

LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

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**ARYL HYDROCARBON RECEPTOR
IN PANCREATIC DUCTAL
ADENOCARCINOMA:
CHEMOTHERAPY RESISTANCE,
EPITHELIAL-MESENCHYMAL
TRANSITION AND CYTOPROTECTION**

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LIST OF ABBREVIATIONS

AHR	– Aryl hydrocarbon receptor
ARE	– AU-rich element
ATP	– Adenosine triphosphate
BAY	– BAY2416964
Bcl-2	– B-cell lymphoma 2
Bcl-xL	– B-cell lymphoma-extra large
cDNA	– Complementary DNA
COX-2 / PTGS2	– Cyclooxygenase-2 / Prostaglandin-endoperoxide synthase 2
CRISPR/Cas9	– Clustered regularly interspaced short palindromic repeats / CRISPR-associated protein 9
DAPI	– 4',6-diamidino-2-phenylindole
DCK	– Deoxycytidine kinase
DMSO	– Dimethyl sulfoxide
DNA	– Deoxyribonucleic acid
ELAVL1 / HuR	– Embryonic lethal abnormal vision-like protein 1 / Human antigen R
ELISA	– Enzyme-linked immunosorbent assay
EMT	– Epithelial–mesenchymal transition
FBS	– Fetal bovine serum
GAPDH	– Glyceraldehyde-3-phosphate dehydrogenase
gDNA	– Genomic DNA
GEM	– Gemcitabine
gRNA	– Guide RNA
HIF-1α	– Hypoxia-inducible factor 1-alpha
HMOX1 / HO-1	– Heme oxygenase 1
hENT1	– Human equilibrative nucleoside transporter 1
HSA	– Highest single agent
ICC	– Immunocytochemistry
ICF	– Immunocytofluorescence
IC50	– Half-maximal inhibitory concentration
IDO1	– Indoleamine 2,3-dioxygenase 1
IL-8	– Interleukin 8

IP	– Immunoprecipitation
JNK	– c-Jun N-terminal kinase
KO	– Knockout
KRAS	– Kirsten rat sarcoma viral oncogene homolog
MAPK	– Mitogen-activated protein kinase
miRNA	– MicroRNA
MMP-9	– Matrix metalloproteinase 9
mRNA	– Messenger RNA
MTT	– 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-κB	– Nuclear factor kappa B
PanIN	– Pancreatic intraepithelial neoplasia
PBS	– Phosphate-buffered saline
PDAC	– Pancreatic ductal adenocarcinoma
PGE2	– Prostaglandin E2
PTGS2 / COX-2	– Prostaglandin-endoperoxide synthase 2 / Cyclooxygenase-2
qRT-PCR	– Quantitative reverse transcription polymerase chain reaction
RBP	– RNA-binding protein
RIP-IP	– RNA immunoprecipitation
RNA	– Ribonucleic acid
RPMI	– Roswell Park Memorial Institute medium
SD	– Standard deviation
sgRNA	– Single-guide RNA
siAHR	– Small interfering RNA targeting AHR
siELAVL1	– Small interfering RNA targeting ELAVL1
siRNA	– Small interfering RNA
TAP	– Tapinarof
TCDD	– 2,3,7,8-tetrachlorodibenzo-p-dioxin
TDO	– Tryptophan 2,3-dioxygenase
TGF-β	– Transforming growth factor beta
TNF-α	– Tumor necrosis factor alpha
TNM	– Tumor, node, metastasis classification
uPA	– Urokinase-type plasminogen activator
VEGF	– Vascular endothelial growth factor

VIM	– Vimentin
WB	– Western blot
XIAP	– X-linked inhibitor of apoptosis protein
XRE / DRE	– Xenobiotic response element / Dioxin response element

INTRODUCTION

Pancreatic cancer remains one of the most aggressive and lethal malignancies worldwide. Despite advances in diagnostic methods, surgical techniques and systemic treatment, patient survival remains poor, largely because the disease is frequently diagnosed at an advanced stage and rapidly develops therapeutic resistance. Pancreatic ductal adenocarcinoma (PDAC), the most common histological subtype, is characterized by early invasion, metastatic spread, dense stromal reaction, marked tumour heterogeneity and limited sensitivity to available therapies. Surgical resection offers the only potentially curative treatment; however, only a minority of patients are eligible for surgery at diagnosis, and disease recurrence is common even after radical treatment. Therefore, there is a critical need to better understand the molecular mechanisms that drive PDAC progression, aggressiveness and resistance to chemotherapy.

