic review article. *Immunol. Lett.* **2020**, *220*, 63–70. [CrossRef]
66. Oliveira, I.; Gomes, R.; Gomides, L.; dos Santos, J.; Carneiro, M.; Ribeiro-Dias, F.; Diniz, D. Interferon-Beta Treatment Differentially Alters TLR2 and TLR4-Dependent Cytokine Production in Multiple Sclerosis Patients. *Neuroimmunomodulation* **2019**, *26*, 77–83. [CrossRef]
67. Oliveira, J.; Busson, M.; Etain, B.; Jamain, S.; Hamdani, N.; Boukouaci, W.; Amokrane, K.; Bennabi, M.; Le Guen, E.; Dargél, A.A.; et al. Polymorphism of Toll-like receptor 4 gene in bipolar disorder. *J. Affect. Disord.* **2014**, *152–154*, 395–402. [CrossRef]
68. Oblak, A.; Jerala, R. Toll-Like Receptor 4 Activation in Cancer Progression and Therapy. *Clin. Dev. Immunol.* **2011**, *2011*, 609579. [CrossRef]
69. Ran, S.; Bhattarai, N.; Patel, R.; Volk-Draper, L. *TLR4*-Induced Inflammation Is a Key Promoter of Tumor Growth, Vascularization, and Metastasis. In *Translational Studies on Inflammation*; Nunes, A.C.F., Ed.; IntechOpen: London, UK, 2020; pp. 133–167. [CrossRef]
70. Hao, B.; Chen, Z.; Bi, B.; Yu, M.; Yao, S.; Feng, Y.; Yu, Y.; Pan, L.; Di, D.; Luo, G.; et al. Role of TLR4 as a prognostic factor for survival in various cancers oncotarget: A meta-analysis. *Oncotarget* **2018**, *9*, 13088–13099. [CrossRef]
71. Jiang, N.; Xie, F.; Chen, L.; Chen, F.; Sui, L. The effect of *TLR4* on the growth and local inflammatory microenvironment of HPV-related cervical cancer in vivo. *Infect. Agents Cancer* **2020**, *15*, 10–12. [CrossRef]

72. Wang, Y.; Weng, Y.; Shi, Y.; Xia, X.; Wang, S.; Duan, H. Expression and Functional Analysis of Toll-like Receptor 4 in Human Cervical Carcinoma. *J. Membr. Biol.* **2014**, *247*, 591–599. [CrossRef]
73. Zhang, H.; Zhang, S. The expression of *Foxp3* and *TLR4* in cervical cancer: Association with immune escape and clinical pathology. *Arch. Gynecol. Obstet.* **2016**, *295*, 705–712. [CrossRef] [PubMed]
74. Pappa, K.; Kontostathi, G.; Lygirou, V.; Zoidakis, J.; Anagnou, N. Novel structural approaches concerning HPV proteins: Insight into targeted therapies for cervical cancer (Review). *Oncol. Rep.* **2018**, *39*, 1547–1554. [CrossRef] [PubMed]
75. Yang, X.; Cheng, Y.; Li, C. The role of TLRs in cervical cancer with HPV infection: A review. *Signal Transduct. Target. Ther.* **2017**, *2*, 17055. [CrossRef] [PubMed]
76. Zhang, K.; Zhou, B.; Wang, Y.; Rao, L.; Zhang, L. The *TLR4* gene polymorphisms and susceptibility to cancer: A systematic review and meta-analysis. *Eur. J. Cancer* **2013**, *49*, 946–954. [CrossRef]
77. Ding, L.; Jiang, Q.; Li, G.; Shen, J.; Du, J.; Lu, X.; Xiong, X. Comprehensive assessment of association between *TLR4* gene polymorphisms and cancer risk: A systematic meta-analysis. *Oncotarget* **2017**, *8*, 100593–100602. [CrossRef]
78. Kutikhin, A.G. Impact of Toll-like receptor 4 polymorphisms on risk of cancer. *Hum. Immunol.* **2011**, *72*, 193–206. [CrossRef]
79. Pandey, N.; Chauhan, A.; Jain, N. TLR4 Polymorphisms and Expression in Solid Cancers. *Mol. Diagn. Ther.* **2018**, *22*, 683–702. [CrossRef]
80. Chauhan, A.; Pandey, N.; Desai, A.; Raithatha, N.; Patel, P.; Choxi, Y.; Kapadia, R.; Khandelwal, R.; Jain, N. Association of *TLR4* and *TLR9* gene polymorphisms and haplotypes with cervicitis susceptibility. *PLoS ONE* **2019**, *14*, e0220330. [CrossRef]
81. Huang, C.; Li, Z.; Zhong, X.; Wang, Y.; Ye, X.; Jing, L.; Huang, S.; Yin, Q.; Miao, Z.; Zhou, Z.; et al. Association between polymorphisms in *TLR4* gene targeted by microRNA-140 and cervical precancerous lesion in south Chinese women: A case control study. *Genet. Mol. Res.* **2017**, *16*, gmr16039831. [CrossRef]
82. Nath, N.; Mishra, P.; Panda, A.K.; Mishra, R. Polymorphisms and haplotypes of *TLR4, TLR9* and *CYP1A1* genes possibly interfere with high-risk human papillomavirus infection and cervical cancer susceptibility in Jharkhand, India. *Int. Immunopharmacol.* **2020**, *88*, 106925. [CrossRef]
83. Pandey, N.O.; Chauhan, A.V.; Raithatha, N.S.; Patel, P.K.; Khandelwal, R.; Desai, A.N.; Choxi, Y.; Kapadia, R.S.; Jain, N.D. Association of *TLR4* and *TLR9* polymorphisms and haplotypes with cervical cancer susceptibility. *Sci. Rep.* **2019**, *9*, 9729. [CrossRef] [PubMed]
84. Pandey, N.; Chauhan, A.; Raithatha, N.; Patel, P.; Khandelwal, R.; Desai, A.; Choxi, Y.; Kapadia, R.; Jain, N. Influence of *TLR4* and *TLR9* polymorphisms and haplotypes on multiple hrHPV infections and HPV16 copy number in cervical cancer and cervicitis. *Microb. Pathog.* **2021**, *159*, 105149. [CrossRef] [PubMed]
85. Huang, H.; Wu, J.; Jin, G.; Zhang, H.; Ding, Y.; Hua, Z.; Zhou, Y.; Xue, Y.; Lu, Y.; Hu, Z.; et al. A 5′-flanking region polymorphism in toll-like receptor 4 is associated with gastric cancer in a Chinese population. *J. Biomed. Res.* **2010**, *24*, 100–106. [CrossRef] [PubMed]
86. Tongtawee, T.; Simawaranon, T.; Wattanawongdon, W.; Dechsukhum, C.; Leeanansaksiri, W. Toll-like receptor 2 and 4 polymorphisms associated with Helicobacter pylori susceptibility and gastric cancer. *Turk. J. Gastroenterol.* **2018**, *30*, 15–20. [CrossRef]
87. Cheng, I.; Plummer, S.J.; Casey, G.; Witte, J.S. Toll-Like Receptor 4 Genetic Variation and Advanced Prostate Cancer Risk. *Cancer Epidemiol. Biomark. Prev.* **2007**, *16*, 352–355. [CrossRef]
88. Luo, B.; Han, L.; Liu, S.; Wang, X.; Shi, Y.; Zhao, Z.; Zhao, C. Toll-like receptor gene polymorphisms and susceptibility to Epstein-Barr virus-associated and -negative gastric carcinoma in Northern China. *Saudi J. Gastroenterol.* **2015**, *21*, 95. [CrossRef]
89. Suzuki, T.; Meguro, A.; Matsushima, M.; Masui, A.; Tsuda, S.; Nakamura, J.; Nishina, R.; Uchida, T.; Yuhara, H.; Igarashi, M.; et al. Investigation of the Association of *TLR2* and *TLR4* Polymorphisms with Susceptibility to Helicobacter pylori-Related Gastrointestinal Diseases. *Open J. Intern. Med.* **2014**, *4*, 52496.
90. Semlali, A.; Jalouli, M.; Parine, N.R.; Al Amri, A.; Arafah, M.; Al Naeem, A.; Abdullah, S.; Rouabhia, M.; Alanazi, M. Toll-like receptor-4 as a predictor of clinical outcomes of estrogen receptor-negative breast cancer in Saudi women. *OncoTargets Ther.* **2017**, *10*, 1207–1216. [CrossRef]
91. Kim, J.; Cho, Y.A.; Choi, I.J.; Lee, Y.S.; Kim, S.Y.; Hwang, J.A.; Cho, S.J.; Kook, M.C.; Kim, C.G.; Kim, Y.W. Effects of Polymorphisms of Innate Immunity Genes and Environmental Factors on the Risk of Noncardia Gastric Cancer. *Cancer Res. Treat.* **2013**, *45*, 313–324. [CrossRef]
92. Proença, M.A. *TLR2* and *TLR4* polymorphisms influence mRNA and protein expression in colorectal cancer. *World J. Gastroenterol.* **2015**, *21*, 7730. [CrossRef]
93. Shui, I.M.; Stark, J.R.; Penney, K.L.; Schumacher, F.R.; Epstein, M.M.; Pitt, M.J.; Stampfer, M.J.; Tamimi, R.M.; Lindstrom, S.; Sesso, H.D.; et al. Genetic variation in the toll-like receptor 4 and prostate cancer incidence and mortality. *Prostate* **2011**, *72*, 209–216. [CrossRef]
94. Chen, Y.C.; Giovannucci, E.; Lazarus, R.; Kraft, P.; Ketkar, S.; Hunter, D.J. Sequence Variants of Toll-Like Receptor 4 and Susceptibility to Prostate Cancer. *Cancer Res.* **2005**, *65*, 11771–11778. [CrossRef]
95. Slattery, M.L.; Herrick, J.S.; Bondurant, K.L.; Wolff, R.K. Toll-like receptor genes and their association with colon and rectal cancer development and prognosis. *Int. J. Cancer* **2011**, *130*, 2974–2980. [CrossRef] [PubMed]
96. Wang, M.H.; Helzlsouer, K.J.; Smith, M.W.; Hoffman-Bolton, J.A.; Clipp, S.L.; Grinberg, V.; De Marzo, A.M.; Isaacs, W.B.; Drake, C.G.; Shugart, Y.Y.; et al. Association of *IL10* and Other immune response- and obesity-related genes with prostate cancer in CLUE II. *Prostate* **2009**, *69*, 874–885. [CrossRef] [PubMed]

97. Lindström, S.; Hunter, D.J.; Grönberg, H.; Stattin, P.; Wiklund, F.; Xu, J.; Chanock, S.J.; Hayes, R.; Kraft, P. Sequence Variants in the *TLR4* and *TLR6-1-10* Genes and Prostate Cancer Risk. Results Based on Pooled Analysis from Three Independent Studies. *Cancer Epidemiol. Biomark. Prev.* **2010**, *19*, 873–876. [CrossRef]
98. Zidi, S.; Verdi, H.; Yilmaz-Yalcin, Y.; Yazici, A.; Gazouani, E.; Mezlini, A.; Atac, F.B.; Yacoubi-Loueslati, B. Involvement of Toll-like receptors in cervical cancer susceptibility among Tunisian women. *Bull. Du Cancer* **2014**, *101*, E31–E35. [CrossRef] [PubMed]
99. Zidi, S.; Sghaier, I.; Gazouani, E.; Mezlini, A.; Yacoubi-Loueslati, B. Evaluation of Toll-Like Receptors 2/3/4/9 Gene Polymorphisms in Cervical Cancer Evolution. *Pathol. Oncol. Res.* **2015**, *22*, 323–330. [CrossRef]
100. Pandey, S.; Mittal, R.D.; Srivastava, M.; Srivastava, K.; Singh, S.; Srivastava, S.; Mittal, B. Impact of Toll-like receptors [TLR] 2 (−196 to −174 del) and TLR 4 (Asp299Gly, Thr399Ile) in cervical cancer susceptibility in North Indian women. *Gynecol. Oncol.* **2009**, *114*, 501–505. [CrossRef] [PubMed]
101. Jin, Y.; Qiu, S.; Shao, N.; Zheng, J. Association of toll-like receptor gene polymorphisms and its interaction with HPV infection in determining the susceptibility of cervical cancer in Chinese Han population. *Mamm. Genome* **2017**, *28*, 213–219. [CrossRef]
102. Ashton, K.A.; Proietto, A.; Otton, G.; Symonds, I.; McEvoy, M.; Attia, J.; Scott, R.J. Toll-Like Receptor (TLR) and Nucleosome-binding Oligomerization Domain (NOD) gene polymorphisms and endometrial cancer risk. *BMC Cancer* **2010**, *10*, 382–387. [CrossRef]
103. Wang, A.C.; Wu, F.X.; Gao, Y.S.; Sheng, X.G. Toll-like receptor 4 single-nucleotide polymorphisms Asp299Gly and Thr399Ile in ovarian cancers. *Oncol. Lett.* **2014**, *8*, 438–440. [CrossRef] [PubMed]
104. Kania, K.D.; Haręża, D.; Wilczyński, J.R.; Wilczyński, M.; Jarych, D.; Malinowski, A.; Paradowska, E. The Toll-like Receptor 4 Polymorphism Asp299Gly Is Associated with an Increased Risk of Ovarian Cancer. *Cells* **2022**, *11*, 3137. [CrossRef] [PubMed]
105. Theodoropoulos, G.E.; Saridakis, V.; Karantanos, T.; Michalopoulos, N.V.; Zagouri, F.; Kontogianni, P.; Lymperi, M.; Gazouli, M.; Zografos, G.C. Toll-like receptors gene polymorphisms may confer increased susceptibility to breast cancer development. *Breast* **2012**, *21*, 534–538. [CrossRef] [PubMed]
106. De Oliveira, J.G.; Rossi, A.F.T.; Nizato, D.M.; Miyasaki, K.; Silva, A.E. Profiles of Gene Polymorphisms in Cytokines and Toll-Like Receptors with Higher Risk for Gastric Cancer. *Dig. Dis. Sci.* **2012**, *58*, 978–988. [CrossRef] [PubMed]
107. Oliveira, J.G.D. (2012). Polymorphisms of the *TLR2* and *TLR4* genes are associated with risk of gastric cancer in a Brazilian population. *World J. Gastroenterol.* **2012**, *18*, 1235. [CrossRef] [PubMed]
108. Garza-Gonzalez, E.; Bosques-Padilla, F.J.; Mendoza-Ibarra, S.I.; Flores-Gutierrez, J.P.; Maldonado-Garza, H.J.; Perez-Perez, G.I. Assessment of the toll-like receptor 4 Asp299Gly, Thr399Ile and interleukin-8 -251 polymorphisms in the risk for the development of distal gastric cancer. *BMC Cancer* **2007**, *7*, 70. [CrossRef]
109. Trejo-de la, O.A.; Torres, J.; Pérez-Rodríguez, M.; Camorlinga-Ponce, M.; Luna, L.F.; Abdo-Francis, J.M.; Lazcano, E.; Maldonado-Bernal, C. *TLR4* single-nucleotide polymorphisms alter mucosal cytokine and chemokine patterns in Mexican patients with Helicobacter pylori-associated gastroduodenal diseases. *Clin. Immunol.* **2008**, *129*, 333–340. [CrossRef]
110. Santini, D.; Angeletti, S.; Ruzzo, A.; Dicuonzo, G.; Galluzzo, S.; Vincenzi, B.; Calvieri, A.; Pizzagalli, F.; Graziano, N.; Ferraro, E.; et al. Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms in gastric cancer of intestinal and diffuse histotypes. *Clin. Exp. Immunol.* **2008**, *154*, 360–364. [CrossRef]
111. Hold, G.L.; Rabkin, C.S.; Chow, W.; Smith, M.G.; Gammon, M.D.; Risch, H.A.; Vaughan, T.L.; McColl, K.E.; Lissowska, J.; Zatonski, W.; et al. A Functional Polymorphism of Toll-Like Receptor 4 Gene Increases Risk of Gastric Carcinoma and Its Precursors. *Gastroenterology* **2007**, *132*, 905–912. [CrossRef]
112. Companioni, O.; Bonet, C.; Muñoz, X.; Weiderpass, E.; Panico, S.; Tumino, R.; Palli, D.; Agnoli, C.; Vineis, P.; Boutron-Ruault, M.C.; et al. Polymorphisms of *Helicobacter pylori* signaling pathway genes and gastric cancer risk in the European prospective investigation into cancer-eurgast cohort. *Int. J. Cancer* **2013**, *134*, 92–101. [CrossRef]
113. Qadri, Q.; Rasool, R.; Afroze, D.; Naqash, S.; Gulzar, G.M.; Yousuf, A.; Siddiqi, M.A.; Shah, Z.A. Study of *TLR4* and *IL-8* Gene Polymorphisms in H.pylori-Induced Inflammation in Gastric Cancer in an Ethnic Kashmiri Population. *Immunol. Investig.* **2013**, *43*, 324–336. [CrossRef] [PubMed]
114. Zhou, Q.; Wang, C.; Wang, X.; Wu, X.; Zhu, Z.; Liu, B.; Su, L. Association between *TLR4* (+896A/G and +1196C/T) Polymorphisms and Gastric Cancer Risk: An Updated Meta-Analysis. *PLoS ONE* **2014**, *9*, e109605. [CrossRef] [PubMed]
115. Reilly, F.; Burke, J.P.; Lennon, G.; Kay, E.W.; McNamara, D.A.; Cullen, G.; Doherty, G.A.; Mulcahy, H.; Martin, S.; Winter, D.C.; et al. A case–control study examining the association of *smad7* and *TLR* single nucleotide polymorphisms on the risk of colorectal cancer in ulcerative colitis. *Color. Dis.* **2021**, *23*, 1043–1048. [CrossRef] [PubMed]
116. Asghari, A.; Nowras, T.; Tavakoli, T.; Fakharian, T.; Razavi, F.E.; Soltaninejad, E.; Naseri, M. Association between rs4986790 and rs4986791 Polymorphisms in *TLR4* with Colorectal Cancer Risk in Iranian Population. *Russ. J. Genet.* **2021**, *57*, 740–744. [CrossRef]
117. Kopp, T.I.; Andersen, V.; Tjonneland, A.; Vogel, U. Polymorphisms in *NFKB1* and *TLR4* and Interaction with Dietary and Life Style Factors in Relation to Colorectal Cancer in a Danish Prospective Case-Cohort Study. *PLoS ONE* **2015**, *10*, e0116394. [CrossRef]
118. Li, X.X.; Sun, G.P.; Meng, J.; Li, X.; Tang, Y.X.; Li, Z.; Wang, M.F.; Liang, G.F.; Lu, X.B. Role of Toll-Like Receptor 4 in Colorectal Carcinogenesis: A Meta-Analysis. *PLoS ONE* **2014**, *9*, e93904. [CrossRef]
119. Moaaz, M.; Youssry, S.; Moaz, A.; Abdelrahman, M. Study of Toll-Like Receptor 4 Gene Polymorphisms in Colorectal Cancer: Correlation with Clinicopathological Features. *Immunol. Investig.* **2020**, *49*, 571–584. [CrossRef]
120. Kutikhin, A.G.; Yuzhalin, A.E.; Volkov, A.N.; Zhivotovskiy, A.S.; Brusina, E.B. Correlation between genetic polymorphisms within IL-1B and TLR4 genes and cancer risk in a Russian population: A case-control study. *Tumor Biol.* **2014**, *35*, 4821–4830. [CrossRef]

121. Weng, P.H.; Huang, Y.L.; Page, J.H.; Chen, J.H.; Xu, J.; Koutros, S.; Berndt, S.; Chanock, S.; Yeager, M.; Witte, J.S.; et al. Polymorphisms of an Innate Immune Gene, Toll-Like Receptor 4, and Aggressive Prostate Cancer Risk: A Systematic Review and Meta-Analysis. *PLoS ONE* **2014**, *9*, e110569. [CrossRef]
122. Winchester, D.A.; Till, C.; Goodman, P.J.; Tangen, C.M.; Santella, R.M.; Johnson-Pais, T.L.; Leach, R.J.; Xu, J.; Zheng, S.L.; Thompson, I.M.; et al. Association between variants in genes involved in the immune response and prostate cancer risk in men randomized to the finasteride arm in the Prostate Cancer Prevention Trial. *Prostate* **2017**, *77*, 908–919. [CrossRef]
123. Kurt, H.; Ozbayer, C.; Bayramoglu, A.; Gunes, H.V.; Degirmenci, R.; Oner, K.S.; Metintas, M. Determination of the Relationship Between rs4986790 and rs4986791 Variants of *TLR4* Gene and Lung Cancer. *Inflammation* **2015**, *39*, 166–171. [CrossRef] [PubMed]
124. Bergmann, C.; Bachmann, H.S.; Bankfalvi, A.; Lotfi, R.; Pütter, C.; Wild, C.A.; Schuler, P.J.; Greve, J.; Hoffmann, T.K.; Lang, S.; et al. Toll-like receptor 4 single-nucleotide polymorphisms Asp299Gly and Thr399Ile in head and neck squamous cell carcinomas. *J. Transl. Med.* **2011**, *9*, 139. [CrossRef] [PubMed]
125. Srivastava, K.; Srivastava, A.; Kumar, A.; Mittal, B. Significant association between toll-like receptor gene polymorphisms and gallbladder cancer. *Liver Int.* **2010**, *30*, 1067–1072. [CrossRef] [PubMed]
126. Gast, A.; Bermejo, J.L.; Claus, R.; Brandt, A.; Weires, M.; Weber, A.; Plass, C.; Sucker, A.; Hemminki, K.; Schadendorf, D.; et al. Association of Inherited Variation in Toll-Like Receptor Genes with Malignant Melanoma Susceptibility and Survival. *PLoS ONE* **2011**, *6*, e24370. [CrossRef]
127. AlKhulaifi, F.M.; Alkhuriji, A.; Mansour, L.; Al-jurayyan, A.; Al-Mulhim, N.M.; Tashkandy, Y.A.; Aldossari, G.S.; Alomar, S. Association between Toll-like receptor 4 polymorphism and Acute Lymphoblastic Leukemia susceptibility in Saudi Arabian patients. *J. King Saud Univ.—Sci.* **2022**, *34*, 101985. [CrossRef]
128. Zhu, L.; Yuan, H.; Jiang, T.; Wang, R.; Ma, H.; Zhang, S. Association of *TLR2* and *TLR4* Polymorphisms with Risk of Cancer: A Meta-Analysis. *PLoS ONE* **2013**, *8*, e82858. [CrossRef]
129. Li, P.; He, C.Y.; Xu, Q.; Sun, L.P.; Ha, M.W.; Yuan, Y. Effect of the −2081G/A Polymorphism of the *TLR4* Gene and Its Interaction with *Helicobacter pylori* Infection on the Risk of Gastric Cancer in Chinese Individuals. *Genet. Test. Mol. Biomark.* **2014**, *18*, 610–615. [CrossRef]
130. Zhao, D.; Wu, Y.H.; Zhao, T.C.; Jia, Z.F.; Cao, D.H.; Yang, N.; Wang, Y.Q.; Cao, X.Y.; Jiang, J. Single-nucleotide polymorphisms in Toll-like receptor genes are associated with the prognosis of gastric cancer and are not associated with Helicobacter pylori infection. *Infect. Genet. Evol.* **2019**, *73*, 384–389. [CrossRef]
131. Oetting, W.S.; Guan, W.; Schladt, D.P.; Leduc, R.E.; Jacobson, P.A.; Matas, A.J.; Chinnakotla, S.; Schröppel, B.; Murphy, B.T.; Israni, A.K. Donor polymorphisms of toll-like receptor 4 associated with graft failure in liver transplant recipients. *Liver Transplant.* **2012**, *18*, 1399–1405. [CrossRef]
132. Shen, Y.; Bu, M.; Zhang, A.; Liu, Y.; Fu, B. Toll-like receptor 4 gene polymorphism downregulates gene expression and involves in susceptibility to bladder cancer. *Tumor Biol.* **2014**, *36*, 2779–2784. [CrossRef]
133. Song, J.; Kim, D.Y.; Kim, C.S.; Kim, H.J.; Lee, D.H.; Lee, H.; Ko, W.; Lee, G. The association between Toll-like receptor 4 (*TLR4*) polymorphisms and the risk of prostate cancer in Korean men. *Cancer Genet. Cytogenet.* **2009**, *190*, 88–92. [CrossRef] [PubMed]

cancers

MDPI

Article

Impact of *RRP1B* Variants on the Phenotype, Progression, and Metastasis of Cervical Cancer

Eglė Balčiūnienė [1,*], Arturas Inčiūra [1], Elona Juozaitytė [1] and Rasa Ugenskienė [1,2]

[1] Institute of Oncology, Lithuanian University of Health Sciences, LT-50161 Kaunas, Lithuania; arturas.inciura@lsmu.lt (A.I.); elona.juozaityte@lsmu.lt (E.J.); rasa.ugenskiene@lsmu.lt (R.U.)
[2] Department of Genetics and Molecular Medicine, Lithuanian University of Health Sciences, LT-50161 Kaunas, Lithuania
* Correspondence: egle.balciuniene@lsmu.lt; Tel.: +370-6821-3898

Simple Summary: Metastasis, a critical aspect of oncologic diseases, is intricately governed by genetic factors. This article delves into the role of the ribosomal RNA processing 1 homolog B (*RRP1B*) gene in metastasis regulation, investigating its implications in human cervical cancer. We analyzed five *RRP1B* polymorphisms in 172 cervical cancer patients to understand their associations with disease characteristics and survival. Certain variations were linked to decreased tumor size, reduced metastasis risk, and improved overall survival, suggesting their potential as markers for predicting prognosis in cervical cancer.