Gemcitabine has remained an important component of PDAC treatment for more than two decades. However, its clinical benefit is limited by intrinsic and acquired chemoresistance. One of the key determinants of gemcitabine efficacy is deoxycytidine kinase (DCK), the rate-limiting enzyme responsible for the intracellular activation of gemcitabine. Reduced DCK expression or impaired DCK regulation can limit gemcitabine activation and contribute to treatment failure. Increasing evidence indicates that DCK expression is regulated not only at the transcriptional level but also through post-transcriptional mechanisms involving RNA-binding proteins. Among these, embryonic lethal abnormal vision-like protein 1 (ELAVL1), also known as HuR, plays an important role by binding and stabilizing target mRNAs, including DCK mRNA. The cytoplasmic localization of ELAVL1 is particularly important for its mRNA-stabilizing function and has been associated with improved gemcitabine response in pancreatic cancer.

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor traditionally known for its role in xenobiotic metabolism and environmental toxin sensing. However, AHR is now recognized as a broader regulator of cancer-related processes, including proliferation, immune modulation, inflammation, epithelial–mesenchymal transition (EMT), invasion and therapy resistance. In PDAC, AHR expression has been associated with tumour progression and poor clinical outcome, but its precise role remains incompletely understood. Importantly, recent evidence suggests that AHR may influence ELAVL1 function by affecting its subcellular localization. Increased AHR activity may promote nuclear retention of ELAVL1, thereby limiting its cytoplasmic function and reducing the post-

transcriptional stabilization of DCK. This mechanism could represent a previously underexplored link between AHR signaling, ELAVL1-mediated RNA regulation and gemcitabine resistance.

In addition to chemoresistance, PDAC aggressiveness is strongly influenced by EMT-related plasticity. EMT enables cancer cells to acquire migratory and invasive properties, contributing to local invasion, lymph node involvement and metastatic dissemination. EMT is regulated by transcription factors such as ZEB1, ZEB2, SNAIL1, SNAIL2 and TWIST, together with changes in epithelial and mesenchymal markers, including E-cadherin and vimentin. AHR has been implicated in EMT regulation in several cancer types, but its contribution to EMT-associated aggressiveness in PDAC remains insufficiently defined. Understanding whether AHR promotes EMT-related changes, migration and invasion may provide important insight into the mechanisms underlying PDAC progression.

AHR may also contribute to tumour adaptation through cytoprotective and inflammatory signaling. Molecules such as heme oxygenase-1 (HO-1) and prostaglandin-endoperoxide synthase 2 (PTGS2/COX-2) are involved in oxidative stress response, inflammation, tumour survival and therapy resistance. PTGS2 activity leads to prostaglandin E2 (PGE2) production, which can support a pro-inflammatory and tumour-promoting microenvironment. Although AHR has been linked to the regulation of these pathways, the relationship between AHR activity, cytoprotective gene expression and PGE2 production in PDAC requires further investigation.

This dissertation focuses on AHR as a potential central regulator connecting several clinically relevant aspects of PDAC biology: chemoresistance, tumour aggressiveness and cytoprotective signaling. The study analyzes AHR, ELAVL1, DCK, EMT-related markers and cytoprotective molecules in PDAC patient tissues and evaluates their associations with clinicopathological features and patient survival. In addition, *in vitro* experiments using PDAC cell lines are used to investigate how genetic and pharmacological modulation of AHR affects gemcitabine sensitivity, ELAVL1 expression and localization, DCK expression, cell proliferation, migration, invasion, EMT-related marker expression and PTGS2/HO-1/PGE2 signaling. This approach corresponds to the thesis aim of analyzing the clinical relevance of AHR in PDAC tissues and investigating their interaction and functional effects on PDAC cell aggressiveness and chemoresistance.