Abstract: Metastasis is a key determinant of cancer progression, influenced significantly by genetic mechanisms. RRP1B, primarily a nucleolar protein, emerges as a suppressor of metastasis, forming alliances with various cellular components and modulating gene expression. This study investigates the involvement of the ribosomal RNA processing 1 homolog B (*RRP1B*) gene in metastasis regulation in cervical cancer. Through a comprehensive analysis of 172 cervical cancer patients, we evaluated five *RRP1B* single nucleotide polymorphisms (SNPs) (rs2838342, rs7276633, rs2051407, rs9306160, and rs762400) for their associations with clinicopathological features and survival outcomes. Significant associations were observed between specific genetic variants and clinicopathological parameters. Notably, the A allele of rs2838342 was associated with reduced odds of advanced tumor size, worse prognosis, and, preliminarily, distant metastasis, while the T allele of rs7276633 correlated with a decreased risk of higher tumor size and worse prognosis. Additionally, the C allele of rs2051407 demonstrated protective effects against larger tumors, metastasis, and adverse prognosis. The rs9306160 C allele exhibited a protective effect against metastasis. The rs762400 G allele was significant for reduced tumor size and metastasis risk. Furthermore, the rs2838342 A allele, rs7276633 T allele, rs2051407 C allele, and rs762400 G allele were associated with improved overall survival, demonstrating their potential significance in predicting prognoses in cervical cancer. Linkage disequilibrium and haplotypes analysis enabled us to evaluate the collective effect of the analyzed SNPs, which was in line with the results of allelic models. Our findings underscore the clinical relevance of *RRP1B* SNPs as prognostic markers in cervical cancer, shedding light on the intricate interplay between genetic factors and disease-progression dynamics. This research provides critical insights for future investigations and underscores the importance of incorporating *RRP1B* SNP detection into prognostic-assessment tools for accurate prediction of disease outcomes in cervical cancer.

Keywords: cervical cancer; *RRP1B*; polymorphisms; genotype; haplotype; metastasis; survival

Citation: Balčiūnienė, E.; Inčiūra, A.; Juozaitytė, E.; Ugenskienė, R. Impact of RRP1B Variants on the Phenotype, Progression, and Metastasis of Cervical Cancer. *Cancers* **2024**, *16*, 1250. https://doi.org/10.3390/cancers16071250

Academic Editor: Jonas Cicenas

Received: 29 January 2024
Revised: 17 March 2024
Accepted: 20 March 2024
Published: 22 March 2024

1. Introduction

Metastasis stands as a paramount and intricate phenomenon in the domain of oncologic diseases. Earlier investigations have ascertained the noteworthy impact of the genetic context in which tumors originate on their proclivity for metastasis. Predictive human gene-expression profiles associated with metastasis exhibit their presence not solely in mouse tumors featuring varying metastatic capacities, but they also display a discernible correlation with the inherent genetic backdrop. It is suggested that the genesis of human metastasis-predictive gene expression signatures may be markedly propelled by the genetic background, eclipsing the influence of acquired somatic mutations [1–6]. The capacity to discern individuals at an elevated risk of disseminated disease precisely during the clinical manifestation of primary cancer holds the potential for a substantial paradigm shift in cancer management.

Employing a meticulously characterized transgenic model of mouse mammary tumorigenesis, the ribosomal RNA-processing 1 homolog B (*RRP1B*/*KIAA0179)* gene has been pinpointed as a potential modifier QTL gene impacting metastasis efficiency [7–9]. RRP1B is primarily identified as a nucleolar protein and is also a nuclear membrane-associated protein, although it has been reported in multiple cellular locations. *The RRP1B* gene is located on Chromosome 21q22.3, and the protein contains 758 amino acids. Previous investigations have revealed that *RRP1B* forms a binding alliance with the metastasis-modulating factor GTPase activator *SIPA1* [10,11]. Simultaneously, in vitro, using mouse and human metastasis gene-expression data, *RRP1B* expression was found to be associated with extracellular matrix gene (ECM) expression and to be a germline regulator of ECM genes, which are recognized as metastasis-predictive components with different regulation in metastasis-prone tumors. The ectopic expression of RRP1B inhibited tumor growth and metastasis in the highly metastatic mouse mammary tumor cell line. The significance of *RRP1B* was underscored by the discovery that germline polymorphisms (SNPs) within the human *RRP1B* consistently correlate with clinical breast cancer outcomes and survival [8,12].

RRP1B upregulation is associated with metastasis suppression. *RRP1B* physically interacts with many nucleosome-binding factors. The primary outcome of transcriptional repression is RRP1B binding to chromatin, and it occupies loci with decreased gene expression. *RRP1B* orchestrates the regulation of metastasis-associated gene expression through its interaction with the transcriptional corepressors tripartite motif-containing protein 28 (TRIM28) and heterochromatin protein 1-α (HP1α) by recruiting chromatin-modifying enzymes. RRP1B influences histone methylation changes [12,13]. RRP1B suppresses metastatic progression while also modulating the expression of alternative mRNA isoforms through interactions with the splicing regulator and oncoprotein SRSF1 [14]. Further experimentation demonstrated that RRP1B interacts with protein phosphatase 1 (PP1), whose functions are implicated in tumorigenesis, the tumor microenvironment, and the metastatic cascade, and it regulates nucleolar phosphorylation signaling [15–17]. *RRP1B* enhances DNA damage-induced apoptosis by functioning as a transcriptional coactivator for proapoptotic target genes under the regulation of the transcriptional activator E2F1 [18].

RRP1B associates with the nucleolar phosphoprotein NPM1, participating in cellular proliferation, growth-suppression pathways, and the apoptotic response to oncogenic stimuli such as DNA damage and hypoxia. NPM1 is implicated in tumorigenesis [12,19,20]. Furthermore, RRP1B interacts with the protein BRD4, a transcriptional and epigenetic regulator that holds a pivotal role in cancer development [21–23]. *RRP1B* can upregulate the expression of claudin-1 by depleting DOCK1 and increase cell viability and motility of claudin-low breast cancer cells [24]. It is proposed that *RRP1B* is targeted by miR-320a and contributes to cancer survival [25].

Various studies underscore the multifaceted nature of how *RRP1B* governs both transcription and metastasis. The dysregulation of *RRP1B* exerts a net effect on multiple

pathways and biological processes, underscoring the complexity of its influence on metastasis and prognostic gene expression.

While the molecular understanding of *RRP1B* as a potential modifier of metastasis is present, there is a scarcity of reports concerning the impact of host genetic factors on various cancer progressions and metastases.

Cervical cancer (CC) ranks among the most frequently diagnosed cancers and stands as the foremost cause of cancer-related mortality in women on a global scale. As reported by the World Health Organization (WHO), it holds the position as the fourth-most prevalent cancer affecting women worldwide. In 2020, the World Health Organization reported an estimated 604,000 new cases and 342,000 deaths worldwide [26]. While cervical cancer remains a leading cause of cancer-related mortality among women in sub-Saharan Africa, with incidence rates as high as 40 per 100,000 women [27], the burden of the disease in Europe is notable for its variance between countries. The Human papillomavirus (HPV) infection is the primary risk factor for cervical cancer, with certain high-risk HPV types, notably HPV 16 and 18, being responsible for the majority of cases [28,29]. Advances in screening methods, such as HPV testing and Pap smears, have significantly improved early detection and prevention efforts, leading to a decrease in cervical cancer incidence and mortality rates in many countries [30]. Vaccination against HPV has emerged as a powerful strategy for cervical cancer prevention, with vaccines targeting the most oncogenic HPV types, demonstrating high efficacy in preventing HPV infection and subsequent cervical lesions [31]. While many countries in Western Europe have implemented organized screening initiatives and achieved substantial reductions in cervical cancer burden, disparities persist in Eastern and Southern Europe, where access to screening services may be limited and screening uptake rates remain suboptimal. Recent data from the European Centre for Disease Prevention and Control (ECDC) indicate that although cervical cancer incidence rates have been declining in most European countries, mortality rates remain a concern, particularly in regions with lower screening coverage and vaccination rates [32]. Despite these challenges, recent advancements in molecular biology and genetics offer promising avenues for improving cervical cancer prevention and treatment. Understanding the genetic determinants of cervical cancer susceptibility, progression, and treatment response is crucial for developing targeted interventions and personalized treatment strategies. Emerging research is focused on further understanding the molecular mechanisms underlying cervical cancer development and progression, as well as identifying novel biomarkers and therapeutic targets to improve patient outcomes.

Previous studies have indicated that inherited polymorphisms are associated with specific tumor characteristics and subsequent outcomes in human cancer. Recognizing the potential impact of germline polymorphisms on disease pathomorphological features and disease progression, we examined five single nucleotide polymorphisms (SNPs) (rs2838342, rs7276633, rs2051407, rs9306160, and rs762400) within the *RRP1B* gene among cervical cancer patients. Our investigation aimed to elucidate their effect on the clinical manifestations and outcomes of the disease.

2. Materials and Methods

2.1. Study Subjects

The retrospective cohort study of adult patients with cervical cancer was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-10 and P1-BE-2-10/2014). All the patients were investigated at the Hospital of Lithuanian University of Health Sciences Kaunas Clinics in Kaunas, Lithuania from October 2014 to August 2020. A total of 172 patients with Stages I–IV cervical cancer were consecutively enrolled, with their diagnoses confirmed through clinical (gynecological and radiological examinations) and histological (cervical biopsies) assessments. Inclusion criteria were the availability of complete data on clinicopathological characteristics and the patient's

written consent to participate. Exclusion criteria encompassed the presence of other malignancies, significant comorbidities, and incomplete medical records, which were used to extract clinical and pathological features, as well as details about the disease course. The blood samples were collected from peripheral veins for further genetic testing. Cancer treatment was administered following institutional guidelines and in accordance with international standards. The follow-up period was extended until November 2020.

2.2. SNP Selection

Genotype information was derived from established online repositories, including The International HapMap Project (http://www.HapMap.org, accessed on 1 September 2023) and the 1000 Genomes Project (http://www.1000genomes.org, accessed on 1 September 2023). The criteria employed for the selection of *RRP1B* Single Nucleotide Polymorphisms (SNPs) were comprehensive. These criteria involved the prerequisite that these SNPs had been previously identified in diverse populations, showcasing associations with the outcomes of various diseases as reported in scientific literature. Moreover, our analysis specifically targeted SNPs that had not been extensively investigated within the context of cervical cancer patients, thereby exploring new avenues of genetic inquiry. Additionally, SNPs under consideration were required to exhibit a minor allele frequency (MAF) equal to or greater than 5% within the European population. This criterion was pivotal in ensuring that the selected SNPs had a sufficiently substantial presence to be statistically significant. Finally, we also considered the potential functional relevance of these SNPs, exploring whether they might be involved in regulating key biological processes. As a point of reference, Table 1 provides a comprehensive listing of the candidate SNPs (rs2838342, rs7276633, rs2051407, rs9306160, and rs762400), their locations, and MAF within the European population data from the 1000 Genomes Project Phase 3 database.

Table 1. SNP genomic region, minor allele frequency (MAF).

Chromosome/ Gene	SNP	Genomic Position in Chromosome	Region/Location	Minor Allele Frequency (MAF) (1000 Genomes)	Highest Population MAF
Chr21/ RRP1B Alias symbols: KIAA0179, Nnp1 RRP1 PPP1R136	rs2838342	43657984 (GRCh38) 45077865 (GRCh37)	Upstream transcript variant, intron variant	0.42/(G)	0.50
	rs7276633	43658919 (GRCh38) 45078800 (GRCh37)	Upstream variant	0.42/(C)	0.49
	rs2051407	43659364 (GRCh38) 45079245 (GRCh37)	Upstream variant	0.37/(T)	0.42
	rs9306160	43687681 (GRCh38) 45107562 (GRCh37)	Missense variant	0.38/(T)	0.44
	rs762400	43693748 (GRCh38) 45113629 (GRCh37)	3′-UTR variant	0.37/(C)	0.50

GRCh37 and GRCh38 are human genome assembly versions by the Genome Reference Consortium.

2.3. Methods

All carcinoma cases were staged according to the guidelines set forth by the International Federation of Gynecology and Obstetrics (FIGO). Tumor grading was determined based on architectural and cytologic (nuclear) criteria. This analysis incorporated clinicopathological features, including age at the time of diagnosis, tumor size (T), lymph node involvement (N), metastasis spread (M), stage, degree of

differentiation (G), response to treatment, presence of disease progression, and patient mortality.

The DNA-extraction process involved the isolation of genetic material from leukocytes in peripheral venous blood samples, which were initially collected in ethylenediaminetetraacetate (EDTA) vacuum tubes and subsequently stored in a laboratory biobank at −20 °C. Genomic DNA extraction was conducted utilizing a genomic DNA purification kit provided by Thermo Fisher Scientific Baltics, based in Vilnius, Lithuania. Genotyping of five selected SNPs within the *RRP1B* gene was conducted at the Institute of Oncology, Lithuanian University of Health Sciences. This was achieved using TaqMan® probe SNP genotyping assays, also sourced from Thermo Fisher Scientific in Lithuania. Molecular genetic analyses were performed employing the real-time polymerase chain reaction (RT-PCR) method, which is designed to amplify specific DNA segments as per the established protocol.

In our comprehensive investigation, we delved into the potential interconnections between SNPs in the genotype and allelic models, and the intricate landscape of tumor clinicopathological features. These attributes encompass the patient's age (categorized into age ≤50 and age >50), the tumor's size (distinguished as T1–T2 and T3–T4), the status of pathological regional lymph nodes (delineated as N0 and N1), the presence of distant metastasis (defined by M0 or M1), the tumor's grade (G1 + G2 or G3), the disease stage (categorized as Stages I–II and Stages III–IV), and the overall disease prognosis (specifically, the worse prognosis: T3–T4 + G3 versus T1–T2 + G1–G2). Furthermore, the study extended its scrutiny to encompass clinical outcomes, specifically progression-free survival (PFS) and overall survival (OS). Within the patient cohort, PFS was computed commencing from the date of diagnosis until the point of local disease spread or the occurrence of distant metastasis/metastasis spread. In parallel, OS was calculated from the date of diagnosis to the date of the patient's demise. Haploview v4.1 software was utilized to assess linkage disequilibrium (LD) among SNPs and generate LD plots (available at http://www.broad.mit.edu/mpg/haploview/, accessed on 5 January 2024). Haplotypes were inferred from the analyzed SNPs using Bayesian methods through the phase software v2.1 (Department of Statistics, University of Washington, Seattle, WA, USA). Finally, we analyzed the associations of haplotypes with clinical manifestations of the disease and survival outcomes. These findings are instrumental in our quest to elucidate the potential genetic factors that may exert influence over these pivotal facets of the disease's clinical intricacies.

2.4. Statistical Analysis

The identified SNPs were subsequently integrated into a comprehensive statistical analysis, encompassing both genotype and allelic models. The statistical evaluation was conducted using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). To investigate the associations between genotypes, alleles, and tumor characteristics, statistical tests, including Pearson's Chi-square and Fisher's Exact tests, were employed. In order to present a robust analysis, both univariate and multivariate models were adopted, with adjustments for age at the time of diagnosis and various cancer clinicopathological features. These models enabled the calculation of odds ratios along with their corresponding 95% confidence intervals (CIs) and *p*-values, using logistic regression. The analysis of differences in PFS and OS involved the performing of hazard ratios (HRs) derived from univariate and multivariate Cox proportional-hazard models. The survival curves were constructed and assessed employing the log-rank test, and the Kaplan–Meier method was used for generating these curves. Throughout the entirety of the analysis, a *p*-value less than 0.05 was deemed statistically significant.

3. Results

3.1. Clinical Characteristics

In the course of our investigation, the study cohort primarily comprised Lithuanian nationals, constituting 90.1% of the participants, with the remaining individuals originating from other European countries. The demographic profile of the subjects exhibited a broad spectrum of ages, spanning a considerable range from 22 to 83 years. When the participants were diagnosed, their mean age stood at 55.4 years, with a standard deviation of 13.5 years, indicating the spectrum of ages represented in this study. An in-depth analysis of the tumor size dimensions unveiled a noteworthy predominance of the T2 category, constituting 48.8% of the cases. Lymph node involvement was documented in 44.8% of the patient cohort. Furthermore, the study uncovered that metastasis to paraaortic lymph nodes was documented in 5.2% of the cases. Distant metastasis was detected in 10 cases, constituting 5.8% of the total. Cancer staging indicated that IIB and IIIC1 were the prevailing stages, representing 32% and 31% of the cases, respectively. This stratification also revealed that lower stages (I–II) accounted for 44.2% of the participants, while the more advanced stages (III–IV) encompassed 55.8% of the study population. Further scrutiny unveiled a distribution of tumor differentiation, with 7.6% classified as well-differentiated (G1), 65.7% as intermediate (G2), and 26.7% as poorly differentiated, thereby illustrating the heterogeneity of tumor grades within the study cohort. Regarding treatment, a significant majority of patients, amounting to 69.2%, underwent standard chemoradiation therapy. The remaining participants underwent surgery followed by radiotherapy or systemic treatment. Importantly, a substantial 70.3% of the patients exhibited a complete response to treatment, while 21.5% showed a partial response. A smaller segment, comprising 8.2%, exhibited either stable disease or progressive disease. Within the context of progression, the median progression-free survival (PFS) was calculated at 13 months, exhibiting a range spanning from a minimum of 1 month to a maximum of 201 months. Over the course of the follow-up period, disease progression was confirmed in 52 cases, impacting 30.2% of the cohort. A substantial majority of those experiencing progression exhibited localized advancement and metastasis in regional lymph nodes, affecting 51 patients, while an additional 18 cases demonstrated progression in paraaortic lymph nodes. The disease also metastasized in 16 patients. Regrettably, 40 events of death occurred during the follow-up period, accounting for 23.3% of the cohort. The median overall survival (OS) spanned from 1 to 201 months, with the midpoint recorded at 16.5 months. Notably, 45.9% of the patients had concurrent chronic diseases, yet the underlying cause of death in all cases was the relentless progression of cancer. Chart 1 offers a comprehensive breakdown of clinicopathological features.

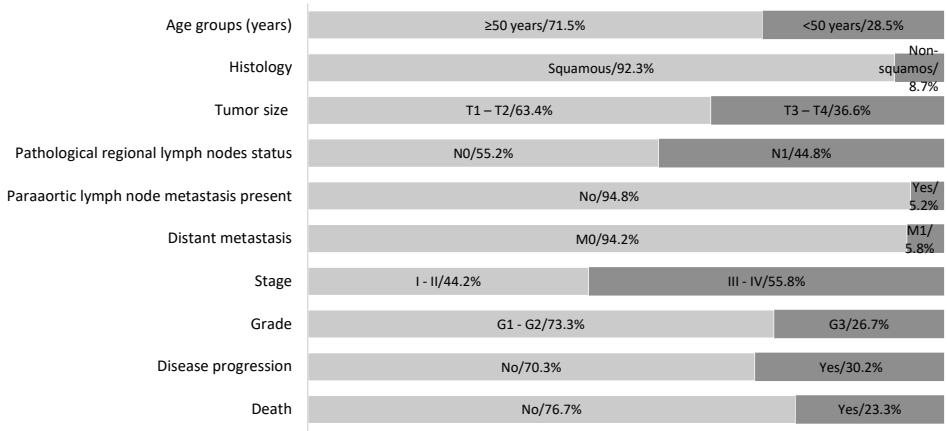

Chart 1. Patient and tumor data from 172 study participants (variables/subgroups/frequencies (%)). T1+T2—smaller tumor size; T3+T4—larger tumor size; N0—no regional lymph node metastasis; N1—positive regional lymph node metastasis; M0—no distant metastasis; M1—positive distant metastasis; G1-G2—well and moderately differentiated tumor; G3—poorly differentiated tumor, Stages I–II—lower stages; Stages III–IV—advanced stages.

3.2. SNP Frequencies

In our study, a total of 172 patients underwent genotyping for a set of five *RRP1* SNPs: rs2838342, rs7276633, rs2051407, rs9306160, and rs762400. Among these, rs9306160 was identified in 169 cases, with three cases excluded due to non-amplification. It is noteworthy that all of the SNPs examined were found to be in accordance with the Hardy–Weinberg equilibrium, as indicated by *p*-values exceeding 0.05. Upon comparing the allele frequencies determined within our cohort to those of the European population data from the 1000 Genomes project, we detected slight yet statistically significant disparities in the minor allele frequencies (MAF) for all of the SNPs, with *p*-values > 0.05. Comprehensive details regarding genotype and allele frequencies can be found in Table 2.

Table 2. The distribution of *RRP1B* genotypes and alleles.

SNP	Sample Size N (Study Cohort)	Genotypes	Genotypes Frequencies		Alleles	Alleles Frequencies (Study Cohort)	Sample Size N (1000 Genomes)	Alleles Frequencies (1000 Genomes)	MAF *p* Value [a]
rs2838342 A>G	172	AA	58/33.7%	0.337	A	0.584	1006	0.589	0.791
		AG	85/49.4%	0.494	G	0.416		0.420	
		GG	29/16.9%	0.169					
rs7276633 T>C	172	TT	59/34.3%	0.343	T	0.587	1006	0.581	0.676
		TC	84/48.8%	0.488	C	0.413		0.420	
		CC	29/16.9%	0.169					
rs2051407 C>T	172	CC	63/36.6%	0.366	C	0.610	1006	0.634	0.282
		CT	84/48.8%	0.489	T	0.390		0.366	
		TT	25/14.5%	0.145					

rs9306160 * C>T	169	CC CT TT	55/32.5% 92/54.4% 22/13.0%	0.325 0.545 0.130	C T	0.598 0.402	1006	0.617 0.383	0.450
rs762400 G>C	172	GG GC CC	63/26.6% 88/51.2% 21/12.2%	0.366 0.512 0.122	G C	0.622 0.378	1006	0.626 0.374	0.804

* Genotypes were determined among 169 patients due to non-amplification in three cases. [a] A chi-squared test for independence analysis for the number of each minor allele in cases and controls.

3.3. Linkage Disequilibrium and Haplotypes Distribution

In our analysis of linkage disequilibrium (LD) among the SNPs in the *RRP1B* gene, we calculated two commonly used measures: D′ and r^2 (Figure 1). For D′, the mean value was approximately 0.949 ± 0.037, indicating a relatively strong LD on average. The range of D′ values varied from a minimum of 0.907 to a maximum of 0.987. Similarly, for r^2, the mean value was approximately 0.802 ± 0.065, suggesting a moderate-to-high degree of LD on average. The range of r^2 values spanned from a minimum of 0.761 to a maximum of 0.953. These findings provide insights into the patterns of LD within the RRP1B gene, highlighting regions of potential genetic linkage and association. Based on the calculated mean values and the range of D′ and r^2 values, it appears that there is a significant level of linkage disequilibrium (LD) among the SNPs in the *RRP1B* gene. The mean values for both D′ and linkage disequilibrium r^2 indicate a relatively strong LD on average, and the range of values suggests consistency in LD across the analyzed SNPs. Therefore, it would be reasonable to conclude that LD between these SNPs in the *RRP1B* gene is indeed strong. Due to the observed strong linkage disequilibrium (LD) among the SNPs within the *RRP1B* gene, it was decided to include all five SNPs in haplotype analysis. This decision was based on the premise that SNPs in strong LD tend to be inherited together as haplotype blocks, allowing for a more comprehensive understanding of the genetic variations within this genomic region. By analyzing haplotypes constructed from these SNPs, we aimed to capture the collective influence of genetic variations on phenotypic traits or disease susceptibility, thereby enhancing the depth of our genetic investigation.

The results revealed a variety of haplotypes present among the tested individuals. Thirteen haplotypes were identified (Table 3). Among the identified haplotypes, the most prevalent was “ATCCG”, accounting for approximately 55% of the total haplotypes observed. Following closely behind, “GCTTC” constituted around 36% of the haplotypes. Other haplotypes, such as “ATCTG”, “ATCTC”, “GCCTG”, etc., were observed at lower frequencies, each comprising less than 10% of the total haplotypes. The diversity in haplotype composition suggests genetic variability within the *RRP1B* gene region among the studied population. Understanding the distribution of these haplotypes can provide valuable insights into genetic susceptibility, disease association, and population genetics within the context of our research objectives.

For further analysis of associations, we focused on the two most common haplotypes observed in our study, namely “ATCCG” and “GCTTC”.

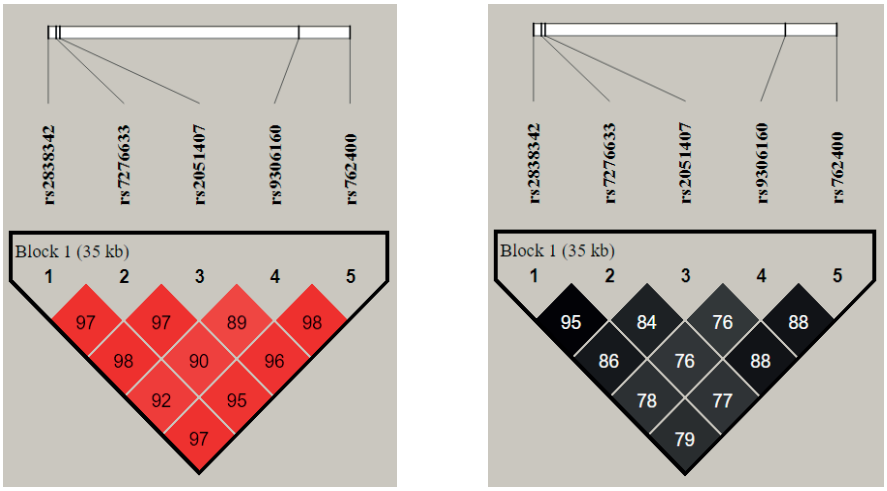

Figure 1. The LD data for *RRP1B* single-nucleotide polymorphisms include numerical values and color coding for both r-squared and D′, providing insights into the linkage disequilibrium. Pairwise linkage disequilibrium (LD) pattern for *RRP1B* polymorphisms in cervical cancer patients. (**a**) The color LD plot indicates the strength of D′, with dark red representing strong LD. (**b**) The color LD plot indicates the strength of r^2, with dark grey representing strong LD.

Table 3. Haplotypes and frequencies.

Haplotype Number	rs2838342 rs7276633 rs2051407 rs9306160 rs762400 Haplotypes	Chromosomes (Counts)	Frequencies (%)
1	ATCCG	190	55.23
2	ATCTG	5	1.45
3	ATCTC	2	0.58
4	ATTCG	1	0.29
5	ACCCG	2	0.58
6	GTCCG	1	0.29
7	GTTTC	1	0.29
8	GCCCG	4	1.16
9	GCCTG	5	1.45
10	GCCTC	1	0.29
11	GCTCG	6	1.74
12	GCTCC	2	0.58
13	GCTTC	124	36.05

Thirteen haplotypes were generated from the analyzed SNPs through the Phase software v2.1.

3.4. Association Analysis

All the examined polymorphisms exhibited statistically significant associations with the clinical manifestations of cervical cancer. However, we did not find any statistically significant associations between SNPs and nodal involvement or tumor differentiation. On the other hand, all the polymorphisms were linked to tumor size or metastasis. Furthermore, some of them appeared to influence cancer stage and prognosis. The tabulated results furnish us with a trove of statistical insights. This meticulous analysis

unveils the intriguing associations between specific SNPs and an array of vital tumor characteristics, offering a multifaceted perspective on the clinical attributes of cervical.

3.4.1. Rs2838342

The analysis of SNP rs2838342 yielded noteworthy results. According to the univariate logistic regression analysis, individuals with the presence of the A allele (A allele +) exhibited a significantly lower odds ratio (OR) of 0.281 (95% CI: 0.122–0.643, p = 0.002) for advanced tumor size (T3–T4) when compared to those with its absence (A −), indicating a significantly reduced likelihood of advanced tumor size (T3–T4). In the multivariate logistic regression analyses across four models, the A allele was consistently associated with significantly lower odds of larger tumor size, with an OR of 0.280 (95% CI: 0.122–0.643, p = 0.003) in the presence of patient age at diagnosis (Model No. 1). Model No. 2 introduced additional covariates (age at diagnosis and tumor differentiation grade) and continued to demonstrate a consistent association between rs2838342 and the tumor size (OR = 0.299, 95% CI: 0.129–0.692, p = 0.005). However, a possible trend emerged where G3 was associated with higher odds of larger tumor size (OR = 1.991, 95% CI: 0.978–4.051, p = 0.058). Model No. 3 expanded the analysis to include the presence of regional lymph node involvement (N1 versus N0). In this model, rs2838342 remained associated with tumor size (OR = 0.244, 95% CI: 0.096–0.619, p = 0.003), but the addition of N1 as a covariate substantially increased the odds of larger tumor size (OR = 7.367, 95% CI: 3.347–16.217, p < 0.001). Model No.4 further extended the analysis to consider the presence of distant metastasis (M1 versus M0), further supporting a significant relationship (OR = 0.266, 95% CI: 0.102–0.691, p = 0.007). Throughout all these models, the association between rs2838342 and tumor size persisted. In summary, these multivariate logistic regression analyses, while adjusting for covariates, reveal a robust and consistent association between the presence of at least one A allele and a lower risk of larger tumor size. In genotypic models, the GG genotype showed an increased odds ratio of 2.160 (95% CI: 0.867–5.380). Nevertheless, this difference did not reach statistical significance (p = 0.098).

The univariate logistic regression analysis revealed that the A allele significantly reduced the odds of having distant metastasis (OR = 0.274, 95% CI: 0.072–1.040, p = 0.044). Conversely, when comparing the presence of at least one G allele to having none (G allele + vs. G −), the OR was 1.199, indicating slightly higher odds of having distant metastasis. However, this association was not statistically significant (p = 0.798). Across all multivariate models for the A allele + versus A − comparison, when adjusting for age, G, N, and tumor size, the OR suggests a potential protective effect of the A allele in reducing the risk of distant metastasis. Unfortunately, statistical significance was limited (all cases p-value > 0.05). These results provide preliminary evidence that the A allele of rs2838342 might play a protective role against the development of distant metastasis.

In the analysis, focused on a worse prognosis group, characterized by T3–T4 tumor stages and the G3 tumor grade, the A allele of rs2838342 significantly reduces the likelihood of a worse prognosis (T3–T4 + G3) compared to those with its absence (A −), with an OR of 0.182. The 95% CI spans from 0.061 to 0.538, and the p-value is a strikingly low 0.002. The GG genotype of rs2838342 presents a notably high OR (3.000) for a worse prognosis (T3–T4 + G3) when compared to the AA genotype. Although the p-value (0.071) suggests a potential association, it did not reach conventional significance levels. Similarly, the G allele of rs2838342 does not significantly impact the likelihood of a worse prognosis, as reflected in the wide 95% CI from 0.274 to 1.847 and a p-value of 0.485.

While the genotypes did not show a significant association in the comparison of positive Stages III–IV versus Stages I–II, allelic comparisons provided additional insights. The A allele demonstrated a substantially lower odds ratio (OR = 0.341, 95% CI =

0.137–0.849, *p*-value = 0.017), suggesting a potential protective effect in the context of advanced cancer stages. Conversely, the G allele did not exhibit a statistically significant association (*p*-value = 0.239).

When considering age as a dichotomous variable (≤50 vs. >50 years), those carrying the AG genotype had a reduced risk of developing cervical cancer before the age of 50 (OR = 0.471, 95% CI: 0.226–0.983, *p* = 0.045).

These findings underscore the potential relevance of the rs2838342 SNP in influencing the progression and severity of cervical cancer, particularly in the transition from early to advanced stages. The protective effect associated with specific genotypes and alleles implies a potential role for rs2838342 as a prognostic marker in cervical cancer patients. All the results are presented in Tables 4 and 5.

Table 4. Univariate and multivariate logistic regression analyses were conducted for rs2838342, adjusting for alleles, and clinicopathological characteristics, with a focus on tumor size and metastasis.

			Univariate			**Multivariate**											
						Model No. 1			**Model No. 2**			**Model No. 3**			**Model No. 4**		
SNP	**Dependent**	**Covariates**	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***
rs2838342	Positive T3–T4	A allele + vs. A -	0.281	0.122–0.643	0.002	0.280	0.122–0.643	0.003	0.299	0.129–0.692	0.005	0.244	0.096–0.619	0.003	0.266	0.102–0.691	0.007
		Age (years)				1.001	0.978–1.025	0.909	1.002	0.979–1.027	0.842	1.028	1.000–1.057	0.054	1.027	0.999–1.056	0.059
		Positive G3 vs. G1 + G2							1.991	0.978–4.051	0.058	1.798	0.826–3.914	0.140	1.687	0.762–3.732	0.197
		Positive N1 vs. N0										7.367	3.347–16.217	<0.001	6.161	2.756–13.771	<0.001
		Positive M1 vs. M0													5.977	0.690–51.748	0.105
rs2838342	Positive M	A allele + vs. A -	0.274	0.072–1.040	0.044	0.272	0.071–1.140	0.057	0.291	0.075–1.127	0.074	0.267	0.063–1.136	0.074	0.521	0.124–2.190	0.374
		Age (years)				0.979	0.932–1.028	0.395	0.980	0.933–1.029	0.417	1.009	0.960–1.061	0.714	0.978	0.927–1.031	0.401
		Positive G3 vs. G1 + G2							1.603	0.416–6.170	0.493	1.293	0.315–5.308	0.721	1.130	0.276–4.633	0.865
		Positive N1 vs. N0										0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4													15.623	1.853–131.722	0.012

Table 5. Univariate logistic regression analysis: assessing odds ratios for the relationships between SNPs and patients' age, cancer stage groups, and disease prognosis.

		Age (Groups): ≤50 vs. >50			**Positive Stage III–IV vs. Stage I–II**			**Positive Worse Prognosis: T3–T4+G3 vs. T1–T2+G1–G2**		
SNP	**Genotype, Alleles**	**OR**	**95% CI**	***p*-Value**	**OR**	**95% CI**	***p*-Value**	**OR**	**95% CI**	***p*-Value**
rs2838342	AG vs. AA	0.471	0.226–0.983	0.045						
	GG vs. AA									
	A allele + vs. A –				0.341	0.137–0.849	0.017	0.182	0.061–0.538	0.002
	G allele + vs. G –									
rs7276633	TC vs. TT									
	CC vs. TT									
	T allele + vs. T –				0.341	0.137–0.849	0.021	0.182	0.061–0.538	0.002
	C allele + vs. C –	2.138	1.080–4.230	0.029						
rs2051407	CT vs. CC									
	TT vs. CC									
	C allele + vs. C –							0.267	0.087–0.823	0.021
	T allele + vs. T –									

3.4.2. Rs7276633

The carriers of the T allele in rs7276633 were significantly associated with a decreased risk of falling into the higher tumor size category (T3–T4), with an odds ratio (OR) of 0.281 (95% CI = 0.122–0.643, p = 0.003). Moving on to the multivariate logistic regression analysis, the findings remain consistent across all four models. In multivariate Model No.1, the presence of the T allele (+) is significantly associated with a reduced risk of having a higher tumor size compared to the absence of the T allele (−). This association is statistically significant with an odds ratio (OR) of 0.280 and a 95% confidence interval (CI) of 0.122–0.643 (p = 0.003). This association persists in Model No.2, demonstrating a significant reduction in the odds of higher tumor size (OR = 0.299, 95% CI: 0.129–0.692, p = 0.005). Models Nos.3 and 4 also support this finding, with ORs of 0.277 and 0.264 (95% CI: 0.125–0.708, p = 0.003; 95% CI: 0.147–0.893, p = 0.007, respectively). Conversely, the C allele of rs7276633 did not exhibit a significant association with tumor size (p = 0.145). Otherwise, the trend of the CC genotype compared to the TT genotype showing an increased risk of higher tumor size remained consistent across all models, although this association was non-significant.

Patients with the presence of the T allele (+) were significantly associated with a reduced risk of higher tumor stage (III–IV) (OR = 0.341, 95% CI: 0.137–0.849, p = 0.021) and worse prognosis (T3–T4 + G3) (OR = 0.182, 95% CI: 0.061–0.538, p = 0.002), while the C allele did not exhibit significant associations with the parameters studied. In conclusion, these findings imply that the T allele of rs7276633 might confer a protective effect against advanced tumor size and prognosis. Moreover, carriers of the C allele were at a higher risk of developing the disease at an age younger than 50 years (OR = 2.138, 95% CI: 1.080–4.230, p = 0.029). The results are presented in Tables 5 and 6.

Table 6. Univariate and multivariate logistic regression analyses were conducted for rs7276633, adjusting for alleles and clinicopathological characteristics, with a focus on tumor size.

			Univariate			Multivariate											
						Model No.1			Model No.2			Model No.3			Model No.4		
SNP	**Dependent**	**Covariates**	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***
rs7276633	Positive T3–T4	T allele + vs. T −	0.281	0.122–0.643	0.003	0.280	0.122–0.643	0.003	0.299	0.129–0.692	0.005	0.277	0.125–0.708	0.003	0.264	0.147–0.893	0.007
		Age (years)				1.001	0.978–1.025	0.909	1.002	0.979–1.027	0.842	1.028	1.000–1.057	0.054	1.027	0.999–1.056	0.059
		Positive G3 vs. G1 + G2							1.991	0.978–4.051	0.058	1.798	0.826–3.914	0.140	1.687	0.762–3.732	0.197
		Positive N1 vs. N0										7.367	3.347–16.217	<0.001	6.161	2.756–13.771	<0.001
		Positive M1 vs. M0													5.977	0.690–51.748	0.105

3.4.3. Rs2051407

There were no significant associations between genotypes and clinicopathomorphological features. However, the presence of the C allele (+) was associated with a decreased risk of having a larger tumor (T3–T4) compared to those without the C allele (C −) (OR 0.393, 95% CI of 0.166–0.929, p = 0.033). This association was consistent and statistically significant across three multivariate analysis models, when the adjustment of age and tumor clinicopthatological features was made (Model No. 1: OR 0.392, 95% CI: 0.166–0.928, p = 0.033; Model No.2: OR 0.414, 95% CI: 0.173–0.992, p = 0.048; Model No. 3: OR 0.354, 95% CI: 0.134–0.930, p = 0.035, respectively). But Model No.4 shows that the association did not reach statistical significance (OR 0.409, 95% CI: 0.149–1.123, p = 0.083).

In the univariate logistic regression analysis, investigating the association between alleles and metastasis, carrying the C allele significantly decreased the chance of having metastasis, with an OR of 0.223 (95% CI: 0.058–0.858) and a *p*-value of 0.019. These findings suggest that the presence of the C allele may serve as a protective factor against metastasis. In multivariate analysis, Models Nos. 1, 2, and 3, showed a consistent association between the presence of the C allele and a reduced risk of metastasis (p = 0.030, p = 0.038, p = 0.037, respectively). However, Model No. 4 did not yield significant results for this polymorphism, when the adjustment of age, G, N, and T was made. Tumor stage (T3–T4) was consistently identified as a significant predictor of metastasis in all models.

The presence of the C allele was associated with a significantly reduced risk of transitioning to a worse prognosis disease (T3–T4 + G3), as evidenced by an OR of 0.267 (95% CI: 0.087–0.823, p = 0.021), suggesting that it may serve as a protective factor. These findings highlight the potential influence of this SNP on the expected prognosis of the disease. The results are presented in Tables 5 and 7.

Table 7. Univariate and multivariate logistic regression analyses were conducted for rs2051407, adjusting for alleles and clinicopathological characteristics, with a focus on tumor size and metastasis.

			Univariate			Multivariate											
						Model No. 1			Model No. 2			Model No. 3			Model No. 4		
SNP	**Dependent**	**Covariates**	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***
rs2051407	Positive T3–T4	C allele + vs. C −	0.393	0.166–0.929	0.033	0.392	0.166–0.928	0.033	0.414	0.173–0.992	0.048	0.354	0.134–0.930	0.035	0.409	0.149–1.123	0.083
		Age (years)				1.002	0.978–1.025	0.890	1.003	0.979–1.027	0.820	1.028	1.000–1.057	0.052	1.027	0.000–1.055	0.060
		Positive G3 vs. G1 + G2							2.067	1.027–4.161	0.042	1.885	0.877–4.050	0.104	1.803	0.827–3.928	0.138
		Positive N1 vs. N0										6.993	3.233–15.125	<0.001	5.795	2.639–12.726	<0.001
		Positive M1 vs. M0													6.116	0.713–52.493	0.099
rs2051407	Positive M	C allele + vs. C −	0.223	0.058–0.858	0.019	0.223	0.058–0.863	0.030	0.236	0.060–0.920	0.038	0.209	0.048–0.913	0.037	0.355	0.083–1.510	0.355
		Age (years)				0.979	0.931–1.029	0.403	0.980	0.932–1.031	0.432	1.007	0.957–1.059	0.801	0.979	0.926–1.034	0.443
		Positive G3 vs. G1 + G2							1.627	0.421–6.285	0.480	1.307	0.315–5.429	0.712	1.088	0.262–4.514	0.908
		Positive N1 vs. N0										0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4													15.475	1.852–129.314	0.011

3.4.4. Rs9306160

The analysis suggests that the rs9306160 SNP may have a significant protective effect against metastasis, as indicated by the statistically significant result for the C allele (+) (OR = 0.179, 95% CI: 0.044–0.721, p = 0.008). There was no significant association between the CT genotype and the presence of metastasis. However, for the TT genotype compared to CC, the OR was 5.889 (95% CI: 0.993–34.906) with a *p*-value close to the significance threshold at 0.051. This implies a potential trend towards an increased risk of metastasis for the TT genotype. In the multivariate logistic regression analysis for metastasis (M), Model No.1 showed that the presence of the C allele (+) significantly reduced risk of metastasis (OR = 0.187, 95% CI: 0.046–0.760, p = 0.019). Model No. 2 continued to show a

protective effect the C allele (+) with an OR of 0.166 (95% CI: 0.039–0.702, $p = 0.015$). In Model No. 3 the C allele (+) still exhibited a protective effect (OR = 0.151, 95% CI: 0.032–0.717, $p = 0.017$). This confirms the significantly reduced risk of metastasis associated with the C allele. However, in Model No.4, the protective effect is not statistically significant, while there is a protective trend. The results are presented in Table 8.

Table 8. Univariate and multivariate logistic regression analyses were conducted for rs9306160, adjusting for alleles and clinicopathological characteristics, with a focus on metastasis.

			Univariate			Multivariate											
						Model No. 1			Model No. 2			Model No. 3			Model No. 4		
SNP	**Dependent**	**Covariates**	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***
rs9306160	Positive M	C allele + vs. C -	0.179	0.044–0.721	0.008	0.187	0.046–0.760	0.019	0.166	0.039–0.702	0.015	0.151	0.032–0.717	0.017	0.262	0.059–1.170	0.079
		Age (years)				0.979	0.929–1.032	0.430	0.981	0.931–1.034	0.479	1.002	0.949–1.059	0.932	0.977	0.921–1.036	0.437
		Positive G3 vs. G1 + G2							2.623	0.629–10.932	0.186	2.193	0.482–9.992	0.310	1.581	0.363–6.897	0.542
		Positive N1 vs. N0										0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4													12.411	1.442–106.842	0.002

3.4.5. Rs762400

This SNP also showed significant results. The G allele was significant for a reduced risk of advanced tumor size (T3–T4) compared to the absence of the G allele (G −) (OR = 0.383, 95% CI: 0.151–0.967, $p = 0.037$). Based on multivariate logistic regression analysis, taking into account age, tumor grade, nodal involvement, and distant metastasis, the association maitains significance in the initial models: Model No. 1 (OR = 0.383, 95% CI: 0.151–0.968, $p = 0.042$), Model No.2 (OR = 0.378, 95% CI: 0.148–0.970, $p = 0.043$), and Model No. 3 (OR = 0.330, 95% CI: 0.115–0.946, $p = 0.039$). However, in Model No. 4, the association was not statistically significant ($p = 0.106$). Thus, the results indicated that the role of other covariates is more important with regard to the impact of the G allele.

Moreover, the univariate logistic regression suggests that individuals carrying the G allele (+) had a significantly lower risk of having metastasis (OR = 0.176, 95% CI: 0.045–0.686, $p = 0.006$). The multivariate analyses reinforce this association, with the presence of the G allele consistently linked to a reduced risk of metastasis. This significance holds in Models Nos. 1, 2, and 3 (OR = 0.165, 95% CI: 0.042–0.659, $p = 0.011$; OR = 0.168, 95% CI: 0.042–0.673, $p = 0.012$; OR = 0.149, 95% CI: 0.032–0.703, $p = 0.016$, respectively). In Model No. 4, while the association between the G allele and metastasis does not reach conventional statistical significance, it still suggests a notable trend towards a reduced risk of metastasis associated with the G allele. Importantly, age and other clinical factors did not demonstrate significant associations with metastasis (Table 9).

Table 9. Univariate and multivariate logistic regression analyses were conducted for rs762400, adjusting for alleles and clinicopathological characteristics, with a focus on tumor size and metastasis.

			Univariate			Multivariate											
						Model No. 1			Model No. 2			Model No. 3			Model No. 4		
SNP	Dependent	Covariates	Odds	95% CI	*p*	Odds	95% CI	*p*	Odds	95% CI	*p*	Odds	95% CI	*p*	Odds	95% CI	*p*
rs762400	Positive T3–T4	G allele + vs. G –	0.383	0.151–0.967	0.037	0.383	0.151–0.968	0.042	0.378	0.148–0.970	0.043	0.330	0.115–0.946	0.039	0.401	0.132–1.216	0.106
		Age (years)				1.001	0.977–1.024	0.963	1.002	0.978–1.026	0.884	1.026	0.998–1.055	0.064	1.025	0.998–1.054	0.072
		Positive G3 vs. G1 + G2							2.173	1.080–4.372	0.029	2.008	0.933–4.320	0.074	1.906	0.877–4.143	0.103
		Positive N1 vs. N0										6.875	3.190–14.820	<0.001	5.717	2.612–12.511	<0.001
		Positive M1 vs. M0													5.895	0.682–50.958	0.107
rs762400	Positive M	G allele + vs. G -	0.176	0.045–0.686	0.006	0.165	0.042–0.659	0.011	0.168	0.042–0.673	0.012	0.149	0.032–0.703	0.016	0.265	0.062–1.135	0.074
		Age (years)				0.974	0.924–1.027	0.327	0.976	0.926–1.029	0.370	0.999	0.946–1.054	0.957	0.977	0.924–1.034	
		Positive G3 vs. G1 + G2							1.796	0.460–7.017	0.400	1.332	0.314–5.647	0.697	1.196	0.289–4.951	0.805
		Positive N1 vs. N0										0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4													14.735	1.757–123.541	0.013

3.4.6. Haplotypes

With the understanding that rs2838342, rs7276633, rs2051407, rs9306160, and rs762400 may not act independently, we opt to explore haplotypes. By analyzing haplotypes, we aim to capture the combined effect of multiple SNPs within the gene, thus providing a more comprehensive understanding of the genetic landscape and its potential implications in our study. We meticulously analyzed the associations between diplotypes and various clinical characteristics. Specifically, we examined the heterozygous diplotype (ATCCG/alternative haplotype) versus the homozygous diplotype (ATCCG/ATCCG), ATCCG haplotype non-carriers versus the homozygous diplotype (ATCCG/ATCCG), heterozygous diplotype (GCTTC/alternative haplotype) versus the homozygous diplotype (GCTTC/GCTTC), GCTTC haplotype non-carriers versus the homozygous diplotype (GCTTC/GCTTC), and heterozygous diplotype (ATCCG/GCTTC) carriers versus non-carriers.