Taken together, this thesis hypothesizes that AHR promotes PDAC progression by integrating multiple biological mechanisms: AHR may contribute to gemcitabine resistance through altered ELAVL1 localization and impaired DCK regulation, enhance tumour aggressiveness through EMT-associated changes, and support cytoprotective inflammatory signaling

through HO-1, PTGS2 and PGE2. By clarifying these relationships, the study aims to provide new insight into the molecular basis of PDAC aggressiveness and treatment resistance, and to evaluate AHR as a potential prognostic biomarker and therapeutic target in pancreatic cancer.

NOVELTY AND RELEVANCE OF THE STUDY

A major clinical challenge of PDAC is the development of resistance to chemotherapy, particularly to gemcitabine-based treatment, together with early invasion, metastatic spread and strong cytoprotective adaptation of tumour cells. Although several molecular mechanisms contributing to PDAC progression and chemoresistance have been described, the regulatory networks connecting transcriptional signalling, post-transcriptional RNA regulation, epithelial–mesenchymal transition and cytoprotective inflammatory pathways remain insufficiently understood.

The novelty of this study lies in the integrated investigation of the aryl hydrocarbon receptor (AHR) as a central regulator of several biologically and clinically relevant mechanisms in PDAC. While AHR has previously been associated with cancer progression, immune modulation and treatment resistance, its functional relationship with ELAVL1-mediated post-transcriptional regulation, DCK-dependent gemcitabine activation, EMT-associated aggressiveness and cytoprotective signalling has not been comprehensively evaluated in pancreatic cancer. This thesis therefore addresses an important research gap by analysing AHR not as an isolated transcription factor, but as a potential molecular link between chemoresistance, tumour aggressiveness and adaptive survival mechanisms.

A particularly novel aspect of this work is the investigation of the AHR–ELAVL1–DCK axis in PDAC chemoresistance. ELAVL1 is known to regulate target mRNAs involved in cancer biology, including DCK, which is essential for gemcitabine activation. This study evaluates whether AHR influences ELAVL1 expression and subcellular localization, and whether modulation of AHR can affect DCK expression and gemcitabine sensitivity. The demonstration that ELAVL1 binds AHR mRNA and that AHR modulation alters ELAVL1 localization provides new insight into the bidirectional regulatory relationship between AHR and ELAVL1. This mechanism may help explain how AHR contributes to impaired gemcitabine response in PDAC cells.

Another important novel component is the combined analysis of AHR expression with EMT-related markers, migration and invasion. EMT is a key process involved in PDAC aggressiveness and metastatic potential, but the contribution of AHR to EMT-associated plasticity in pancreatic cancer remains incompletely defined. By integrating patient tissue expression data with functional in vitro experiments, this study investigates whether AHR expression is associated with EMT-related transcriptional changes and

whether AHR inhibition or silencing can reduce migratory and invasive behaviour of PDAC cells.

The study is also relevant because it evaluates the role of AHR in cytoprotective and inflammatory signalling pathways. By analysing PTGS2, HO-1 and downstream PGE2 production, this work explores whether AHR contributes to adaptive tumour cell survival and pro-inflammatory signalling in PDAC. These pathways are important because they may support tumour persistence, therapy resistance and interaction with the tumour microenvironment.

The clinical relevance of the study is strengthened by the analysis of PDAC patient tissues and clinicopathological parameters. The evaluation of AHR, ELAVL1, DCK, EMT-related markers and cytoprotective molecules in tumour tissues allows the identification of associations with lymph node involvement and patient survival. These findings support the potential use of AHR-related molecular patterns as prognostic or predictive biomarkers in PDAC. Moreover, the functional experiments suggest that AHR inhibition may represent a potential therapeutic strategy to improve gemcitabine sensitivity, reduce tumour aggressiveness and suppress cytoprotective inflammatory signalling.