Significantly, GCTTC haplotype non-carriers exhibited a greater protective effect against advanced tumor size (T3–T4) and metastasis compared to those with the homozygous diplotype (GCTTC/GCTTC) (OR = 0.367, 95% CI: 0.136–0.992, $p = 0.038$; OR = 0.098, 95% CI: 0.016–0.578, $p = 0.010$, respectively). This finding suggests a potential role of genetic variations represented by the GCTTC haplotype in promoting aggressive tumor behavior. For patients with advanced tumor stages (III–IV versus I–II) and worse prognosis (T3–T4 + G3 versus T1–T2 + G1–G2), individuals lacking the ATCCG haplotype showed a significantly higher likelihood of exhibiting advanced tumor stages and being in the worse prognosis group compared to those with the homozygous diplotype (ATCCG/ATCCG) (OR = 1.250, 95% CI: 0.454–3.444, $p = 0.032$; OR = 2.100, 95% CI: 0.638–6.916, $p = 0.048$, respectively) (Table 10).

Table 11 presents the results of multivariate logistic regression analyses focusing on diplotypes, with adjustments made for clinicopathological characteristics, particularly emphasizing tumor size and metastasis. Model 1: In the initial model, we adjusted for age

(years) as an additional covariate. The association between GCTTC haplotype non-carriers and reduced odds of advanced tumor size remained significant (OR = 0.393, 95% CI: 0.188–0.822, p = 0.039). This underscores the robustness of our initial findings, indicating that age did not substantially alter the observed relationship between haplotype status and tumor size. Model 2: Further adjustments were made by including tumor grade (G3 versus G1 + G2) in the analysis. Despite this additional adjustment, the association between GCTTC haplotype non-carriers and decreased odds of advanced tumor size remained statistically significant (OR = 0.392, 95% CI: 0.185–0.827, p = 0.041). This suggests that the observed association is independent of tumor grade, emphasizing the potential importance of genetic factors in influencing tumor progression. Model 3: Despite the inclusion of nodal status in the analysis, the association between GCTTC haplotype non-carriers and reduced odds of advanced tumor size remained statistically significant (OR = 0.391, 95% CI: 0.173–0.884, p = 0.041). Model 4: Finally, we included metastasis (M1 versus M0) as an additional covariate in the analysis. The association between GCTTC haplotype non-carriers and advanced tumor size showed a trend towards significance (OR = 0.380, 95% CI: 0.166–0.869, p = 0.046). On the focus on metastasis, in Model No.1, GCTTC haplotype non-carriers exhibit a substantial protective effect against metastasis (OR = 0.101, 95% CI 0.017–0.598, p = 0.012). This suggests a potential role of genetic variations represented by the GCTTC haplotype in influencing metastatic propensity, even after adjusting for age. In Model No.2, which includes additional adjustments for tumor grade (G3 versus G1 + G2), the protective effect against metastasis remains significant (OR = 0.095, 95% CI 0.016–0.577, p = 0.011), further emphasizing the independent nature of this association. Model No.3 incorporates adjustments for lymph node involvement (N1 versus N0) along with age and tumor grade. Despite these additional adjustments, the protective effect against metastasis among GCTTC haplotype non-carriers persists (OR = 0.075, 95% CI 0.011–0.534, p = 0.010), highlighting the robustness of the observed association. Finally, in Model No.4, which includes adjustments for tumor stage (T3–T4), in addition to age, tumor grade, and lymph node involvement, the protective effect against metastasis remains significant (OR = 0.150, 95% CI 0.023–0.965, p = 0.048). This suggests that the influence of genetic variations represented by the GCTTC haplotype on metastatic propensity is independent of tumor size and other clinicopathological factors.

Overall, the consistent significance of the protective effect across all models underscores the potential importance of genetic variations represented by diplotypes in predicting tumor size and metastasis in cervical cancer patients, irrespective of traditional clinicopathological factors.

Table 10. Univariate logistic regression analysis: assessing odds ratios for the relationships between diplotypes and patients' age and tumor characteristics.

Cinical Characteristics	Diplotypes														
	Heterozygous Diplotype (ATCCG/Alternative Hap) vs. Homozygous Diplotype (ATCCG/ATCCG)			ATCCG Haplotype Non-Carriers vs. Homozygous Diplotype (ATCCG/ATCCG)			Heterozygous Diplotype (GCTTC/Alternative Hap) vs. Homozygous Diplotype (GCTTC/GCTTC)			GCTTC Haplotype Non-Carriers vs. Homozygous Diplotype (GCTTC/GCTTC)			Heterozygous Diplotype (ATCCG/GCTTC) Carriers vs. Non-Carriers		
	OR	95% CI	*p*-Value	OR	95% CI	*p*-Value	OR	95% CI	*p*-Value	OR	95% CI	*p*-Value	OR	95% CI	*p*-Value
Positive T3–T4 vs. T1–T2	0.383	0.183–0.800	0.077	1.773	0.703–4.471	0.225	0.506	0.185–1.387	0.186	0.367	0.136–0.992	0.038	0.424	0.220–0.817	0.090
Positive N1 vs. N0	*	*	*	*	*	*	0.831	0.311–2.221	0.712	1.222	0.449–3.326	0.694	0.671	0.364–1.238	0.202
Positive M1 vs. M0	1.022	0.180–5.790	0.980	3.538	0.606–20.653	0.160	0.250	0.056–1.110	0.068	0.098	0.016–0.578	0.010	0.313	0.064–1.517	0.149
Positive G3 vs. G1 + G2	0.699	0.317–1.538	0.373	1.406	0.534–3.705	0.491	1.271	0.417–3.876	0.673	0.923	0.290–2.935	0.892	0.908	0.458–1.801	0.783
Age (groups): ≤50 vs. >50	2.278	1.070–4.846	0.073	1.529	0.578–4.040	0.392	0.750	0.225–2.495	0.639	0.458	0.138–1.527	0.204	1.840	0.919–3.684	0.085

Positive stage III–IV vs. stage I–II	1.002	0.169–1.733	0.091	1.250	0.454–3.444	0.032	0.429	0.150–1.222	0.113	0.612	0.210–1.787	0.369	0.494	0.267–0.912	0.084
Positive worse prognosis: T3–T4 + G3 vs. T1–T2 + G1–G2	0.212	0.064–0.707	0.162	2.100	0.638–6.916	0.048	0.465	0.117–1.855	0.278	0.354	0.126–2.098	0.354	0.305	0.104–0.895	0.101

* OR could not be estimated because of zero value within a cell.

Table 11. Multivariate logistic regression analyses were conducted for diplotypes, adjusting for clinicopathological characteristics, with a focus on tumor size and metastasis.

			Multivariate											
			Model No. 1			**Model No. 2**			**Model No. 3**			**Model No. 4**		
SNPs	**Dependent**	**Covariates**	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***	**Odds**	**95% CI**	***p***
rs2838342 rs7276633 rs2051407 rs9306160 rs762400	Positive T3–T4	GCTTC haplotype non-carriers vs. homozygous diplotype (GCTTC/GCTTC)	0.393	0.188–0.822	0.039	0.392	0.185–0.827	0.041	0.391	0.173–0.884	0.041	0.380	0.166–0.869	0.046
		Age (years)	1.004	0.981–1.032	0.614	1.006	0.983–1.034	0.567	1.031	1.002–1.063	0.131	1.032	1.002–1.064	0.132
		Positive G3 vs. G1 + G2				2.007	0.971–4.147	0.061	1.842	0.831–4.067	0.131	0.579	0.257–1.299	0.185
		Positive N1 vs. N0							0.000	0.000	0.996	0.000	0.000	0.996
		Positive M1 vs. M0										6.508	0.719–58.708	0.096
rs2838342 rs7276633 rs2051407 rs9306160 rs762400	Positive M	GCTTC haplotype non-carriers vs. homozygous diplotype (GCTTC/GCTTC)	0.101	0.017–0.598	0.012	0.095	0.016–0.577	0.011	0.075	0.011–0.534	0.010	0.150	0.023–0.965	0.048
		Age (years)	0.980	0.927–1.035	0.462	0.983	0.931–1.039	0.544	1.005	0.952–1.062	0.850	0.982	0.926–1.041	0.545
		Positive G3 vs. G1 + G2				2.051	0.515–8.170	0.309	1.657	0.377–7.294	0.504	1.324	0.312–5.579	0.702
		Positive N1 vs. N0							0.000	0.000	0.996	0.000	0.000	0.996
		Positive T3–T4										8.404	0.915–77.157	0.060

3.5. Survival Analysis

The influence of the SNPs on survival, both progression-free survival (PFS) and overall survival (OS), was assessed using genotype and allelic models. In Cox's univariate and multivariate models for PFS and OS, we assessed the impact of SNPs on survival outcomes. No significant link between SNP's genotypes or alleles and PFS was detected. In the case of SNP Rs9306160, the survival analysis did not yield differences for the genotypes and alleles.

But the effect of four SNPs (rs2838342, rs7276633, rs2051407, rs762400) on OS has been identified as important.

The results indicate that for SNP rs2838342, there were no statistically significant associations between genotypes and survival outcomes. However, the presence of the A allele displayed a considerably lower hazard, signifying a potential protective role. This observation is particularly noteworthy, as the *p*-value of 0.031 indicates that the A allele may significantly contribute to improved OS outcomes (HR = 0.465, 95% CI: 0.232–0.931). Utilizing Cox's multivariate models, an effect of the A allele was sustained even after adjusting for age at diagnosis (HR = 0.462, 95% CI: 0.231–0.926, $p = 0.030$, Model No. 1). When scrutinizing the influence of age at diagnosis and broader tumor characteristics (tumor T, N, G) in Model No. 2, we observed that the impact of the A allele on OS has now become statistically insignificant. We must note that the effect of tumor size was significant on the survival outcome, revealing an HR of 7.463 (T3–T4 vs. T1–T2, $p < 0.001$), reflecting its substantial impact on OS.

Similarly, for SNP rs7276633, the TC and CC genotypes did not show significant differences in survival when compared to TT, but the presence of the T allele was associated with better OS (HR = 0.465, CI: 0.232–0.931, $p = 0.031$). The T allele's protective effect persisted, with an adjusted HR of 0.462 (95% CI: 0.231–0.926, $p = 0.030$) after accounting for age-related factors (Model No. 1). These findings underscore the significance of allelic effects in influencing overall survival. Regrettably, Model No.2 did not produce statistically significant results when adjusting for tumor T, N, G, and patients' age. The impact of tumor size on OS was significant, with an HR of 7.463 (95% CI: 3.195–17.432, $p < 0.001$).

Next, our attention turned to SNP rs2051407. Like previous SNPs, the different genotypes showed no substantial differences in survival outcomes. However, the C allele was a factor in modulating Patient OS. The C allele carriers had a decreased risk of dying faster (HR = 0.418, CI: 0.204–0.858, $p = 0.017$). In multivariate Cox's regression analysis, the C allele remains a factor for longer overall survival (HR = 0.404, 95% CI: 0.196–0.832, $p = 0.014$), when adjusting for the age of patients (Model No. 1). Unfortunately, Model No.2 did not yield statistically significant results, with a significant effect of tumor size on overall survival persisting (HR = 7.484, 95% CI: 3.227–17.355, $p < 0.001$).

Finally, our analysis extended to SNP rs762400. In the univariate model, patients with the CC genotype, compared to the GG genotype, exhibited a significant impact on OS, with an HR of 2.550 (95% CI: 1.098–5.923, $p = 0.030$), indicating an elevated risk of adverse outcomes for individuals carrying this genotype. This result remained significant in multivariate Model No.1, controlling for patient age (HR = 2.476, 95% CI: 1.064–5.758, $p = 0.035$). Exploring the interplay of tumor characteristics and age at diagnosis in Model No. 2, advanced tumor size (T3–T4 versus T1–T2) once again emerged as a significant predictor, displaying a substantial HR of 7.546 (95% CI: 3.250–17.520, $p < 0.001$). In this model, the CC genotype still increases the risk for shorter OS, but the significance level (p) is >0.05. Moreover, the scrutiny of SNP rs762400 showcased that the allelic model does not contradict the results of the genotypic model. The presence of the G allele emerged as a significant protective factor. The holders of G allele were less likely to have shorter OS when compared to the non-carriers (HR = 0.374, CI: 0.177–0.788, $p = 0.010$). The presence of the G allele (+) was associated with an HR of 0.370 (95% CI: 0.176–0.781, $p = 0.009$) in Model No. 1, after adjusting for age, indicating a substantially reduced risk of adverse OS outcomes linked to this genetic variant. In multivariate Model No. 2, advanced tumor size (T3–T4) exhibited a significant HR of 7.496 (95% CI: 3.235–17.373, $p < 0.001$). These results underline the considerable impact of tumor characteristics on OS outcomes, and once again, the significant influence of the G allele for OS was not observed.

In our analysis, using Cox's univariate model for progression-free survival (PFS) and overall survival (OS), we observed interesting trends in the association between *RRP1B* haplotypes and patient outcomes. Specifically, the ATCCG haplotype non-carriers versus the homozygous diplotype (ATCCG/ATCCG) showed an elevated hazard ratio (HR) for both PFS and OS, indicating a potential link between this haplotype and poorer survival outcomes. However, statistical significance was not achieved in this comparison.

Conversely, the heterozygous diplotype of GCTTC/alternative haplotype, compared to the homozygous diplotype (GCTTC/GCTTC), displayed a significantly decreased HR for OS (HR = 0.274, 95% CI: 0.120–0.626, $p = 0.002$), suggesting a possible protective effect associated with this haplotype. Similarly, GCTTC haplotype non-carriers compared to the homozygous diplotype (GCTTC/GCTTC) also exhibited a significantly decreased HR for OS (HR = 0.298, 95% CI: 0.128–0.695, $p = 0.005$), indicating a potentially favorable impact on survival outcomes.

In our comprehensive analysis using Cox's multivariate models for overall survival (OS), we meticulously examined the adjusted associations between diplotypes, age at diagnosis, and various tumor characteristics. Focusing on diplotypes, particularly the comparison between the heterozygous diplotype (GCTTC/alternative hap) and the

homozygous diplotype (GCTTC/GCTTC), our findings consistently demonstrated significantly decreased odds of overall survival (OS) across both Model Nos. 1 and 2 (OR = 0.259, 95% CI: 0.113–0.597, p = 0.002; OR = 0.372, 95% CI: 0.153–0.904, p = 0.029, respectively). This suggests a potential protective effect associated with certain diplotypes, indicating their relevance as prognostic indicators in cervical cancer. Similarly, when comparing GCTTC haplotype non-carriers to the homozygous diplotype (GCTTC/GCTTC), we observed notably reduced odds of OS in both Model Nos. 1 and 2 (OR = 0.303, 95% CI: 0.130–0.708, p = 0.006; OR = 0.363, 95% CI: 0.151–0.871, p = 0.023, respectively). This reinforces the importance of haplotype status in predicting survival outcomes, further highlighting the potential clinical significance of genetic variations represented by diplotypes.

In summary, our multivariate analysis within the Cox regression framework unraveled the intricate relationships between genetic variations, age at diagnosis, and tumor characteristics, providing a nuanced understanding of their combined impact on overall survival in this particular context. Tumor characteristics played a significant role, unveiling HRs and reflecting their substantial impact on OS.

All the results are presented in Tables 12–15. Kaplan–Meier analysis was performed to generate survival curves for genotypes, alleles, and haplotypes showing significant associations with overall survival (OS) (Figures 2–6).

Table 12. Cox's univariate model for PFS and OS.

		Progression-Free Survival			Overall Survival		
SNP	**Genotype/Allele**	**HR**	**95% CI**	***p*-Value**	**HR**	**95% CI**	***p*-Value**
rs2838342	AG vs. AA	0.560	0.303–1.036	0.065	0.742	0.358–1.539	0.423
	GG vs. AA	1.155	0.545–2.447	0.706	1.824	0.827–4.025	0.137
	A allele + vs. A −	1.572	0.784–3.151	0.202	0.465	0.232–0.931	0.031
	G allele + vs. G −	0.682	0.391–1.187	0.176	0.990	0.516–1.899	0.977
rs7276633	TC vs. TT	0.577	0.312–1.066	0.079	0.763	0.368–1.582	0.467
	CC vs. TT	1.176	0.555–2.490	0.673	1.854	0.840–4.090	0.126
	T allele + vs. T −	0.636	0.317–1.275	0.202	0.465	0.232–0.931	0.031
	C allele + vs. C −	0.700	0.402–1.219	0.208	1.015	0.529–1.946	0.964
rs2051407	CT vs. CC	0.556	0.302–1.026	0.060	0.813	0.397–1.667	0.573
	TT vs. CC	1.314	0.604–2.842	0.488	2.144	0.959–4.793	0.063
	C allele + vs. C −	0.568	0.275–1.175	0.127	0.418	0.204–0.858	0.017
	T allele + vs. T −	0.689	0.397–1.194	0.184	1.081	0.568–2.056	0.812
rs9306160	CT vs. CC	0.647	0.352–1.187	0.160	0.856	0.418–1.751	0.669
	TT vs. CC	1.357	0.595–3.092	0.468	2.213	0.943–5.193	0.068
	C allele + vs. C −	0.613	0.286–1.314	0.208	0.498	0.229–1.084	0.079
	T allele + vs. T −	0.705	0.399–1.245	0.228	0.915	0.476–1.758	0.789
rs762400	GC vs. GG	0.613	0.336–1.117	0.110	0.917	0.451–1.866	0.811
	CC vs. GG	1.443	0.642–3.240	0.375	2.550	1.098–5.923	0.030
	G allele + vs. G −	0.537	0.251–1.147	0.108	0.374	0.177–0.788	0.010
	C allele + vs. C −	0.734	0.422–1.275	0.272	1.178	0.613–2.263	0.624

Table 13. Cox's multivariate models for overall survival: adjusted ratios for associations between SNPs, age at diagnosis, and tumor characteristics.

	Variables	**Overall Survival**		
		HR	**95% CI**	***p*-Value**
rs2838342	A allele + vs. A −	0.462	0.231–0.926	0.030
Model No. 1	Age at diagnosis	1.011	0.987–1.037	0.366
	A allele + vs. A −	0.802	0.382–1.686	0.561
rs2838342	Age at diagnosis	1.020	0.995–1.046	0.110
Model No. 2	T3–T4 vs. T1–T2	7.463	3.195–17.432	<0.001
	N1 vs. N0	1.874	0.907–3.872	0.090
	G3 vs. G1–G2	0.710	0.346–1.457	0.350
rs7276633	T allele + vs. T −	0.462	0.231–0.926	0.030
Model No. 1	Age at diagnosis	1.011	0.987–1.037	0.366
rs7276633	T allele + vs. T −	0.802	0.382–1.686	0.561
Model No. 2	Age at diagnosis	1.029	0.995–1.046	0.110
	T3–T4 vs. T1–T2	7.463	3.195–17.432	<0.001
	N1 vs. N0	1.874	0.907–3.872	0.090
	G3 vs. G1–G2	0.710	0.346–1.457	0.350
rs2051407	C allele + vs. C −	0.404	0.196–0.832	0.014
Model No. 1	Age at diagnosis	1.013	0.988–1.039	0.297
Rs2051407	C allele + vs. C −	0.604	0.285–1.281	0.189
Model No. 2	Age at diagnosis	1.022	0.997–1.048	0.082
	T3–T4 vs. T1–T2	7.484	3.227–17.355	<0.001
	N1 vs. N0	1.824	0.892–3.732	0.100
	G3 vs. G1–G2	0.698	0.346–1.405	0.313
rs762400	GC vs. GG	0.858	0.416–1.767	0.677
Model No. 1	CC vs. GG	2.476	1.064–5.758	0.035
	Age at diagnosis	1.013	0.987–1.040	0.325
rs762400	GC vs. GG	1.083	0.521–2.248	0.831
Model No.2	CC vs. GG	1.865	0.785–4.431	0.158
	Age at diagnosis	1.021	0.996–1.047	0.100
	T3–T4 vs. T1–T2	7.546	3.250–17.520	<0.001
	N1 vs. N0	1.814	0.882–3.731	0.105
	G3 vs. G1–G2	0.719	0.358–1.448	0.356
rs762400	G allele + vs. G −	0.370	0.176–0.781	0.009
Model No. 1	Age at diagnosis	1.012	0.987–1.038	0.356
rs762400	G allele + vs. G −	0.560	0.261–1.203	0.137
Model No. 2	Age at diagnosis	1.021	0.996–1.047	0.100
	T3–T4 vs. T1–T2	7.496	3.235–17.373	<0.001
	N1 vs. N0	1.798	0.879–3.677	0.108
	G3 vs. G1–G2	0.728	0.365–1.452	0.367

Table 14. Cox's univariate model for PFS and OS.

RRP1B Haplotypes	Progression-Free Survival			Overall Survival		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Heterozygous diplotype (ATCCG/alternative haplotype) vs. homozygous diplotype (ATCCG/ATCCG)	0.990	0.523–1.873	0.975	0.843	0.397–1.790	0.656
ATCCG haplotype non-carriers vs. homozygous diplotype (ATCCG/ATCCG)	2.244	0.991–5.080	0.052	2.121	0.910–4.943	0.081
Heterozygous diplotype (GCTTC/alternative haplotype) vs. homozygous diplotype (GCTTC/GCTTC)	0.485	0.208–1.132	0.094	0.274	0.120–0.626	0.002
GCTTC haplotype non-carriers vs. homozygous diplotype (GCTTC/GCTTC)	0.434	0.190–0.993	0.051	0.298	0.128–0.695	0.005
Heterozygous diplotype (ATCCG/GCTTC) carriers vs. non-carriers	0.872	0.479–1.588	0.655	0.694	0.362–1.331	0.271

Table 15. Cox's multivariate models for overall survival: adjusted ratios for associations between diplotypes, age at diagnosis, and tumor characteristics.

SNPs	Covariates	Model No. 1			Model No.2		
		Odds	95% CI	*p*	Odds	95% CI	*p*
rs2838342 rs7276633 rs2051407 rs9306160 rs762400	Heterozygous diplotype (GCTTC/alternative hap) vs. homozygous diplotype (GCTTC/GCTTC)	0.259	0.113–0.597	0.002	0.372	0.153–0.904	0.029
	GCTTC haplotype non-carriers vs. homozygous diplotype (GCTTC/GCTTC)	0.303	0.130–0.708	0.006	0.363	0.151–0.871	0.023
	Age (years)	1.013	0.987–1.039	0.334	1.027	1.000–1.054	0.050
	G3 vs. G1–G2				0.760	0.374–1.547	0.449
	N1 vs. N0				1.913	0.933–3.922	0.076
	T3–T4 vs. T1–T2				7.412	3.196–17.188	<0.001

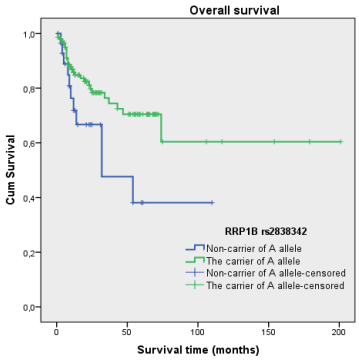

Figure 2. Kaplan–Meier survival curves were generated for patients with cervical cancer based on the rs2838342 polymorphism in the allelic model, illustrating differences in OS (n = 172). The y-axis displays the probability of survival, while the x-axis represents the duration in months from the diagnosis of cervical cancer, verifying the occurrence date of the event of interest (OS). Each vertical step in the curve signifies events (i.e., deaths), and right-censored patients are denoted by a vertical mark in the curve at the censoring time. Carrying the A allele of rs2838342 was associated with an increased likelihood of longer overall survival (HR = 0.465, 95% CI: 0.232–0.931, p = 0.031).

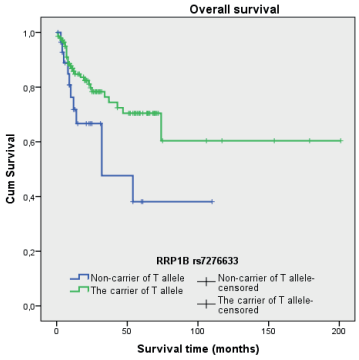

Figure 3. Kaplan–Meier survival curves for OS in patients with cervical cancer, according to rs7276633 polymorphism (n = 169). Carrying the T allele in rs7276633 increased the possibility for longer OS (HR = 0.465, CI: 0.232–0.931, p = 0.031).

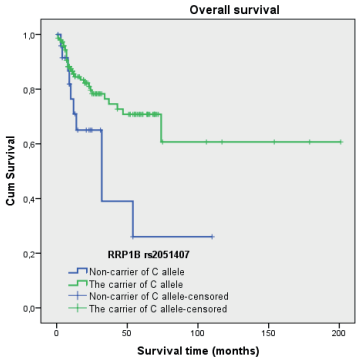

Figure 4. Kaplan–Meier survival curves for OS in patients with cervical cancer, according to rs2051407 polymorphism (n = 172). Carriers of the rs2051407 C allele had an increased chance of longer OS in comparison with non-carriers (HR = 0.418, CI: 0.204–0.858, p = 0.017).

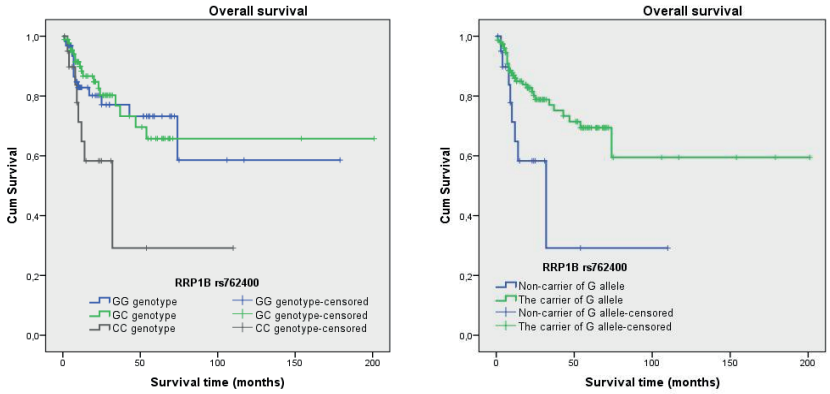

Figure 5. Kaplan–Meier survival curves for OS in patients with cervical cancer, according to rs762400 polymorphism (n = 172). Rs762400 CC genotype increased the risk for shorter OS compared to patients with the GG genotype (HR = 2.550, 95% CI: 1.098–5.923, p = 0.030). Individuals carrying the G allele exhibited a heightened likelihood of longer OS compared to those without the G allele (HR = 0.374, CI: 0.177–0.788, p = 0.010).

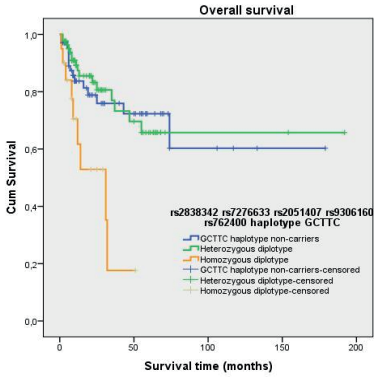

Figure 6. Kaplan–Meier survival curves for OS in patients with cervical cancer stratified by diplotypes at rs2838342, rs7276633, rs2051407, rs9306160, and rs762400, with a focus on the GCTTC haplotype (n = 172). The heterozygous diplotype (GCTTC/alternative haplotype), compared to the homozygous diplotype (GCTTC/GCTTC), displayed a significantly decreased hazard ratio (HR = 0.274, 95% CI: 0.120–0.626, p = 0.002), suggesting a possible protective effect associated with this haplotype. Similarly, GCTTC haplotype non-carriers compared to the homozygous diplotype (GCTTC/GCTTC) also exhibited a significantly decreased hazard ratio for OS (HR = 0.298, 95% CI: 0.128–0.695, p = 0.005).

4. Discussion

Despite prevention programs and vaccination efforts, cervical cancer continues to pose a significant global public health challenge. Mortality rates vary across different regions of the world, with the majority of deaths occurring in low- and middle-income countries [27,33]. With various risk factors such as human papillomavirus, sexual

activity, oral contraceptives, immunosuppression, family history, and various molecular factors (including HOX, PI3K/AKT/mTOR, EGFR, PDGFR, VEGF genes) influencing cervical cancer progression and metastasis [34], a comprehensive understanding of genome variations and biological characteristics, set against the backdrop of environmental modifications, will enhance the accuracy of disease diagnosis and treatment. This knowledge will pave the way for more precise, personalized, and effective therapeutic protocols tailored to individual patients.

In our study, all examined SNPs exhibited significant associations with clinicopathological features of cervical cancer. Rs2838342, rs2051407, and rs762400 were linked to tumor size (T) and metastasis (M), while rs7276633 was associated with tumor size and rs9306160 was associated with metastasis. When analyzing the prognosis of the disease, considering tumor size and differentiation, significant results were observed in cases involving rs2838342, rs7276633, and rs2051407. Additionally, rs2838342 and rs7276633 were associated with the stage of the disease and patients' age groups. Based on these abundant and trending findings, it can be anticipated that *RRP1B* SNPs play a role in influencing the aggressiveness of cervical cancer and and the risk of metastasis.

Regrettably, our data could not be compared with that of other researchers, as we were unable to find publications specifically investigating and analyzing *RRP1B* polymorphisms in cervical cancer cases. Evaluating the results of rs2838342, rs7276633, rs2051407, and rs762400 polymorphisms poses particular difficulties. In some cases, explaining the lack of correspondence between the genotypic model and the allelic model in the associations with the clinical characteristics of the tumor is challenging, especially due to the absence of published results from studies analyzing these SNPs. The analysis of these four polymorphisms clearly delineated the tendency of the more common allele to enhance overall survival. However, further replication of these findings is still needed.

A review of the global literature focused on the expression levels of *RRP1B*.

Crawford et al. conducted research on breast cancer. Expression of *RRP1B*, and the activity of *RRP1B* expression, was investigated to be higher in low-metastatic mice inbred strains with mammary cancer compared to high-metastatic strains. Additionally, the variation in *RRP1B* expression within a highly metastatic mouse mammary tumor cell line was found to modify progression. Ectopic Expression of *RRP1B* reduced tumor growth and metastatic potential. Expression of this gene also predicted survival in human breast cancer. A significant difference in overall survival for the groups with good and poor prognosis, predicted by the *RRP1B* activation signature, was observed across various datasets [8,12]. *RRP1B* has been represented as a likely biomarker for early gastric cancer. The expression level of *RRP1B* was significantly reduced in 76 early gastric cancer tissues compared with normal cases in the Chinese study [35]. The other study involved the analysis of 54 pairs of laryngeal tumor and adjacent normal tissues, it was revealed that *RRP1B* is significantly downexpressed in laryngeal squamous cell carcinoma [36]. There is a potential link between ALY (Aly/REF export factor), RRP1B, and metastasis in oral squamous cell carcinoma (OSCC). A knockdown of ALY reduces invasiveness and migration in OSCC cells, accompanied by an increase in *RRP1B* expression. Elevated RRP1B, alongside CD82, in ALY knockdown cells indicates that *RRP1B* may play a key role in regulating OSCC cellular invasiveness and migration [37].

Several studies have been conducted to evaluate the influence of *RRP1B* in non-oncological diseases. *RRP1B* is one of the genes regulating AREG (Amphiregulin) in endothelial cells, with HIF-1α playing a role in their upregulation in hypoxia. Silencing *RRP1B* reduces inflammation and apoptosis, highlighting its potential significance in pulmonary hypertension pathology [38]. It has been identified that *RRP1B* participates in the pathogenetic process of sepsis by regulating the activation and differentiation of lymphocytes. [39]. Based on a large-scale genome-wide association study, *RRP1B* is associated with a significant signal of blood pressure regulation [40]. The *RRP1B* gene was associated with blood pressure response to specific antihypertensive drugs, particularly atenolol [41]. The expression of *RRP1B* was analyzed in leucocytes of

individuals with Down's syndrome (DS). The results indicated that *RRP1B* showed significant upregulation in DS patients compared to the normal population [42].

It is interesting that data from the Cancer Genome Atlas (TCGA) project suggest the expression level of *RRP1B* is not a prognostic factor in cervical cancer survival analysis (https://tcga-data.nci.nih.gov/docs/publications/cesc_2016/, accessed on 15 December 2023).

There are few studies evaluating the associations of *RRP1B* rs9306160 polymorphisms with cancer risk or clinical data.

In our study, we found that the C allele of rs9306160 is more common and may have a significant protective effect against metastasis ($p = 0.008$). The variant T allele did not show statistically significant results, but the TT genotype increased the risk for metastasis (p-value close to the significance at 0.051). Unfortunately, we did not obtain significant associations between rs9306160 and clinical features such as lymph node metastasis, tumor differentiation, or survival rates. But if we consider that a frequent allele is a sign of a better prognosis, then when analyzing the results of other authors' studies, the data differ.

Crawford et al.'s study with breast cancer outcomes was conducted in two cohorts: one from Orange County and another from the Greater Baltimore Area. Consistent findings were observed between the cohorts, although some differences could be attributed to cohort characteristics. They found a significant association between the variant A allele of rs9306160 and disease stage in a Caucasian cohort. The A allele was more prevalent in patients with localized disease compared to those with advanced regional or metastatic disease. The variant allele showed significant associations with various tumor characteristics, including estrogen receptor (ER) and progesterone receptor (PR) status, the presence of lymph node disease, and tumor grade. It was more frequent in patients with ER-positive and PR-positive tumors, as well as in those with well-to-moderately differentiated tumors. Carriers of the variant allele had better breast cancer-specific survival compared to homozygous carriers of the common allele (G/G). This survival advantage was more pronounced in patients with ER-positive tumors [8].

Another study involved 1863 Dutch patients with operable primary breast cancer from Rotterdam, The Netherlands. The investigation identified a significant association of variants in rs9306160 with metastasis-free survival (MFS) ($p = 0.012$). Specifically, the study revealed a connection between the T allele of the *RRP1B* SNP (rs9306160) and a more favorable prognosis in MFS among breast cancer patients. Carrying the T allele (CT or TT genotypes) of rs9306160 was associated with a positive outcome in terms of MFS. Remarkably, this association maintained significance even in multivariate analysis, indicating that the T allele functions as an independent prognostic factor. Notably, the association with patients' survival was confined to estrogen receptor-positive, lymph node-negative (ER+/LN−) patients ($p = 0.011$). Furthermore, combining the genotypes of two genes (*SIPA1* and *RRP1B*) demonstrated a significant ability to discriminate patients with poor metastasis-free survival (HR: 0.40, 95% CI: 0.24 to 0.68, $p = 0.001$). It is important to acknowledge the study's limitations, as the observed association was significant only for a specific subgroup (ER+/LN− patients) and not for other patient subgroups (ER+/LN+, ER−/LN+, ER−/LN−). The study was conducted within a Dutch patient population, and to establish broader applicability, the results may require validation in diverse populations [43].

On the other hand, the study of Nanchari et al., which included 493 breast cancer cases and 558 age-matched healthy female controls, could reflect a guideline for the results we obtained. The TT genotype and T allele frequencies of the *RRP1B* rs9306160 (1307T>C) polymorphism were significantly elevated in breast cancer cases compared to controls. The presence of the T allele conferred a 1.75-fold increased risk for breast cancer development. The TT genotype was associated with a higher risk under codominant and recessive models. Moreover, the TT genotype frequency was significantly elevated in obese patients, patients with advanced disease, and those with increased tumor size. The

T allele was associated with positive lymph node status and Her2-negative receptor status. In silico analysis of RNA secondary structures near the SNP site indicated that the T allele may result in a less stable mRNA structure compared to the C allele, potentially affecting functional interactions. The study suggests that the TT genotype may increase the risk for both breast cancer development and progression. It acknowledges deviations from the Hardy–Weinberg equilibrium and suggests the possibility of selective forces influencing genotype frequencies over generations. Additionally, the study highlights discrepancies in results compared to other cohorts, possibly due to ethnic variations. The C allele was more frequent in both controls and breast cancer cases, indicating that the C allele was more prevalent in both groups. However, there were differences in allele frequencies between controls and breast cancer cases. It is important to note that the findings are specific to the population studied (Southern Indian) and may not be directly applicable to other populations [44].

Earlier research from Lithuania characterized a group of young Lithuanian patients with breast cancer. Consistent with our findings, the prevalence of the C allele of rs9306160 (c.436T4C) was higher, constituting 59.5% in the allelic model. The study revealed a statistically significant association between rs9306160 and tumor grade (G). Specifically, the T allele was significantly linked to G3 tumor grade (high-grade tumors), indicating a higher probability of G3 grade in carriers of the T allele. This association remained significant after adjustments, including age at diagnosis, tumor receptor status, tumor size, and lymph node involvement, suggesting an independent effect of the polymorphism on this breast cancer characteristic. The C allele was associated with ER-positive status, implying a higher likelihood of positive ER in individuals with the CC genotype or carriers of the C allele. Therefore, these findings support the notion of the T allele as a worse prognostic factor [45].

Moreover, a case-control study involving 100 Iraqi women (75 with confirmed breast cancer and 25 with normal breast tissue) could also corroborate the observed trend in our results. The results indicated a higher frequency of the CC genotype in the control group. The homozygous TT genotype was associated with histologic grade, and this association remained significant across all grades. Among cancer patients with a high-grade variant, T alleles were more prevalent compared to those in low-grade conditions. Furthermore, the (TT) genotype was more frequently observed in breast cancer cases with metastatic lymph node involvement compared to cases without lymph node involvement [46].

Our extended haplotype analysis of the investigated SNPs revealed that GCTTC haplotype non-carriers, predominantly consisting of ATCCG haplotypes, were less likely to exhibit advanced tumor size and metastasis. These findings were consistent with the results obtained from allelic models. The same trend was also noted in survival assessments. Consequently, we posit that these haplotypes could serve as independent markers.

In the present study, we examined the associations between five functional SNPs in the *RRP1B* gene and the clinicopathological profiles and survival rates in a cohort of Lithuanian women with cervical cancer. Our study is the first to analyze *RRP1B* SNPs for assessing the clinicopathological features and progression of CC. It establishes a link between SNPs in *RRP1B* and CC, suggesting these genetic variants as predictive biomarkers for prognosticating the development of the disease in the future. The study boasts several strengths, including a comprehensive dataset comprising genetic data, tumor phenotype information, and survival data. However, certain limitations warrant consideration. Notably, the absence of comparable studies on associations between these polymorphisms and clinicopathological characteristics of CC prevents a direct comparison of our results. Additionally, the limited sample size may have influenced the robustness of our findings. Furthermore, a notable weakness is the absence of a control group, hindering the assessment of CC risk.

Our investigation indicates a potential link between *RRP1B* polymorphisms and the pathomorphological features of cervical cancer, as well as disease outcomes. The

association of these genetic variations with the aggressiveness of cervical cancer underlines the importance of considering germline factors in understanding cancer behavior. This observation opens avenues for further research to elucidate the mechanistic basis of *RRP1B*'s involvement in metastatic processes and its clinical implications. While *RRP1B* may not traditionally be classified as an oncogene, we believe that its inclusion in our investigation offers a unique opportunity to uncover novel facets of the disease's molecular underpinnings. Importantly, our decision to study *RRP1B* stems from a comprehensive approach aimed at elucidating the full spectrum of genetic factors contributing to cervical cancer development and progression. We recognize that the complexity of cancer biology extends beyond well-established oncogenes, and exploring genes like *RRP1B* allows us to broaden our understanding of the disease.

5. Conclusions

All investigated *RRP1B* polymorphisms (rs2838342, rs7276633, rs2051407, rs9306160, and rs762400) in our study have the potential to serve as markers for clinical characteristics and prognosis in cervical cancer. Among these, three (rs2051407, rs9306160, and rs762400) were found to be significant in relation to metastasis, while rs2838342 showed potential association with metastasis. Rs2838342, rs7276633, rs2051407, and rs762400 showed the assotiations with survival outcomes. Haplotypes analysis was in line with the allelic models. These results highlight the intricate interplay between genetic factors and clinical dynamics in the progression of tumors. Nevertheless, it is crucial to acknowledge that certain comparisons did not attain statistical significance, possibly owing to the relatively small sample size. Considering the clinical context is imperative, it is essential to interpret the results cautiously, especially for genotypes or alleles with borderline significance levels. Our results offer insights for subsequent studies on cervical cancer and other cancer types, examining these polymorphisms to ascertain their functionality. In the future, SNP detection in *RRP1B* may serve as a predictive tool for assessing the clinical manifestations and prognoses of cervical cancer. While evidence is accumulating regarding the significance of genetic variation in the etiology and development of cervical cancer, research exploring the role of metastasis-related gene variants in cervical cancer is still in its early stages. Further investigations are required to validate these observations and gain a comprehensive understanding of the underlying biological mechanisms.

Author Contributions: Conceptualization, E.B., A.I., R.U. and E.J.; Methodology, R.U. and A.I.; Formal analysis, E.B., A.I., R.U. and E.J.; Investigation, E.B. and R.U.; Resources, E.B. and A.I.; Data Curation, E.B; Writing—Original draft preparation, E.B.; Writing—Review and Editing, A.I., R.U., and E.J.; Visualization, E.B.; Supervision, A.I., R.U. and E.J.; Project Administration, Supervision, A.I., R.U. and E.J.; Funding Acquisition, E.B. and R.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The retrospective cohort study of adult patients with cervical cancer was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-10 and P1-BE-2-10/2014), approved on 7 May 2014.

Informed Consent Statement: All subjects participating in the study provided informed consent.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors express their gratitude to the patients, nurses, and colleagues who collaborated in the laboratory, employing the real-time PCR technique for this study.

Conflicts of Interest: The authors report no declaration of interest.

References

1. Lifsted, T.; Voyer, T.L.; Williams, M.; Muller, W.J.; Klein-Szanto, A.J.; Buetow, K.H.; Hunter, K.W. Identification of inbred mouse strains harboring genetic modifiers of mammary tumor age of onset and metastatic progression. *Int. J. Cancer* **1998**, *77*, 640–644. https://doi.org/10.1002/(sici)1097-0215(19980812)77:4.
2. Hunter, K.W.; Welch, D.R.; Liu, E.T. Genetic background is an important determinant of metastatic potential. *Nat. Genet.* **2003**, *34*, 23–24. https://doi.org/10.1038/ng0503-23b.
3. Steeg, P.S. Metastasis suppressors alter the signal transduction of cancer cells. *Nat. Rev. Cancer* **2003**, *3*, 55–63. https://doi.org/10.1038/nrc967.
4. Hunter, K.W. Allelic diversity in the host genetic background may be an important determinant in tumor metastatic dissemination. *Cancer Lett.* **2003**, *200*, 97–105. https://doi.org/10.1016/s0304-3835(03)00420-8.
5. Schadt, E.E.; Monks, S.A.; Drake, T.A.; Lusis, A.J.; Che, N.; Colinayo, V.; Ruff, T.G.; Milligan, S.B.; Lamb, J.; Cavet, G.; et al. Genetics of gene expression surveyed in maize, mouse and man. *Nature* **2003**, *422*, 297–302. https://doi.org/10.1038/nature01434.
6. Yang, H.; Crawford, N.P.; Lukes, L.; Finney, R.; Lancaster, M.; Hunter, K.W. Metastasis predictive signature profiles pre-exist in normal tissues. *Clin. Exp. Metastasis* **2005**, *22*, 593–603. https://doi.org/10.1007/s10585-005-6244-6.
7. Lancaster, M.; Rouse, J.; Hunter, K.W. Modifiers of mammary tumor progression and metastasis on mouse Chromosomes 7, 9, and 17. *Mamm. Genome* **2005**, *16*, 120–126. https://doi.org/10.1007/s00335-004-2432-y.
8. Crawford, N.P.; Qian, X.; Ziogas, A.; Papageorge, A.G.; Boersma, B.J.; Walker, R.C.; Lukes, L.; Rowe, W.; Zhang, J.; Ambs, S.; et al. RRP1B, a new candidate susceptibility gene for breast cancer progression and metastasis. *PLOS Genet.* **2007**, *3*, e214. https://doi.org/10.1371/journal.pgen.0030214.
9. Crawford, N.P.; Walker, R.C.; Lukes, L.; Officewala, J.S.; Williams, R.W.; Hunter, K.W. The Diasporin Pathway: A tumor progression-related transcriptional network that predicts breast cancer survival. *Clin. Exp. Metastasis* **2008**, *25*, 357–369. https://doi.org/10.1007/s10585-008-9146-6.
10. Park, Y.; Zhao, X.; Lesueur, F.; Lowy, D.R.; Lancaster, M.; Pharoah, P.D.; Qian, X.; Hunter, K.W. Sipa1 is a candidate for underlying the metastasis efficiency modifier locus Mtes1. *Nat. Genet.* **2005**, *37*, 1055–1062. https://doi.org/10.1038/ng1635.
11. Crawford, N.P.; Ziogas, A.; Peel, D.; Hess, J.; Anton-Culver, H.; Hunter, K.W. Germline polymorphisms in SIPA1 are associated with metastasis and other indicators of poor prognosis in breast cancer. *Breast Cancer Res.* **2006**, *8*, R16. https://doi.org/10.1186/bcr1389.
12. Crawford, N.P.; Yang, H.; Mattaini, K.; Hunter, K.W. The metastasis efficiency modifier ribosomal RNA processing 1 Homolog B (RRP1B) is a chromatin-associated factor. *J. Biol. Chem.* **2009**, *284*, 28660–28673. https://doi.org/10.1074/jbc.m109.023457.
13. Lee, M.; Dworkin, A.M.; Lichtenberg, J.; Patel, S.J.; Trivedi, N.; Gildea, D.; Bodine, D.M.; Crawford, N.P. Metastasis-Associated Protein Ribosomal RNA Processing 1 Homolog B (RRP1B) Modulates Metastasis through Regulation of Histone Methylation. *Mol. Cancer Res.* **2014**, *12*, 1818–1828. https://doi.org/10.1158/1541-7786.mcr-14-0167.
14. Lee, M.; Dworkin, A.M.; Gildea, D.; Trivedi, N.; Moorhead, G.B.G.; Crawford, N.P. RRP1B is a metastasis modifier that regulates the expression of alternative mRNA isoforms through interactions with SRSF1. *Oncogene* **2013**, *33*, 1818–1827. https://doi.org/10.1038/onc.2013.133.
15. Felgueiras, J.; Jerónimo, C.; Fardilha, M. Protein phosphatase 1 in tumorigenesis: Is it worth a closer look? *Biochim. Biophys. Acta (BBA)-Rev. Cancer* **2020**, *1874*, 188433. https://doi.org/10.1016/j.bbcan.2020.188433.
16. Srivastava, G.; Bajaj, R.; Kumar, G.S.; Gaudreau-Lapierre, A.; Nicolas, H.; Chamousset, D.; Kreitler, D.; Peti, W.; Trinkle-Mulcahy, L.; Page, R. The ribosomal RNA processing 1B:protein phosphatase 1 holoenzyme reveals non-canonical PP1 interaction motifs. *Cell Rep.* **2022**, *41*, 111726. https://doi.org/10.1016/j.celrep.2022.111726.
17. Chamousset, D.; De Wever, V.; Moorhead, G.B.G.; Chen, Y.; Boisvert, F.; Lamond, A.I.; Trinkle-Mulcahy, L. RRP1B Targets PP1 to Mammalian Cell Nucleoli and Is Associated with Pre-60S Ribosomal Subunits. *Mol. Biol. Cell* **2010**, *21*, 4212–4226. https://doi.org/10.1091/mbc.e10-04-0287.
18. Paik, J.C.; Wang, B.; Liu, K.; Lue, J.K.; Lin, W. Regulation of E2F1-induced apoptosis by the nucleolar protein RRP1B. *J. Biol. Chem.* **2010**, *285*, 6348–6363. https://doi.org/10.1074/jbc.m109.072074.
19. Grisendi, S.; Mecucci, C.; Falini, B.; Pandolfi, P.P. Nucleophosmin and cancer. *Nat. Rev. Cancer* **2006**, *6*, 493–505. https://doi.org/10.1038/nrc1885.
20. Okuwaki, M.; Saito, S.; Hirawake-Mogi, H.; Nagata, K. The interaction between nucleophosmin/NPM1 and the large ribosomal subunit precursors contribute to maintaining the nucleolar structure. *Biochim. Biophys. Acta (BBA)-Mol. Cell Res.* **2021**, *1868*, 118879. https://doi.org/10.1016/j.bbamcr.2020.118879.
21. Crawford, N.P.; Alsarraj, J.; Lukes, L.; Walker, R.C.; Officewala, J.S.; Yang, H.H.; Lee, M.P.; Ozato, K.; Hunter, K.W. Bromodomain 4 activation predicts breast cancer survival. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6380–6385. https://doi.org/10.1073/pnas.0710331105.
22. Alsarraj, J.; Faraji, F.; Geiger, T.; Mattaini, K.; Williams, M.; Wu, J.J.; Ha, N.; Merlino, T.; Walker, R.C.; Bosley, A.D.; et al. BRD4 Short Isoform Interacts with RRP1B, SIPA1 and Components of the LINC Complex at the Inner Face of the Nuclear Membrane. *PLoS ONE* **2013**, *8*, e80746. https://doi.org/10.1371/journal.pone.0080746.
23. Donati, B.; Lorenzini, E.; Ciarrocchi, A. BRD4 and Cancer: Going beyond transcriptional regulation. *Mol. Cancer* **2018**, *17*, 164. https://doi.org/10.1186/s12943-018-0915-9.
24. Chiang, S.K.; Chang, W.C.; Chen, S.; Chang, L. DOCK1 Regulates Growth and Motility through the RRP1B-Claudin-1 Pathway in Claudin-Low Breast Cancer Cells. *Cancers* **2019**, *11*, 1762. https://doi.org/10.3390/cancers11111762.