Taken together, this dissertation provides new evidence that AHR may act as a central regulator of PDAC progression by linking ELAVL1-mediated post-transcriptional regulation, gemcitabine chemoresistance, EMT-associated aggressiveness and cytoprotective signalling. These findings may contribute to a better understanding of PDAC biology and support further investigation of AHR as a potential biomarker and therapeutic target in pancreatic cancer. This directly reflects the thesis aim and objectives, which focus on AHR, ELAVL1, DCK, EMT-related markers, PTGS2, HO-1 and PGE2 in patient tissues and PDAC cell models.

1. AIM AND OBJECTIVES

Aim

To investigate the clinical relevance and functional role of aryl hydrocarbon receptor (AHR) in pancreatic ductal adenocarcinoma, with emphasis on its involvement in ELAVL1/DCK-mediated gemcitabine chemoresistance, EMT-associated tumour aggressiveness and cytoprotective PTGS2/HO-1/PGE2 signalling.

Objectives

1. To evaluate the expression of AHR, ELAVL1, DCK, EMT-related markers and cytoprotective molecules in pancreatic ductal adenocarcinoma tissues, and to assess their associations with clinicopathological features.
2. To determine how AHR and ELAVL1 interact in PDAC cells and how AHR modulation affects ELAVL1 localization, DCK expression, cell viability and gemcitabine sensitivity.
3. To evaluate the role of AHR in EMT-associated PDAC cell aggressiveness by investigating EMT-related marker expression, migration and invasion.
4. To determine the effect of AHR modulation on cytoprotective and inflammatory signalling by analysing PTGS2, HO-1 and downstream PGE2 production.

2. LITERATURE REVIEW

2.1. Pancreatic cancer – a global health challenge

2.1.1. Epidemiology and burden of pancreatic cancer

Pancreatic cancer represents one of the most devastating malignancies worldwide, characterized by exceptionally poor prognosis and limited therapeutic options. Cancer of the pancreas remains one of the deadliest cancer types, causing more than 331,000 deaths per year globally and ranking as the seventh leading cause of cancer death in both sexes combined [1]. Based on GLOBOCAN 2022 estimates, pancreatic cancer caused 467,409 new deaths and 510,992 new cases were reported. Pancreatic cancer could become one of the leading causes of cancer-related death by 2030, surpassed only by lung cancer [2,3].

The incidence of pancreatic cancer varies significantly across geographical regions, with the highest rates observed in developed countries. For a long time pancreatic cancers 5-year survival was less than 10% [4]. Despite significant advances in modern medical technology and improvements in survival rates of many cancer types, pancreatic cancer still maintains a highly lethal malignancy with a low 5-year survival rate and difficulty early detection [5]. The overall 5-year survival rate, irrespective of stage at diagnosis, increased from 6% for patients diagnosed in 2004 to 14% for those diagnosed in 2017, representing a doubling of survival over this period [6]. However, this improvement primarily reflects enhanced survival among patients diagnosed with localized disease, as survival rates for distant disease only increased from 2% to 4% during the same period [6]. This minimal advancement in treatment outcomes for individuals identified with distant disease at diagnosis highlights the persistent challenge of treating advanced-stage pancreatic cancer.

2.1.2. Clinical characteristics and diagnostic challenges

Pancreatic ductal adenocarcinoma (PDAC) is the most common histologic subtype of pancreatic cancer, accounting for the vast majority of cases. Because patients seldom exhibit symptoms until an advanced stage of the disease, pancreatic cancer remains one of the most lethal malignant neoplasms [2]. The special anatomical location of the pancreas means that the development of pancreatic cancer is usually diagnosed at a late stage with obvious clinical symptoms, making early detection particularly challenging [5].

At diagnosis, approximately 75% of patients are already in stage III-IV, reflecting the aggressive nature of the disease and the difficulty in early detection [7]. The distribution of localized and regional disease stages has remained largely consistent over study periods, with only 30.9% of patients presenting in the localized and regional stage in which surgical treatment is possible [8]. This late-stage presentation significantly limits treatment options and contributes to poor outcomes.