25. Chang, J.; Wang, F.; Chapin, W.; Huang, R.S. Identification of MicroRNAs as Breast Cancer Prognosis Markers through the Cancer Genome Atlas. *PLoS ONE* **2016**, *11*, e0168284. https://doi.org/10.1371/journal.pone.0168284.
26. World Health Organization (WHO). Cervical Cancer. 2023. Available online: https://www.who.int/news-room/fact-sheets/detail/cervical-cancer (accessed on 7 January 2024).
27. Arbyn, M.; Weiderpass, E.; Bruni, L.; de Sanjosé, S.; Saraiya, M.; Ferlay, J.; Bray, F. Estimates of incidence and mortality of cervical cancer in 2018: A worldwide analysis. *Lancet Glob. Health* **2020**, *8*, e191–e203.
28. Pappa, K.; Kontostathi, G.; Lygirou, V.; Zoidakis, J.; Anagnou, N. Novel structural approaches concerning HPV proteins: Insight into targeted therapies for cervical cancer (Review). *Oncol. Rep.* **2018**, *39*, 1547–1554. https://doi.org/10.3892/or.2018.6257.
29. De Sanjosé, S.; Brotons, M.; Pavón, M.Á. The natural history of human papillomavirus infection. *Best Pract. Res. Clin. Obstet. Gynaecol.* **2018**, *47*, 2–13. https://doi.org/10.1016/j.bpobgyn.2017.08.015.
30. Drolet, M.; Bénard, É.; Pérez, N.; Brisson, M.; Ali, H.; Boily, M.; Baldo, V.; Brassard, P.; Brotherton, J.; Callander, D.; et al. Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: Updated systematic review and meta-analysis. *Lancet* **2019**, *394*, 497–509. https://doi.org/10.1016/s0140-6736(19)30298-3.
31. Small, W.; Bacon, M.; Bajaj, A.; Chuang, L.; Fisher, B.J.; Harkenrider, M.M.; Jhingran, A.; Kitchener, H.C.; Mileshkin, L.; Viswanathan, A.N.; et al. Cervical cancer: A global health crisis. *Cancer* **2017**, *123*, 2404–2412. https://doi.org/10.1002/cncr.30667.
32. European Centre for Disease Prevention and Control (ECDC). Cervical Cancer Screening in the EU/EEA: Quality Assurance and Organization. 2021. Available online: https://www.ecdc.europa.eu/en/publications-data/cervical-cancer-screening-eueea-quality-assurance-and-organization (accessed on 8 January 2024).
33. Gültekin, M.; Ramírez, P.T.; Broutet, N.; Hutubessy, R. World Health Organization call for action to eliminate cervical cancer globally. *Int. J. Gynecol. Cancer* **2020**, *30*, 426–427. https://doi.org/10.1136/ijgc-2020-001285.
34. Aziz, S.Q.; Aziz, M. Cervical cancer metastasis. In *Introduction to Cancer Metastasis*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 77–94. https://doi.org/10.1016/b978-0-12-804003-4.00005-0.
35. Lu, Q.; Takata, Y.; Ou, X.; Cao, D.; Xie, T.; Chen, X. Potential lncRNA diagnostic biomarkers for early gastric cancer. *Mol. Med. Rep.* **2017**, *16*, 9545–9552. https://doi.org/10.3892/mmr.2017.7770.
36. Chen, H.; Yuan, X.; Zhou, L.; Huang, J.; Tao, L.; Cheng, L.; Tian, J. Cisplatin and paclitaxel target significant long noncoding RNAs in laryngeal squamous cell carcinoma. *Med. Oncol.* **2014**, *31*, 246. https://doi.org/10.1007/s12032-014-0246-7.
37. Saito, Y.; Kasamatsu, A.; Yamamoto, A.; Shimizu, T.; Yokoe, H.; Sakamoto, Y.; Ogawara, K.; Shiiba, M.; Tanzawa, H.; Uzawa, K. ALY as a potential contributor to metastasis in human oral squamous cell carcinoma. *J. Cancer Res. Clin. Oncol.* **2012**, *139*, 585–594. https://doi.org/10.1007/s00432-012-1361-5.
38. Florentin, J.; Zhao, J.; Tai, Y.; Sun, W.; Ohayon, L.; O'Neil, S.; Arunkumar, A.; Zhang, X.; Zhu, J.; Aaraj, Y.A.; et al. Loss of Amphiregulin drives inflammation and endothelial apoptosis in pulmonary hypertension. *Life Sci. Alliance* **2022**, *5*, e202101264. https://doi.org/10.26508/lsa.202101264.
39. Li, H.; Yang, L. Identification of novel immune infiltration-related biomarkers of sepsis based on bioinformatics analysis. *Cell. Mol. Biol.* **2023**, *69*, 205–209. https://doi.org/10.14715/cmb/2023.69.12.33.
40. Wain, L.V.; Vaez, A.; Jansen, R.C.; Joehanes, R.; Van Der Most, P.J.; Erzurumluoglu, A.M.; O'Reilly, P.F.; Cabrera, C.; Warren, H.R.; Rose, L.M.; et al. Novel blood pressure locus and gene discovery using Genome-Wide association study and expression data sets from blood and the kidney. *Hypertension* **2017**, *70*, e4–e19. https://doi.org/10.1161/hypertensionaha.117.09438.
41. Iniesta, R.; Campbell, D.; Venturini, C.; Faconti, L.; Singh, S.; Irvin, M.R.; Cooper-DeHoff, R.M.; Johnson, J.A.; Turner, S.T.; Arnett, D.K.; et al. Gene variants at LOCI related to blood pressure account for variation in response to antihypertensive drugs between black and white individuals. *Hypertension* **2019**, *74*, 614–622. https://doi.org/10.1161/hypertensionaha.118.12177.
42. Salemi, M.; Barone, C.; Romano, C.; Zolezzi, F.; Romano, C.; Scavuzzo, C.; Salluzzo, R.; Scillato, F.; Signorelli, M.; Kapetis, D.; et al. Gene expression profiling and qRT-PCR expression of RRP1B, PCNT, KIF21A and ADRB2 in leucocytes of Down's syndrome subjects. *J. Genet.* **2012**, *93* (Suppl. S1), 18–23. https://doi.org/10.1007/s12041-012-0132-z.
43. Hsieh, S.; Look, M.P.; Sieuwerts, A.M.; Foekens, J.A.; Hunter, K.W. Distinct inherited metastasis susceptibility exists for different breast cancer subtypes: A prognosis study. *Breast Cancer Res.* **2009**, *11*. https://doi.org/10.1186/bcr2412.
44. Nanchari, S.R.; Cingeetham, A.; Meka, P.; Surekha, D.; Tipirisetti, N.; Padala, C.; Annamaneni, S.; Hanumanth, S.R.; Digumarthi, R.R.; Satti, V. Rrp1B gene polymorphism (1307T>C) in metastatic progression of breast cancer. *Tumor Biol.* **2014**, *36*, 615–621. https://doi.org/10.1007/s13277-014-2613-6.
45. Ugenskienė, R.; Myrzaliyeva, D.; Jankauskaitė, R.; Gedminaitė, J.; Jančiauskienė, R.; Šepetauskienė, E.; Juozaitytė, E. The contribution of SIPA1 and RRP1B germline polymorphisms to breast cancer phenotype, lymph node status and survival in a group of Lithuanian young breast cancer patients. *Biomarkers* **2016**, *21*, 363–370. https://doi.org/10.3109/1354750x.2016.1141989.
46. Al-Husseini RM, A.; Hussain, R.A.; Abed, A.M. Assess the contribution of ribosomal RNA processing 1B gene polymorphisms in breast cancer in Iraqi patients. *Ann. Biol.* **2020**, *36*, 26–33.

SUPPLEMENTS

Supplementary Picture 1. Ethics Committee Approval.

KAUNO REGIONINIS BIOMEDICININIŲ TYRIMŲ ETIKOS KOMITETAS

LSMUL KK, Fiziologijos ir farmakologijos institutas (Klinikinės farmakologijos padalinys), Eivenių g.2, LT-50009 Kaunas, tel. (+370) 37 32 68 89;el.paštas: kaunorbtek@lsmuni.lt

LEIDIMAS ATLIKTI BIOMEDICININĮ TYRIMĄ

2014-05-07 Nr. BE-2-10

Biomedicininio tyrimo pavadinimas:	**"Onkologinių ligų tiriamosios medžiagos biobankas"**
Protokolo Nr.:	**01 BB**
Data:	**2013-12-17**
Versija:	**I**
Asmens informavimo forma bei Informuoto asmens sutikimo forma data:	**2013-12-17 Versija nr. I**
Pagrindinis tyrėjas:	**Prof. Elona Juozaitytė**
Biomedicininio tyrimo vieta: Įstaigos pavadinimas: Adresas:	**LSMU MA MF Onkologijos institutas** Mickevičiaus g. 9, LT-44307, Kaunas

Išvada:

Kauno regioninio biomedicininių tyrimų etikos komiteto posėdžio, įvykusio **2014 m. gegužės 7 d.** (protokolo Nr. BE-10-4) sprendimu pritarta biomedicininio tyrimo vykdymui.

Mokslinio eksperimento vykdytojai įsipareigoja: (1) nedelsiant informuoti Kauno Regioninį biomedicininių Tyrimų Etikos komitetą apie visus nenumatytus atvejus, susijusius su studijos vykdymu, (2) iki sausio 15 dienos – pateikti metinį studijos vykdymo apibendrinimą bei, (3) per mėnesį po studijos užbaigimo, pateikti galutinį pranešimą apie eksperimentą.

Kauno regioninio biomedicininių tyrimų etikos komiteto nariai			
Nr.	Vardas, Pavardė	Veiklos sritis	Dalyvavo posėdyje
1.	Prof. Romaldas Mačiulaitis	Klinikinė farmakologija	taip
2.	Prof. Edgaras Stankevičius	Fiziologija, farmakologija	taip
3.	Doc. Eimantas Peičius	Filosofija	taip
4.	Dr. Ramunė Kasperavičienė	Kalbotyra	taip
5.	Med. dr. Jonas Andriuškevičius	Chirurgija	taip
6.	Agnė Krušinskaitė	Teisė	taip
7.	Prof. Skaidrius Miliauskas	Pulmonologija, vidaus ligos	taip
8.	Med. dr. Rokas Bagdonas	Chirurgija	ne
9.	Eglė Vaižgelienė	Visuomenės sveikata	taip

Kauno regioninis biomedicininių tyrimų etikos komitetas dirba vadovaudamasis etikos principais nustatytais biomedicininių tyrimų Etikos įstatyme, Helsinkio deklaracijoje, vaistų tyrinėjimo Geros klinikinės praktikos taisyklėmis.

Pirmininkas Prof. Romaldas Mačiulaitis

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS * KAUNO REGIONINIS BIOMEDICININIŲ TYRIMŲ ETIKOS KOMITETAS

Supplementary Figure 1. *Cervical cancer questionnaire.*

KLAUSIMYNAS

GIMDOS KAKLELIO VĖŽYS

1) Gydytojo vardas, pavardė, parašas:

.....

2) Data: ☐☐☐☐ ☐☐ ☐☐
metai mėnuo diena

DOKUMENTINĖ DALIS

3) Paciento vardas, pavardė: .....

4) Adresas: .....

.....

5) Telefonas: ☐☐☐☐☐☐☐☐☐☐☐☐

6) Gyvenamoji aplinka (*pažymėti* ☑): ☐ Kaimas ☐ Miestas

7) Svoris (kg): ☐☐☐

8) Ūgis (cm): ☐☐☐

9) Paciento kortelės numeris: ☐☐☐☐☐☐☐☐☐☐

10) Asmens kodas: ☐☐☐☐☐☐☐☐☐☐☐☐

11) Paciento numeris (*pateiktas atskirame lape*): ☐☐☐☐☐☐

12) Gimimo data: ☐☐☐☐ ☐☐ ☐☐
metai mėnuo diena

13) Lytis (*pažymėti* ☑): ☐ Vyras ☐ Moteris

14) Paciento tautybė (*pažymėti* ☑):
☐ Lietuvių ☐ Baltarusų
☐ Latvių ☐ Suomių
☐ Lenkų ☐ Žydų/Izraelitų
☐ Estų ☐ Totorių
☐ Rusų ☐ Vokiečių
☐ Ukrainiečių ☐ Kita.....

15) Tėvo tautybė (*pažymėti* ☑):
☐ Lietuvių ☐ Baltarusų
☐ Latvių ☐ Suomių
☐ Lenkų ☐ Žydų/Izraelitų
☐ Estų ☐ Totorių
☐ Rusų ☐ Vokiečių
☐ Ukrainiečių ☐ Kita.....

16) Motinos tautybė (*pažymėti* ☑):

☐ Lietuvių ☐ Baltarusų
☐ Latvių ☐ Suomių
☐ Lenkų ☐ Žydų/Izraelitų
☐ Estų ☐ Totorių
☐ Rusų ☐ Vokiečių
☐ Ukrainiečių ☐ Kita.....

KLINIKINIAI DUOMENYS

17) Diagnozė nustatyta pirmą kartą: ☐☐☐☐ ☐☐ ☐☐
metai mėnuo diena

18) Gimdos kaklelio vėžio klasifikacija pagal TNM sistemą (*įrašyti klinikinis (c) ar patologinis (p) TNM*)

T kategorija

N kategorija

M kategorija

19) Naviko histologinė forma (*pažymėti* ☑):

☐ Plokščialąstelinė karcinoma
☐ Adenokarcinoma
☐ Kita (*patikslinti*).....

20) Naviko diferenciacijos laipsnis (*pažymėti* ☑):

☐ G1 ☐ G3
☐ G2 ☐ G4

21) Naviko invazija į kraujagysles (V) (*pažymėti* ☑):

☐ V0
☐ V1

22) Naviko invazija į limfagysles (L) (*pažymėti* ☑):

☐ L0
☐ L1

23) Ar vėžys išplitęs į kitus organus (*pažymėti* ☑)?

☐ TAIP ☐ NE
☐ Kepenis ☐ Lokalus
☐ Plaučius ☐ Kita .....
☐ Pilvo ertmės organus
.....
.....
☐ Kitus organus.....

24) Kiti onkologiniai susirgimai (*pažymėti* ☑):

☐ TAIP ☐ NE

Jei TAIP, nurodykite (*pažymėti* ☑):

☐ Kraujo vėžys ☐ Storosios žarnos vėžys
☐ Stemplės vėžys ☐ Skrandžio vėžys
☐ Kepenų vėžys ☐ Kiaušidžių vėžys
☐ Šlapimo pūslės vėžys ☐ Gimdos vėžys
☐ Krūties vėžys ☐ Prostatos vėžys
☐ Plaučių vėžys ☐ Kita .....

25) Chirurginis gydymas (*pažymėti* ☑): ☐ TAIP ☐ NE

Data: ☐☐☐☐ ☐☐ ☐☐
metai mėnuo diena

arba amžius (metais): ☐☐☐

26) Operacija (*pažymėti* ☑): ☐ Radikali ☐ Neradikali

Operacijos tipas: .....

pTNM .....

27) Ar sergate lėtinėmis ligomis (*pažymėti* ☑): ☐ TAIP ☐ NE

Jei TAIP, nurodykite kokiomis .....
.....

Kada ši liga buvo diagnozuota? ☐☐☐☐ ☐☐ ☐☐
metai mėnuo diena

28) Rūkymas (*pažymėti* ☑): ☐ Rūkantis ☐ Nerūkantis ☐ Rūkęs anksčiau

Jei rūkote/rūkėte anksčiau: Kiek metų: nuo..... iki .....
Kiek cigarečių per dieną: .....

29) Alkoholio vartojimas (*pažymėti* ☑):
☐ Vartojantis kasdien
☐ Vartojantis, bet nereguliariai
☐ Negeriantis

Jei vartojate: Kiek metų: nuo..... iki .....

Jūs išgeriate – 2 taures (200 ml) vyno arba 1 butelį alaus arba 100 ml stipriųjų gėrimų (degtinės, konjako, romo ar kt.) (*pažymėti* ☑):
☐ Kiekvieną dieną ☐ 1 kartą per 3 mėnesius
☐ 1 kartą per savaitę ☐ 1 kartą per 6 mėnesius
☐ 1 kartą per mėnesį ☐ 1 kartą per 1 metus

30) Adjuvantinis ar neoadjuvantinis gydymas	Gydymo rūšis	Gydymo laikotarpis	
	.....	.....	
31) Gydomoji chemoterapija (chemoterapijos rūšis)	Gydymo rūšis	Gydymo laikotarpis	Atsakas (PA, DA, SL, PL)
I eilė	.....	.....	.....
II eilė	.....	.....	.....
III eilė	.....	.....	.....
32) Biologinė terapija	Biologinės terapijos rušis	Gydymo laikotarpis	Atsakas (PA, DA, SL, PL)
	.....	.....	.....
33) Spindulinė terapija	Biologinės terapijos rušis	Gydymo laikotarpis	Atsakas (PA, DA, SL, PL)
	.....	.....	.....

Supplementary Figure 2. *Patient informed consent form.*

Informuotojo asmens sutikimo forma

Protokolo numeris, versija: **Nr. 02 BB, versija II, 2015.10.10**

Planuojamo tyrimo pavadinimas: **„Onkologinių ligų tiriamosios medžiagos biobankas“**

Tiriamojo vardas, pavardė: .....

Adresas: .....

Perskaičiau pateiktą informaciją apie tyrimą *„Onkologinių ligų tiriamosios medžiagos biobankas“,* kurio užsakovas yra LSMU MA MF Onkologijos institutas. Gavau šių dokumentų po vieną kopiją: tiriamųjų informavimo lapą ir šią informuotojo asmens sutikimo formą. Turėjau galimybę aptarti tyrimą su tyrėju ir užduoti jam klausimus.

Suprantu, kad šiuo biomediciniinio tyrimo metu bus kaupiamas biobankas t.y. pacientų biologinė medžiaga ir medicininė informacija, ji tiriama analizuojama ir panaudojama moksliniams tyrimams. Šio biomedicininiu tyrimu siekiama gerinti vėžio biologijos žinias, kurias tikimasi ateityje pritaikyti klinikinėje praktikoje. Suprantu, kad iš paimtos medžiagos bus atliekami specialūs tyrimai, apie kuriuos man buvo paaiškinta. Taip pat buvau supažindintas (-a) su visais galimais šalutiniais reiškiniais ar komplikacijomis.

Suprantu, kad mano dalyvavimas tyrime ir mano individualūs tyrimo duomenys liks paslaptyje. Pasinaudoti šiais duomenimis galės tik tyrėjas, arba (kai reikės) tyrimą finansavęs rėmėjas.
Esu informuotas/-a, kad už dalyvavimą tyrime ir su tyrimu susijusios išlaidos nebus apmokamos.
Sąmoningai ir laisva valia sutinku dalyvauti tyrime, kuris man buvo išaiškintas.

Tiriamasis (arba atsakingas asmuo):

.....
(Vardas, pavardė) (Parašas) (Data)

Aš, tyrėjas, atsakingas už šį tyrimą, patvirtinu, kad paaiškinau anksčiau minėtam asmeniui būsimojo tyrimo esmę ir tikslą.

Tyrėjas:

.....
(Vardas, pavardė) (Parašas) (Data)

Jeigu Jums iškilo problemų dėl šio tyrimo, prašome pranešti:
Tyrėjui med. dr. Rasai Ugenskienei Tel.: 8 37 787317
arba
Kauno regioniniam biomediciniinių tyrimų etikos komitetui Tel.: 8 37 326889

Supplementary Table 1. *Univariate logistic regression analysis of the associations between TLR4 SNPs rs10759932, rs1927906, rs11536865, rs11536898, rs10983755, rs4986790, rs4986791, rs11536897, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate											
		Positive T3–T4 *vs.* T1–T2			Positive N1 *vs.* N0			Positive M1 *vs.* M0			Positive G3 *vs.* G1 + G2		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs10759932	TC *vs.* TT	1.276	0.639–2.551	0.490	1.272	0.647–2.499	0.486	2.158	0.554–8.413	0.268	1.110	0.520–2.370	0.787
	CC *vs.* TT	1.881	0.256–13.834	0.535	1.327	0.181–9.734	0.781	7.733	0.678–88.176	0.099	2.903	0.392–21.495	0.297
	T allele+ *vs.* T–	0.570	0.078–4.150	0.574	0.806	0.111–5.861	0.831	0.170	0.016–1.800	0.097	0.355	0.049–2.596	0.290
	C allele+ *vs.* C–	1.317	0.6673–2.577	0.421	1.276	0.662–2.460	0.467	2.522	0.697–9.122	0.147	1.210	0.584–2.504	0.608
rs1927906	TC *vs.* TT	1.033	0.489–2.183	0.932	0.668	0.318–1.403	0.567	1.543	0.379–6.278	0.545	1.919	0.887–4.154	0.098
	CC *vs.* TT	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	T allele+ *vs.* T–	2.758	2.261–3.364	0.187	2.250	1.903–2.660	0.265	1.062	1.023–1.103	0.803	3.800	2.957–4.883	0.097
	C allele+ *vs.* C–	1.107	0.530–2.310	0.787	0.716	0.345–1.484	0.368	1.500	0.369–6.097	0.325	2.056	0.962–4.398	0.060
rs11536898	CA *vs.* CC	1.405	0.671–2.944	0.368	1.543	0.746–3.191	0.242	4.735	1.204–18.626	0.026	1.576	0.722–3.439	0.253
	AA *vs.* CC	2.898	0.469–17.989	0.253	2.083	0.337–12.898	0.430	7.812	0.704–86.710	0.094	0.758	0.082–7.030	0.807
	C allele+ *vs.* C–	0.374	0.061–2.300	0.271	0.530	0.086–3.258	0.487	0.228	0.023–2.254	0.169	1.475	0.161–13.555	0.730
	A allele+ *vs.* A–	1.529	0.757–3.090	0.235	1.597	0.798–3.197	0.184	5.068	1.357–18.918	0.008	1.463	0.689–3.106	0.320

Supplementary Table 1. *Continued.*

SNP	Genotypes, alleles	Univariate											
		Positive T3–T4 *vs.* T1–T2			Positive N1 *vs.* N0			Positive M1 *vs.* M0			Positive G3 *vs.* G1 + G2		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs10983755	AG *vs.* GG	1.088	0.340–3.482	0.887	1.483	0.477–4.613	0.496	1.389	0.162–11.900	0.764	2.550	0.809–8.035	0.110
	AA *vs.* GG	*	*	*	*	*	*	*	*	*	*	*	*
	G allele+ *vs.* G–	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele+ *vs.* A–	1.088	0.340–3.482	0.887	1.483	0.477–4.613	0.494	1.389	0.162–11.900	0.763	2.550	0.809–8.035	0.110
rs4986790	AG *vs.* AA	1.152	0.467–2.841	0.758	0.501	0.195–1.289	0.152	1.667	0.331–8.388	0.536	1.600	0.628–4.077	0.325
	GG *vs.* AA	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele+ *vs.* A–	2.758	2.261–3.364	0.187	2.250	1.903–2.660	0.265	1.062	1.023–1.103	0.803	3.800	2.957–4.883	0.267
	G allele+ *vs.* G–	1.280	0.532–3.082	0.581	0.572	0.231–1.419	0.225	1.591	0.317–7.986	0.570	1.800	0.727–4.455	0.199
rs4986791	TC *vs.* CC	1.305	0.542–3.143	0.553	0.581	0.234–1.441	0.241	1.580	0.315–7.929	0.580	1.486	0.588–3.756	0.402
	TT *vs.* CC	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	C allele+ *vs.* C–	2.758	2.261–3.364	0.187	2.250	1.903–2.660	0.265	1.062	1.023–1.103	0.803	3.800	2.957–4.883	0.267
	T allele+ *vs.* T–	1.435	0.608–3.389	0.408	0.653	0.271–1.573	0.340	1.511	0.302–7.567	0.613	1.672	0.682–4.103	0.258
rs11536897	AG *vs.* GG	0.682	0.128–3.623	0.653	0.922	0.200–4.251	0.917	0.000	0.000	1.000	1.100	0.206–5.877	0.911
	AA *vs.* GG	*	*	*	*	*	*	*	*	*	*	*	*
	G allele+ *vs.* G–	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele+ *vs.* A–	0.682	0.128–3.623	0.651	0.922	0.200–4.251	0.917	0.939	0.904–0.977	0.502	1.100	0.206–5.877	0.911

T3–T4, T1–T2 – tumor size, N1 – pathological regional lymph nodes, N0 – no pathological regional lymph nodes, M1 – distant metastases, M0 – no distant metastases, G1 + G2, G3 – tumor grade. *OR could not be estimated because of zero value within a cell.

Supplementary Table 2. *Univariate logistic regression analysis of the associations between TLR4 gene SNPs rs10759932, rs1927906, rs11536865, rs11536898, rs10983755, rs4986790, rs4986791, rs11536897, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate								
		Positive stage III–IV *vs.* stage I–II			Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2			Age (years): ≤ 50 *vs.* > 50		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs10759932	TC *vs.* TT	1.088	0.551–2.148	0.808	1.143	0.396–3.298	0.805	1.575	0.793–3.134	0.195
	CC *vs.* TT	0.806	0.110–5.911	0.832	4.000	0.237–67.473	0.336	5.854	0.590–58.052	0.131
	T allele+ *vs.* T–	1.270	0.175–9.232	0.813	0.259	0.016–4.304	0.346	0.195	0.020–1.915	0.120
	C allele+ *vs.* C–	1.062	0.549–2.055	0.857	1.273	0.463–3.500	0.640	1.734	0.891–3.377	0.104
rs1927906	TC *vs.* TT	0.655	0.317–1.350	0.251	2.159	0.785–5.940	0.136	0.896	0.393–1.178	0.642
	CC *vs.* TT	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	T allele+ *vs.* T–	1.800	1.574–2.058	0.372	0.000	0.000	1.000	2.672	2.201–3.243	0.378
	C allele+ *vs.* C–	0.691	0.338–1.414	0.310	2.429	0.907–6.506	0.078	0.900	0.429–1.891	0.852
rs11536898	CA *vs.* CC	1.123	0.541–2.334	0.755	1.725	0.579–5.140	0.328	1.227	0.588–2.563	0.586
	AA *vs.* CC	1.225	0.198–7.582	0.827	2.156	0.184–25.271	0.541	0.422	0.046–3.885	0.446
	C allele+ *vs.* C–	0.838	0.136–5.146	0.848	0.524	0.045–6.045	0.604	2.485	0.272–22.734	0.651
	A allele+ *vs.* A–	1.135	0.564	2.281	1.776	0.631–4.997	0.277	1.103	0.544–2.240	0.856
rs10983755	AG *vs.* GG	1.291	0.405–4.119	0.666	2.430	0.535–11.030	0.250	1.453	0.466–4.528	0.520
	AA *vs.* GG	*	*	*	*	*	*	*	*	*
	G allele+ *vs.* G–	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele+ *vs.* A–	1.291	0.405–4.119	0.666	2.430	0.535–11.030	0.250	1.453	0.366–4.528	0.520
rs4986790	AG *vs.* AA	0.570	0.235–1.383	0.214	2.005	0.616–6.533	0.248	0.876	0.349–2.199	0.778
	GG *vs.* AA	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele+ *vs.* A–	1.800	1.574–2.058	0.372	0.000	0.000	1.000	0.000	0.000	1.000
	G allele+ *vs.* G–	0.627	0.264–1.492	0.289	2.406	0.781–7.416	0.126	0.986	0.405–2.402	0.975

Supplementary Table 2. *Continued.*

SNP	Genotypes, alleles	Univariate								
		Positive stage III–IV *vs.* stage I–II			**Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2**			**Age (years): ≤ 50 *vs.* > 50**		
		OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
rs4986791	TC *vs.* CC	0.635	0.267–1.510	0.304	2.005	0.616–6.533	0.248	0.812	0.327–2.022	0.655
	TT *vs.* CC	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	C allele+ *vs.* C–	1.800	1.574–2.058	0.372	0.000	0.000	1.000	0.000	0.000	1.000
	T allele+ *vs.* T–	0.692	0.296–1.620	0.395	2.406	0.781–7.416	0.126	0.914	0.378–2.208	0.842
rs11536897	AG *vs.* GG	0.581	0.126–2.677	0.486	0.000	0.000	1.000	0.000	0.000	1.000
	AA *vs.* GG	*	*	*	*	*	*	*	*	*
	G allele+ *vs.* G–	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	A allele+ *vs.* A–	0.581	0.126–2.677	0.481	*	*	*	*	*	*

*OR could not be estimated because of zero value within a cell.

Supplementary Table 3. *Univariate logistic regression analysis of the associations between RRP1B SNPs rs2838342, rs7276633, rs2051407, rs9306160, rs762400, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate											
		Positive T3–T4 *vs.* T1–T2			Positive N1 *vs.* N0			Positive M1 *vs.* M0			Positive G3 *vs.* G1 + G2		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs2838342	AG *vs.* AA	0.406	0.197–0.836	0.054	0.592	0.302–1.163	0.128	0.671	0.131–3.445	0.632	0.882	0.407–1.910	0.750
	GG *vs.* AA	2.160	0.867–5.380	0.098	0.871	0.357–2.126	0.762	2.933	0.610–14.097	0.179	1.752	0.676–4.543	0.249
	A allele+ *vs.* A–	0.281	0.122–0.643	0.002	0.844	0.379–1.877	0.677	0.274	0.072–1.040	0.044	0.530	0.229–1.230	0.136
	G allele+ *vs.* G–	0.660	0.659–1.106	0.209	0.655	0.347–1.237	0.191	1.199	0.298–4.820	0.798	1.071	0.522–2.195	0.852
rs7276633	TC *vs.* TT	0.371	0.180–0.766	0.067	0.556	0.283–1.091	0.088	0.691	0.135–3.550	0.658	0.917	0.424–1.983	0.825
	CC *vs.* TT	2.077	0.837–5.156	0.115	0.843	0.346	0.707	2.987	0.622–14.348	0.172	1.793	0.692	0.229
	T allele + *vs.* T–	0.281	0.122–0.643	0.003	0.844	0.379–1.877	0.677	0.274	0.072–1.040	0.057	3.800	0.229–1.230	0.140
	C allele + *vs.* C -	0.618	0.324–1.180	0.145	0.620	0.329–1.169	0.140	1.233	0.307–4.953	0.768	1.109	0.541–2.272	0.777
rs2051407	CT *vs.* CC	0.608	0.304–1.215	0.159	0.627	0.324–1.213	0.165	0.360	0.064–2.029	0.247	0.979	0.462–2.077	0.956
	TT *vs.* CC	1.935	0.758–4.939	0.168	0.894	0.354–2.260	0.813	2.810	0.644–12.252	0.169	1.652	0.611–4.466	0.322
	C allele+ *vs.* C–	0.393	0.166–0.929	0.033	0.859	0.367–2.008	0.725	0.223	0.058–0.858	0.019	0.598	0.244–1.467	0.258
	T allele+ *vs.* T–	0.814	0.429–1.543	0.527	0.681	0.365–1.271	0.227	0.859	0.233–3.169	0.820	1.116	0.551–2.260	0.762
rs9306160	CT *vs.* CC	0.578	0.287–1.163	0.124	0.603	0.307–1.183	0.141	0.893	0.145–5.520	0.903	0.860	0.408–1.812	0.692
	TT *vs.* CC	1.670	0.617–4.519	0.313	0.747	0.277–2.015	0.565	5.889	0.993–34.906	0.051	0.914	0.303–2.757	0.832
	C allele+ *vs.* C–	0.432	0.180–1.035	0.055	0.790	0.333–1.876	0.593	0.179	0.044–0.721	0.008	1.498	0.525–4.278	0.448
	T allele+ *vs.* T–	0.708	0.363–1.384	0.312	0.667	0.346–1.286	0.226	1.591	0.319–7.932	0.568	0.678	0.332–1.388	0.287
rs762400	GC *vs.* GG	0.578	0.287–1.163	0.124	1.066	0.397–2.864	0.900	0.213	0.043–1.043	0.056	0.851	0.282–2.566	0.775
	CC *vs.* GG	1.670	0.617–4.519	0.313	0.762?	0.293–1.981	0.577	0.150	0.031–0.732	0.059	0.938	0.326–2.697	0.905
	G allele+ *vs.* G–	0.383	0.151–0.967	0.037	0.877	0.352–2.190	0.779	0.176	0.045–0.686	0.006	0.901	0.327–2.482	0.840
	C allele+ *vs.* C–	0.905	0.477–1.720	0.761	0.754	0.404–1.406	0.373	1.373	0.342–5.508	0.654	1.116	0.551–2.260	0.762

T3–T4, T1–T2 – tumor size, N1 – pathological regional lymph nodes, N0 – no pathological regional lymph nodes, M1 – distant metastases, M0 – no distant metastases, G1 + G2, G3 – tumor grade.

Supplementary Table 4. *Univariate logistic regression analysis of the associations between RRP1B SNPs rs2838342, rs7276633, rs2051407, rs9306160, rs762400, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate								
		Positive stage III–IV *vs.* stage I–II			Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2			Age (years): ≤ 50 *vs.* > 50		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs2838342	AG *vs.* AA	0.494	0.250–0.977	0.053	0.300	0.091–0.985	0.057	0.471	0.226–0.983	0.045
	GG *vs.* AA	1.921	0.705–5.233	0.202	3.000	0.910–9.889	0.071	0.623	0.236–1.647	0.341
	A allele+ *vs.* A–	0.341	0.137–0.849	0.017	0.182	0.061–0.538	0.002	1.055	0.433–2.573	0.906
	G allele+ *vs.* G–	0.679	0.356–1.295	0.239	0.712	0.274–1.847	0.485	0.508	0.256–1.006	0.052
rs7276633	TC *vs.* TT	0.468	0.237–0.925	0.059	0.300	0.091–0.985	0.057	0.427	0.204–0.893	0.064
	CC *vs.* TT	1.869	0.687–5.084	0.221	3.000	0.910–9.889	0.071	0.596	0.227–1.570	0.295
	T allele+ *vs.* T–	0.341	0.137–0.849	0.021	0.182	0.061–0.538	0.002	1.055	0.433–2.573	0.906
	C allele+ *vs.* C–	0.650	0.341–1.237	0.189	0.712	0.274–1.847	0.485	2.138	1.080–4.230	0.029
rs2051407	CT *vs.* CC	0.598	0.309–1.159	0.128	0.524	0.177–1.555	0.244	0.543	0.265–1.114	0.096
	TT *vs.* CC	1.692	0.617–4.638	0.307	2.679	0.779–9.216	0.118	0.549	0.192–1.572	0.264
	C allele+ *vs.* C–	0.440	0.173–1.116	0.078	0.267	0.087–0.823	0.021	1.309	0.489–3.503	0.592
	T allele+ *vs.* T–	0.748	0.399–1.404	0.366	0.877	0.340–2.268	0.785	0.545	0.277–1.071	0.078
rs9306160	CT *vs.* CC	0.547	0.276–1.085	0.084	0.500	0.177–1.416	0.192	0.583	0.283–1.204	0.145
	TT *vs.* CC	1.224	0.428–3.506	0.706	1.543	0.407–5.850	0523	0.656	0.221–1.947	0.448
	C allele+ *vs.* C–	0.479	0.187–1.226	0.119	0.455	0.167–1.759	0.308	0.788	0.313–1.983	0.614
	T allele+ *vs.* T–	0.649	0.332–1.270	0.205	0.552	0.210–1.454	0.229	0.681	0.337–1.376	0.284
rs762400	GC *vs.* GG	0.569	0.195–1.662	0.303	0.450	0.118–1.718	0.243	0.659	0.326–1.332	0.246
	CC *vs.* GG	0.400	0.142–1.126	0.083	0.313	0.118–1.179	0.086	0.439	0.131–1.465	0.180
	G allele+ *vs.* G–	0.463	0.170–1.257	0.124	0.369	0.108–1.262	0.410	1.804	0.575–5.662	0.312
	C allele+ *vs.* C–	0.829	0.443–1.553	0.558	0.922	0.358–2.371	0.886	0.614	0.312–1.207	0.157

Supplementary Table 5. *Univariate logistic regression analysis of the associations between SIPA1 SNPs rs931127, rs3741378, rs746429, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate											
		Positive T3-T4 *vs.* T1-2			Positive N1 *vs.* N0			Positive M1 *vs.* M0			Positive G3 *vs.* G1+G2		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs931127	AG *vs.* AA	1.370	0.697–2.694	0.361	1.432	0.746–2.748	0.281	2.791	0.560–13.907	0.210	1.093	0.528–2.260	0.810
	GG *vs.* AA	1.289	0.464–3.585	0.626	1.125	0.415–3.048	0.817	1.575	0.136–18.299	0.717	0.882	0.280–2.777	0.831
	A allele+ *vs.* A–	0.931	0.363–2.386	0.882	1.092	0.435–2.746	0.851	1.268	0.152–10.543	0.826	1.193	0.411–3.464	0.746
	G allele+ *vs.* G–	1.354	0.708–2.590	0.359	1.366	0.731–2.551	0.327	2.545	0.524–12.375	0.232	1.050	0.522–2.111	0.892
rs3741378	CT *vs.* CC	0.827	0.359–1.903	0.655	0.794	0.355–1.773	0.573	0.494	0.060–4.056	0.512	1.030	0.421–2.519	0.948
	TT *vs.* CC	0.551	0.056–5.440	0.610	1.190	0.163–8.695	0.864	0.000	0.000	0.999	2.833	0.385–20.862	0.307
	C allele+ *vs.* C–	1.755	0.179–17.237	0.625	0.806	0.111–5.861	0.831	1.063	1.024–1.105	0.615	0.355	0.049–2.596	0.290
	T allele+ *vs.* T–	0.837	0.375–1.864	0.662	0.762	0.351–1.651	0.490	0.451	0.055–3.693	0.447	1.244	0.540–2.864	0.607
rs746429	GA *vs.* GG	0.842	0.403–1.760	0.648	1.223	0.593–2.520	0.586	1.012	0.241–4.249	0.987	0.329	0.147–0.736	0.007
	AA *vs.* GG	0.780	0.318–1.913	0.587	0.995	0.415–2.386	0.991	0.378	0.038–3.796	0.409	0.692	0.279–1.716	0.427
	G allele+ *vs.* G–	1.145	0.537–2.438	0.726	1.149	0.555–2.380	0.708	2.664	0.327–21.718	0.342	0.737	0.335–1.618	0.446
	A allele+ *vs.* A–	0.823	0.409–1.655	0.585	1.150	0.579–2.286	0.689	0.817	0.202–3.303	0.776	0.424	0.205–0.880	0.019

T3–T4, T1–T2 – tumor size, N1 – pathological regional lymph nodes, N0 – no pathological regional lymph nodes, M1 – distant metastases, M0 – no distant metastases, G1+G2, G3 – tumor grade.

Supplementary Table 6. *Univariate logistic regression analysis of the associations between SIPA1 SNPs rs931127, rs3741378, rs746429, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate								
		Positive stage III–IV *vs.* stage I–II			Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2			Age (years): ≤ 50 *vs.* > 50		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs931127	AG *vs.* AA	1.190	0.622–2.278	0.599	1.128	0.419–3.038	0.811	1.327	0.641–2.749	0.446
	GG *vs.* AA	0.943	0.352–2.526	0.907	1.091	0.251–4.748	0.908	1.531	0.526–4.457	0.434
	A allele+ *vs.* A–	1.171	0.469–2.922	0.735	0.978	0.249–3.842	0.974	0.771	0.291–2.042	0.600
	G allele+ *vs.* G–	1.137	0.612–2.113	0.685	1.120	0.437–2.869	0.812	1.366	0.680–2.744	0.381
rs3741378	CT *vs.* CC	0.747	0.339–1.647	0.470	1.375	0.470–4.022	0.561	1.050	0.443–2.489	0.912
	TT *vs.* CC	0.747	0.102–5.457	0.774	2.062	0.176–24.185	0.564	0.000	0.000	0.999
	C allele+ *vs.* C–	1.270	0.175–9.232	0.813	0.524	0.045–6.045	0.604	0.000	0.000	0.999
	T allele+ *vs.* T–	0.694	0.324–1.486	0.346	1.543	0.554–4.295	0.407	0.765	0.319–1.836	0.549
rs746429	GA *vs.* GG	1.174	0.571–2.414	0.662	0.255	0.088–0.739	0.012	0.435	0.200–0.945	0.036
	AA *vs.* GG	1.081	0.454–2.573	0.860	0.412	0.112–1.511	0.181	0.611	0.243–1.534	0.294
	G allele+ *vs.* G–	1.029	0.499–2.124	0.938	1.170	0.352–3.892	0.798	0.972	0.439–2.154	0.943
	A allele+ *vs.* A–	1.145	0.579–2.267	0.697	0.296	0.114–0.769	0.012	0.484	0.236–0.996	0.049

Supplementary Table 7. *Univariate logistic regression analysis of the associations between SRSF1 SNPs rs8819, rs34592492, rs11654058, rs2233908, rs2585828, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate											
		Positive T3-T4 *vs.* T1-2			Positive N1 *vs.* N0			Positive M1 *vs.* M0			Positive G3 *vs.* G1+G2		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs8819	CT *vs.* CC	0.641	0.305–1.348	0.241	1.541	0.767–3.093	0.224	1.254	0.309–5.080	0.752	1.246	0.580–2.678	0.573
	TT *vs.* CC	0.000	0.000	0.999	0.335	0.036–3.084	0.334	0.000	0.000	0.999	0.719	0.077–6.670	0.771
	C allele+ *vs.* C–	1.606	1.427–1.807	0.084	3.341	0.366–30.525	0.258	1.064	1.024–1.105	0.573	1.475	0.161–13.555	0.730
	T allele+ *vs.* T–	0.550	0.653–1.025	0.106	1.340	0.687–2.614	0.391	1.114	0.276–4.498	0.679	1.184	0.564–2.484	0.655
rs34592492	GC *vs.* GG	0.557	0.172–1.810	0.653	1.279	0.457–3.584	0.639	1.225	0.143–10.471	0.853	0.927	0.283–3.036	0.900
	CC *vs.* GG	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	G allele+ *vs.* G–	2.758	2.261–3.364	0.187	2.250	1.903	0.265	19.000	10.060–35.886	0.000	3.800	2.957–4.883	0.267
	C allele+ *vs.* C–	0.687	0.234–2.078	0.515	1.439	0.527–3.928	0.475	2.450	0.476–12.605	0.269	1.159	0.385–3.490	0.794
rs11654058	TC *vs.* TT	0.000	0.000	0.999	1.749	0.771–3.967	0.181	0.543	0.066	4.467	0.904	0.355–2.296	0.831
	CC *vs.* TT	0.000	0.000	0.999	0.000	0.000	0.999	0.000	0.000	0.999	1.355	0.119–15.381	0.806
	T allele+ *vs.* T–	1.594	1.419–1.791	0.184	1.837	1.600–2.109	0.116	1.063	1.023–1.104	0.664	0.726	0.064–8.200	0.795
	C allele+ *vs.* C–	0.443	0.179–1.097	0.073	1.399	0.642–3.049	0.397	0.489	0.060–4.007	0.496	0.943	0.389–2.287	0.896
rs2233908	GA *vs.* GG	0.641	0.305–1.348	0.653	1.541	0.767–3.084	0.224	1.254	0.309–5.080	0.752	1.246	0.580–2.678	0.573
	AA *vs.* GG	0.000	0.000	0.999	0.335	0.036–3.084	0.334	0.000	0.000	0.999	0.719	0.077–6.670	0.771
	G allele+ *vs.* G–	1.606	1.427–1.807	0.084	3.341	0.366–30.525	0.258	1.064	1.024–1.105	0.573	1.475	0.161–13.555	0.730
	A allele+ *vs.* A–	0.550	0.265–1.142	0.106	1.340	0.687–2.614	0.391	1.114	0.276–4.498	0.879	1.184	0.564–2.484	0.655
rs2585828	AG *vs.* AA	0.641	0.305–1.348	0.241	0.335	0.767–3.093	0.224	1.254	0.309–5.080	0.752	1.246	0.580–2.678	0.573
	GG *vs.* AA	0.000	0.000	0.999	0.335	0.036–3.084	0.334	0.000	0.000	0.999	0.719	0.077–6.670	0.771
	A allele+ *vs.* A–	1.606	1.427–1.807	0.084	3.341	0.366–30.525	0.258	1.064	1.024–1.105	0.573	1.475	0.161–13.555	0.731
	G allele+ *vs.* G–	0.550	0.265–1.142	0.106	1.340	0.687–2.614	0.391	1.114	0.276–4.498	0.879	1.184	0.564–2.484	0.655

T3–T4, T1–2 – tumor size, N1 – pathological regional lymph nodes, N0 – no pathological regional lymph nodes, M1 – distant metastases, M0 – no distant metastases, G1 + G2, G3 – tumor grade.

Supplementary Table 8. *Univariate logistic regression analysis of the associations between SRSF1 SNPs rs8819, rs34592492, rs11654058, rs2233908, rs2585828, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate								
		Positive stage III–IV *vs.* stage I–II			Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2			Age (years): ≤ 50 *vs.* > 50		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs8819	CT *vs.* CC	1.071	0.531–2.162	0.847	0.905	0.317–2.582	0.853	1.418	0.676–2.975	0.355
	TT *vs.* CC	0.193	0.021–1.775	0.146	0.000	0.000	0.999	0.000	0.000	0.999
	C allele+ *vs.* C–	5.278	0.577–48.236	0.102	0.000	0.000	0.999	0.000	0.000	0.999
	T allele+ *vs.* T–	0.912	0.467–1.781	0.787	0.771	0.274–2.174	0.623	1.203	0.582–2.489	0.618
rs34592492	GC *vs.* GG	0.782	0.279–2.189	0.639	0.407	0.049–3.400	0.407	0.841	0.257–2.748	0.774
	CC *vs.* GG	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	G allele+ *vs.* G–	1.800	1.574–2.058	0.372	0.000	0.000	1.000	0.000	0.000	1.000
	C allele+ *vs.* C–	0.879	0.322–2.399	0.802	0.815	0.163–4.062	0.803	1.051	0.350–3.158	0.929
rs11654058	TC *vs.* TT	1.213	0.530–2.776	0.648	0.772	0.233–2.557	0.672	1.453	0.617–3.422	0.393
	CC *vs.* TT	0.000	0.000	0.999	0.000	0.000	0.999	0.000	0.000	0.999
	T allele+ *vs.* T–	2.315	1.947–2.752	0.052	0.000	0.000	0.999	0.000	0.000	0.999
	C allele+ *vs.* C–	0.953	0.436–2.082	0.904	0.695	0.212–2.281	0.548	1.245	0.538–2.881	0.608
rs2233908	GA *vs.* GG	1.071	0.531–2.162	0.847	0.905	0.317–2.582	0.853	1.418	0.676–2.975	0.355
	AA *vs.* GG	0.193	0.021–1.775	0.146	0.000	0.000	0.999	0.000	0.000	0.999
	G allele+ *vs.* G–	5.278	0.577–48.236	0.102	0.000	0.000	0.999	0.000	0.000	0.999
	A allele+ *vs.* A–	0.912	0.467–1.781	0.787	0.771	0.274–2.174	0.623	1.203	0.582–2.489	0.618
rs2585828	AG *vs.* AA	1.071	0.531–2.162	0.847	0.905	0.317–2.582	0.853	1.418	0.676–2.975	0.355
	GG *vs.* AA	0.193	0.021–1.775	0.146	0.000	0.000	0.999	0.000	0.000	0.999
	A allele+ *vs.* A–	5.278	0.577–48.236	0.102	0.000	0.000	0.999	0.000	0.000	0.999
	G allele+ *vs.* G–	0.912	0.467–1.781	0.787	0.771	0.274–2.174	0.623	1.203	0.582–2.489	0.618

Supplementary Table 9. *Univariate logistic regression analysis of the associations between HOTAIR SNPs rs12826786, rs7958904, rs920778, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate											
		Positive T3–T4 *vs.* T1–T2			Positive N1 *vs.* N0			Positive M1 *vs.* M0			Positive G3 *vs.* G1+G2		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs12826786	CT *vs.* CC	0.792	0.410–1.531	0.488	0.982	0.517–1.867	0.957	0.459	0.111–1.909	0.285	1.012	0.494–2.075	0.973
	TT *vs.* CC	0.587	0.204–1.684	0.322	1.129	0.428–2.983	0.806	0.567	0.064–4.987	0.609	0.844	0.273–2.606	0.768
	C allele+ *vs.* C–	1.516	0.556–4.130	0.416	0.877	0.352–2.190	0.779	1.268	0.152–10.543	0.826	1.193	0.411–3.464	0.746
	T allele+ *vs.* T–	0.745	0.398–1.391	0.355	1.012	0.552–1.858	0.968	0.482	0.131–1.775	0.273	0.975	0.493–1.927	0.942
rs7958904	CG *vs.* GG	0.950	0.487–1.854	0.881	1.208	0.627–2.327	0.572	0.543	0.140–2.109	0.378	1.759	0.824–3.755	0.144
	CC *vs.* GG	0.594	0.204–1.728	0.339	1.154	0.433–3.072	0.775	0.543	0.060–4.922	0.587	1.109	0.344–3.571	0.863
	G allele+ *vs.* G–	1.634	0.605–4.418	0.333	0.969	0.394–2.380	0.945	1.340	0.162–11.125	0.786	1.279	0.443–3.691	0.649
	C allele + *vs.* C–	0.870	0.457–1.655	0.671	1.197	0.638–2.244	0.575	0.543	0.151–1.954	0.350	1.615	0.775–3.369	0.201
rs920778	AG *vs.* GG	1.600	0.570–4.494	0.372	1.047	0.410–2.674	0.924	1.000	0.106–9.420	1.000	1.587	0.532–4.735	0.408
	AA *vs.* GG	1.684	0.579–4.902	0.339	0.867	0.326–2.307	0.775	1.842	0.203–16.701	0.587	0.902	0.208–2.905	0.863
	G allele + *vs.* G–	0.870	0.457–1.655	0.671	1.197	0.638–2.244	0.575	0.543	0.151–1.954	0.350	1.615	0.775–3.369	0.201
	A allele + *vs.* A–	1.634	0.605–4.418	0.333	0.969	0.394–2.380	0.945	1.340	0.162–11.125	0.786	1.279	0.443–3.691	0.649

T3–T4, T1–T2 – tumor size, N1 – pathological regional lymph nodes, N0 – no pathological regional lymph nodes, M1 – distant metastases, M0 – no distant metastases, G1+G2, G3 – tumor grade.

Supplementary Table 10. *Univariate logistic regression analysis of the associations between HOTAIR SNPs rs12826786, rs7958904, rs920778, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate								
		Positive stage III–IV *vs.* stage I–II			Positive worse prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2			Age (years): ≤ 50 *vs.* > 50		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs12826786	CT *vs.* CC	1.018	0.536–1.935	0.957	1.008	0.373–2.725	0.987	1.049	0.544–2.021	0.887
	TT *vs.* CC	1.073	0.403–2.855	0.887	0.846	0.197–3.628	0.822	0.821	0.296–2.282	0.706
	C allele+ *vs.* C–	0.941	0.374–2.364	0.896	1.187	0.308–4.577	0.803	1.247	0.475–3.273	0.653
	T allele+ *vs.* T–	1.029	0.561–1.890	0.925	0.969	0.377–2.488	0.947	0.996	0.535–1.857	0.991
rs7958904	CG *vs.* GG	1.211	0.630–2.330	0.566	1.764	0.609–5.113	0.296	1.720	0.867–3.413	0.121
	CC *vs.* GG	1.055	0.397–2.799	0.915	1.107	0.241–5.076	0.896	1.293	0.465–3.596	0.622
	G allele+ *vs.* G–	1.061	0.432–2.606	0.898	1.296	0.339–4.959	0.705	1.073	0.424–2.716	0.882
	C allele+ *vs.* C–	1.178	0.630–2.202	0.608	1.597	0.570–4.471	0.373	1.627	0.841–3.146	0.148
rs920778	AG *vs.* GG	1.149	0.449–2.940	0.773	1.593	0.398–6.377	0.510	1.330	0.506–3.493	0.563
	AA *vs.* GG	0.948	0.357–2.517	0.915	0.903	0.197–4.141	0.896	0.773	0.278–2.150	0.622
	G allele+ *vs.* G–	1.178	0.630–2.202	0.608	1.597	0.570–4.471	0.373	1.627	0.841–3.146	0.148
	A allele+ *vs.* A–	1.061	0.432–2.606	0.898	1.296	0.339–4.959	0.705	1.073	0.424–2.716	0.882

Supplementary Table 11. *Univariate logistic regression analysis of the associations between MALAT1 SNPs rs619586, rs664589, rs3200401, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate											
		Positive T3–T4 *vs.* T1–T2			Positive N1 *vs.* N0			Positive M1 *vs.* M0			Positive G3 *vs.* G1 + G2		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs619586	AG *vs.* AA	1.780	0.429–7.378	0.427	1.247	0.301–5.156	0.761	0.000	0.000	0.999	0.378	0.045–3.157	0.369
	GG *vs.* AA	*	*	*	*	*	*	*	*	*	*	*	*
	A allele+ *vs.* A–	*	*	*	*	*	*	*	*	*	*	*	*
	G allele+ *vs.* G–	1.780	0.429–7.378	0.427	1.247	0.301–5.156	0.761	0.000	0.000	0.999	0.378	0.045–3.157	0.369
rs664589	GC *vs.* CC	2.437	0.527–11.262	0.254	1.704	0.370–7.855	0.494	0.000	0.000	0.999	1.091	0.204–5.829	0.919
	GG *vs.* CC	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	C allele+ *vs.* C–	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	G allele+ *vs.* G–	3.046	0.703–13.204	0.137	2.130	0.493–9.209	0.312	0.000	0.000	0.999	0.909	0.177–4.673	0.909
rs3200401	CT *vs.* CC	0.733	0.340–1.581	0.428	1.676	0.811–3.467	0.163	2.471	0.660–9.254	0.179	1.560	0.717–3.394	0.262
	TT *vs.* CC	0.000	0.000	0.999	0.000	0.000	0.999	0.000	0.000	0.999	0.000	0.000	0.999
	C allele+ *vs.* C–	0.000	0.000	0.999	0.000	0.000	0.999	0.000	0.000	0.999	0.000	0.000	0.999
	T allele+ *vs.* T–	0.681	0.318–1.458	0.322	1.500	0.737–3.051	0.263	2.333	0.624–8.719	0.208	1.444	0.669–3.120	0.349

T3–T4, T1–T2 – tumor size, N1 – pathological regional lymph nodes, N0 – no pathological regional lymph nodes, M1 – distant metastases, M0 – no distant metastases, G1 + G2, G3 – tumor grade.

Supplementary Table 12. *Univariate logistic regression analysis of the associations between MALAT1 SNPs rs619586, rs664589, rs3200401, and tumor characteristics.*

SNP	Genotypes, alleles	Univariate								
		Positive stage III–IV *vs.* stage I–II			Positive Worse Prognosis: T3–T4 + G3 *vs.* T1–T2 + G1–G2			Age (years): ≤ 50 *vs.* > 50		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
rs619586	AG *vs.* AA	1.337	0.309–5.781	0.697	0.932	0.099–8.764	0.951	0.534	0.105–2.730	0.451
	GG *vs.* AA	*	*	*	*	*	*	*	*	*
	A allele+ *vs.* A–	*	*	*	*	*	*	*	*	*
	G allele+ *vs.* G–	1.337	0.309–5.781	0.697	0.932	0.099–8.764	0.951	0.534	0.105–2.730	0.451
rs664589	GC *vs.* CC	2.056	0.388–10.902	0.397	2.635	0.413–16.794	0.305	0.641	0.121–3.406	0.602
	GG *vs.* CC	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	C allele+ *vs.* C–	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
	G allele+ *vs.* G–	2.467	0.483–12.584	0.278	2.653	0.413–16.794	0.305	0.534	0.104–2.730	0.451
rs3200401	CT *vs.* CC	1.239	0.594–2.586	0.568	1.207	0.418–3.491	0.728	1.417	0.682–2.943	0.350
	TT *vs.* CC	0.000	0.000	0.999	0.000	0.000	0.999	0.000	0.000	0.999
	C allele+ *vs.* C–	0.000	0.000	0.999	0.000	0.000	0.999	0.000	0.000	0.999
	T allele+ *vs.* T–	1.093	0.535–2.234	0.806	1.092	0.381–3.130	0.869	1.294	0.629-2.658	0.484

*OR could not be estimated because of zero value within a cell.

Supplementary Table 13. *Cox's univariate muodel: TLR4 genotypes and alleles for PFS and OS.*

SNP	Genotype/allele	Progression-free survival			Overall survival		
		HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
rs10759932	TC *vs.* TT	0.884	0.472–1.653	0.699	0.818	0.382–1.752	0.606
	CC *vs.* TT	2.918	0.894–9.530	0.049	3.340	1.006–11.095	0.048
	T allele+ *vs.* T–	0.331	0.103–1.067	0.048	0.284	0.087–0.928	0.037
	C allele+ *vs.* C–	1.012	0.564–1.816	0.967	1.012	0.509–2.010	0.973
rs1927906	TC *vs.* TT	0.975	0.498–1.910	0.975	0.695	0.306–1.576	0.383
	CC *vs.* TT	2.584	0.352–18.949	0.350	3.081	0.417–22.761	0.383
	T allele+ *vs.* T–	0.385	0.053–2.807	0.346	0.301	0.041–2.216	0.239
	C allele+ *vs.* C–	1.028	0.537–1.971	0.933	0.770	0.354–1.673	0.509
rs11536898	CA *vs.* CC	1.103	0.586–2.073	0.762	1.294	0.636–2.633	0.476
	AA *vs.* CC	3.926	1.201–12.837	0.024	5.057	1.522–16.802	0.008
	C allele+ *vs.* C–	0.261	0.081–0.844	0.025	0.212	0.065–0.691	0.010
	A allele+ *vs.* A–	1.274	0.707–2.295	0.420	1.545	0.803–2.971	0.193
rs10983755	AG *vs.* GG	0.508	0.123–2.097	0.349	0.341	0.043–2.290	0.253
	AA *vs.* GG	*	*	*	*	*	*
	G allele+ *vs.* G–	0.000	0.000	1.000	0.000	0.000	1.000
	A allele+ *vs.* A–	0.508	0.123–2.097	0.349	0.341	0.043–2.290	0.253
rs4986790	AG *vs.* AA	1.482	0.716–3.069	0.290	1.062	0.444–2.542	0.892
	GG *vs.* AA	2.767	0.378–20.275	0.316	3.346	0.453–24.696	0.236
	A allele+ *vs.* A–	0.385	0.053–2.807	0.346	0.301	0.041–2.216	0.239
	G allele+ *vs.* G–	1.554	0.774–3.123	0.215	1.178	0.520–2.669	0.695
rs4986791	TC *vs.* CC	1.426	0.689–2.952	0.339	1.029	0.430–2.461	0.950
	TT *vs.* CC	2.752	0.376–20.165	0.319	3.331	0.451–24.582	0.238
	C allele+ *vs.* C–	1.499	0.746–3.010	0.256	0.301	0.041–2.216	0.239
	T allele+ *vs.* T–	0.385	0.053–2.807	0.346	1.142	0.504–2.587	0.750
rs11536897	AG *vs.* GG	0.425	0.058–3.084	0.397	1.314	0.316–5.454	0.707
	AA *vs.* GG	*	*	*	*	*	*
	G allele+ *vs.* G–	0.000	0.000	1.000	0.000	0.000	1.000
	A allele+ *vs.* A–	0.425	0.058–3.084	0.397	1.314	0.316–5.454	0.707

*OR could not be estimated because of zero value within a cell.

Supplementary Table 14. *Cox's univariate model for TLR4, SRSF1, and HOTAIR haplotypes in PFS and OS.*

Gene/ haplotype	Diplolotypes	Progression-free survival			Overall survival		
		HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
TLR4/ACT	ACT haplotype non-carriers *vs.* homozygous diplotype (ACT/ACT)	0.632	0.086–4.649	0.652	3.020	0.409–22.320	0.279
	Heterozygous diplotype (ACT/alternative hap) *vs.* homozygous diplotype (ACT/ACT)	1.183	0.600–2.333	0.627	0.705	0.311–1.599	0.403
SRSF1/ CTGA	CTGA haplotype non-carriers *vs.* homozygous diplotype (CTGA/CTGA)	1.836	0.245–13.733	0.554	0.000	0.000	0.972
	Heterozygous diplotype (CTGA/alternative hap) *vs.* homozygous diplotype (CTGA/CTGA)	0.686	0.364–1.295	0.245	0.744	0.354–1.566	0.436
HOTAIR/ CGG	CGG haplotype non-carriers *vs.* (Heterozygous diplotype (CGG/alternative hap)	1.171	0.665–2.062	0.585	1.231	0.661–2.290	0.512
HOTAIR/ CGA	CGA haplotype non-carriers *vs.* homozygous diplotype (CGA/CGA)	0.691	0.386-1.240	0.215	0.593	0.317–1.110	0.102
	Heterozygous diplotype (CGA/alternative hap) *vs.* homozygous diplotype (CGA/CGA)	*	*	*	0.000	0.000	0.976
HOTAIR/ TCA	TCA haplotype non-carriers *vs.* (Heterozygous diplotype (TCA /alternative hap)	1.077	0.614–1.888	0.795	1.119	0.594–2.107	0.728

*OR could not be estimated because of zero value within a cell.

Supplementary Table 15. *Cox's univariate model for RRP1B rs2838342, rs7276633, rs2051407, rs9306160, and rs762400 in PFS and OS.*

SNP	Genotype/ allele	Progression-free survival			Overall survival		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value
rs2838342	AG *vs.* AA	0.560	0.303–1.036	0.065	0.742	0.358–1.539	0.423
	GG *vs.* AA	1.155	0.545–2.447	0.706	1.824	0.827–4.025	0.137
	A allele+ *vs.* A–	1.572	0.784–3.151	0.202	0.465	0.232–0.931	0.031
	G allele+ *vs.* G–	0.682	0.391–1.187	0.176	0.990	0.516–1.899	0.977
rs7276633	TC *vs.* TT	0.577	0.312–1.066	0.079	0.763	0.368–1.582	0.467
	CC *vs.* TT	1.176	0.555–2.490	0.673	1.854	0.840–4.090	0.126
	T allele+ *vs.* T–	0.636	0.317–1.275	0.202	0.465	0.232–0.931	0.031
	C allele+ *vs.* C–	0.700	0.402–1.219	0.208	1.015	0.529–1.946	0.964
rs2051407	CT *vs.* CC	0.556	0.302–1.026	0.060	0.813	0.397–1.667	0.573
	TT *vs.* CC	1.314	0.604–2.842	0.488	2.144	0.959–4.793	0.063
	C allele+ *vs.* C–	0.568	0.275–1.175	0.127	0.418	0.204–0.858	0.017
	T allele+ *vs.* T–	0.689	0.397–1.194	0.184	1.081	0.568–2.056	0.812
rs9306160	CT *vs.* CC	0.647	0.352–1.187	0.160	0.856	0.418–1.751	0.669
	TT *vs.* CC	1.357	0.595–3.092	0.468	2.213	0.943–5.193	0.068
	C allele+ *vs.* C–	0.613	0.286–1.314	0.208	0.498	0.229–1.084	0.079
	T allele+ *vs.* T–	0.705	0.399–1.245	0.228	0.915	0.476–1.758	0.789
rs762400	GC *vs.* GG	0.613	0.336–1.117	0.110	0.917	0.451–1.866	0.811
	CC *vs.* GG	1.443	0.642–3.240	0.375	2.550	1.098–5.923	0.030
	G allele+ *vs.* G–	0.537	0.251–1.147	0.108	0.374	0.177–0.788	0.010
	C allele+ *vs.* C–	0.734	0.422–1.275	0.272	1.178	0.613–2.263	0.624

Supplementary Table 16. *Cox's univariate model for SIPA1 rs746429, rs931127, and rs3741378 in PFS and OS.*

SNP	Genotype/ allele	Progression-free survival			Overall survival		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value
rs746429	GA *vs.* GG	1.103	0.568–2.144	0.771	1.460	0.678–3.147	0.334
	AA *vs.* GG	0.876	0.374–2.056	0.762	1.076	0.399–2.897	0.886
	G allele+ *vs.* G–	1.219	0.591–2.512	0.592	1.208	0.533–2.735	0.651
	A allele+ *vs.* A–	1.037	0.548–1.961	0.912	1.352	0.642–2.846	0.427
rs931127	AG *vs.* AA	1.068	0.574–1.988	0.835	0.975	0.497–1.911	0.940
	GG *vs.* AA	1.313	0.539–3.197	0.548	0.823	0.271–2.505	0.732
	A allele+ *vs.* A–	0.793	0.355–1.768	0.570	1.196	0.425–3.365	0.735
	G allele+ *vs.* G–	1.112	0.612–2.018	0.728	0.947	0.494–1.819	0.871
rs3741378	CT *vs.* CC	0.735	0.329–1.640	0.452	0.955	0.422–2.160	0.912
	TT *vs.* CC	0.907	0.125–6.603	0.923	0.000	0.000	0.976
	C allele+ *vs.* C–	1.049	0.145–7.610	0.963	21.233	0.003–152176.036	0.500
	T allele+ *vs.* T–	0.801	0.375–1.711	0.567	0.895	0.396–2.024	0.790

Supplementary Table 17. *Cox's univariate model for SRSF1 rs8819, rs34592492, rs11654058, rs2233908, and rs2585828 in PFS and OS.*

SNP	Genotype/ allele	Progression-free survival			Overall survival		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value
rs8819	CT *vs.* CC	1.070	0.577–1.984	0.831	0.774	0.368–1.629	0.500
	TT *vs.* CC	0.660	0.090–4.827	0.683	0.000	0.000	0.973
	C allele+ *vs.* C–	1.542	0.212–11.189	0.669	21.204	0.010–46672.572	0.437
	T allele+ *vs.* T–	1.027	0.562–1.876	0.931	0.687	0.326–1.446	0.323
rs34592492	GC *vs.* GG	0.576	0.243–1.364	0.210	0.772	0.237–2.515	0.667
	CC *vs.* GG	2.869	0.378–21.759	0.308	19.947	2.489–159.836	0.005
	G allele+ *vs.* G–	0.328	0.043–2.483	0.022	0.043	0.005–0.348	0.003
	C allele+ *vs.* C–	1.649	0.760–3.578	0.206	0.977	0.346–2.755	0.965
rs11654058	TC *vs.* TT	1.001	0.469–2.137	0.998	0.687	0.269–1.757	0.434
	CC *vs.* TT	0.000	0.000	0.974	0.000	0.000	0.977
	T allele+ *vs.* T–	20.978	0.006–72840.189	0.464	20.977	0.003–161440.498	0.505
	C allele+ *vs.* C–	0.869	0.407–1.855	0.716	0.600	0.235–1.533	0.286
rs2233908	GA *vs.* GG	1.070	0.577–1.984	0.831	0.774	0.368–1.629	0.500
	AA *vs.* GG	0.660	0.090–4.827	0.683	0.000	0.000	0.973
	G allele+ *vs.* G–	1.542	0.212–11.189	0.669	21.204	0.020–46672.572	0.437
	A allele+ *vs.* A–	1.027	0.562–1.876	0.931	0.687	0.326–1.446	0.323
rs2585828	AG *vs.* AA	1.070	0.577–1.984	0.831	0.774	0.368–1.629	0.500
	GG *vs.* AA	0.660	0.090–4.827	0.683	0.000	0.000	0.973
	A allele+ *vs.* A–	1.542	0.212–11.189	0.669	21.204	0.010–46672.572	0.437
	G allele+ *vs.* G–	1.027	0.562–1.876	0.931	0.687	0.326–1.446	0.323

Supplementary Table 18. *Cox's univariate model for HOTAIR rs12826786, rs7958904, and rs920778 in PFS and OS.*

SNP	Genotype/ allele	Progression-free survival			Overall survival		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value
rs12826786	CT *vs.* CC	0.848	0.480–1.498	0.570	0.708	0.369–1.358	0.299
	TT *vs.* CC	0.410	0.093–1.797	0.237	0.477	0.142–1.605	0.232
	C allele+ *vs.* C–	2.260	0.525–9.726	0.274	1.777	0.546–5.779	0.339
	T allele+ *vs.* T–	0.785	0.451–1.367	0.393	0.658	0.354–1.226	0.188
rs7958904	CG *vs.* GG	0.726	0.403–1.307	0.286	0.668	0.350–1.275	0.221
	CC *vs.* GG	0.350	0.076–1.606	0.177	0.405	0.119–1.383	0.149
	G allele+ *vs.* G–	2.260	0.525–9.726	0.274	1.958	0.602–6.372	0.264
	C allele+ *vs.* C–	0.691	0.386–1.240	0.215	0.615	0.329–1.150	0.128
rs920778	AG *vs.* GG	2.071	0.474–9.060	0.333	1.648	0.487–5.581	0.422
	AA *vs.* GG	2.854	0.623–13.077	0.177	2.468	0.723–8.423	0.149
	G allele+ *vs.* G–	0.691	0.386–1.240	0.215	0.615	0.329–1.150	0.128
	A allele+ *vs.* A–	2.260	0.525–9.726	0.274	1.958	0.602–6.372	0.264

Supplementary Table 19. *Cox's univariate model for MALAT1 rs619586, rs664589, and rs3200401 in PFS and OS.*

SNP	Genotype/Allele	Progression-free survival			Overall survival		
		HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
rs619586	AG *vs.* AA	0.696	0.215–2.257	0.546	1.401	0.431–4.558	0.575
	GG *vs.* AA	*	*	*	*	*	*
	A allele+ *vs.* A–	*	*	*	*	*	*
	G allele+ *vs.* G–	0.696	0.215–2.257	0.546	1.401	0.431–4.558	0.575
rs664589	GC *vs.* CC	1.318	0.316–5.489	0.705	1.368	0.328–5.701	0.667
	GG *vs.* CC	3.093	0.408–23.462	0.275	12.212	1.594–93.558	0.016
	C allele+ *vs.* C–	0.328	0.043–2.483	0.280	0.083	0.11–0.637	0.017
	G allele+ *vs.* G–	1.624	0.497–5.304	0.422	1.936	0.594–6.316	0.273
rs3200401	TC *vs.* CC	1.638	0.757–3.542	0.210	0.786	0.347–1.778	0.563
	TT *vs.* CC	*	*	*	0.000	0.000	0.977
	C allele+ *vs.* C–	*	*	*	20.553	0.000–16524928.940	0.663
	T allele+ *vs.* T–	1.638	0.757–3.542	0.210	0.748	0.331–1.694	0.487

*OR could not be estimated because of zero value within a cell.

CURRICULUM VITAE

Name, Surname:	Eglė Balčiūnienė
Address:	Oncology and Hematology department, Hospital of Lithuanian University of Health Sciences Kauno klinikos, Eivenių 2, LT-50161 Kaunas, Lithuania
E-mail:	egle.balciuniene@lsmu.lt

Work experience

2015–till now	Radiation Oncologist at the Hospital of Lithuanian University of Health Sciences Kauno klinikos, Oncology and Hematology department, Eivenių 2, LT-50161 Kaunas, Lithuania.
2012–till now	Medical doctor at the Hospital in Šakiai, Bažnyčios 37, LT-71123 Šakiai, Lithuania.

Education

2018–2024	PhD student, Lithuanian University of Health Sciences
2011–2015	Resident of Radiation Oncology at the Hospital of Lithuanian University of Health Sciences, Oncology and Hematology department, Eivenių 2, LT-50161 Kaunas, Lithuania.
2009/2010	Internship of the medical studies program at Alytus S. Kudirka Hospital, Ligoninės 12, LT-62114 Alytus, Lithuania.
2003–2009	Medicine master degree, Kaunas University of Medicine.
2003	Graduated from Kaunas Aušra Gymnasium.

ACKNOWLEDGEMENTS

I would like to extend my heartfelt thanks to my main supervisor, Prof. Arturas Inčiūra, for the opportunities provided, his invaluable support in planning the study, his help in involving patients in the research, and his humor during difficult times.

I am also deeply grateful to Prof. Rasa Ugenskienė for her assistance in conducting all the research, performing the genetic studies, managing finances, and offering valuable advice.

My sincere thanks go to Prof. Elona Juozaitytė for her unwavering support, encouragement, and guidance throughout this journey.

I also wish to thank the entire laboratory team and my oncology colleagues for their help with the research and their insightful suggestions.

Additionally, I am profoundly grateful to the patients, without whom this research would not have been possible.

Finally, I want to express my deepest appreciation to my husband and children for their understanding, encouragement, and support. I apologize for the time and attention that I have taken away from them during this process. I am sorry, I cannot freeze time.