The clinical progression of pancreatic cancer is characterized by rapid metastatic spread, with liver metastasis being the most common site and associated with worse prognosis [9]. The absence of effective screening methods for the general population further compounds the challenge of early diagnosis. While screening of tightly targeted groups, especially those with family history, is being evaluated using newer techniques, general population screening is not considered useful for detecting the disease at its early stage [2].

2.1.3. Current treatment strategies and limitations

Surgical resection remains the only potentially curative option for pancreatic cancer; however, only 15-20% of patients have resectable disease at diagnosis [10]. The current common treatment pathways for PDAC include surgery in combination with neoadjuvant and adjuvant chemotherapy, and palliative chemotherapy in unresectable and metastatic disease [11]. For patients with advanced or metastatic disease, combination chemotherapy regimens such as FOLFIRINOX (folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin), NALIRIFOX, and combined gemcitabine/nanoparticle albumin-bound paclitaxel offer survival benefits, although toxicity remains a concern [10].

Gemcitabine (2',2'-difluoro-2'-deoxycytidine, dFdC) has been the main chemotherapy for pancreatic cancer for over two decades and remains a cornerstone of PDAC treatment in all stages of the disease [12,13]. However, its efficacy is limited by suboptimal clinical effects primarily caused by molecular mechanisms limiting cellular uptake and activation, as well as the development of chemoresistance within weeks of treatment initiation [13].

The proportion of surgical treatment within the first 4 months after diagnosis was relatively higher in gallbladder and extrahepatic bile duct cancers than in pancreatic cancer, reflecting the advanced stage at diagnosis and limited surgical candidacy of many pancreatic cancer patients [14]. However, these gains remain insufficient, and there is an urgent need for novel therapeutic approaches to improve patient outcomes.

2.1.4. Molecular basis of pancreatic cancer development

PDAC arises through a well-characterized stepwise progression from pancreatic intraepithelial neoplasia (PanIN) precursor lesions, accompanied by sequential accumulation of oncogenic mutations (Fig. 2.1) [15–17].

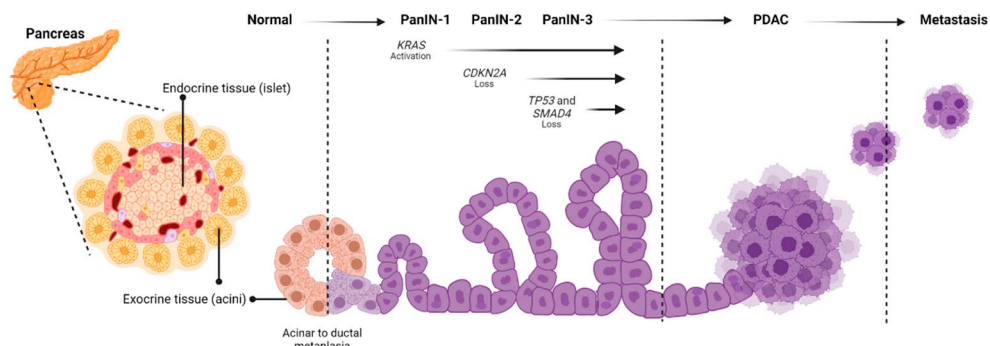


Fig. 2.1. Model of pancreatic cancer progression

Progression of normal pancreatic glandular epithelial cells to infiltrating cancer cells and the mutations involved in this process [17].

PDAC arises from a complex interplay of genetic and epigenetic alterations that drive tumor initiation, progression, and metastasis. Core genetic drivers identified in human tumors include activating mutations in *KRAS*, inactivation of the *TP53* tumor suppressor, and loss of *SMAD4*, which collectively promote uncontrolled proliferation, evasion of apoptosis, and altered signaling [18]. These foundational mutations occur early in tumorigenesis and are present in the majority of PDAC cases, with *KRAS* mutations being almost ubiquitous.

Beyond these canonical mutations, PDAC genomes frequently exhibit alterations in other oncogenes and tumor suppressors, as well as dysregulation of key signaling pathways such as TGF- β , Notch, Wnt/ β -catenin, and Hedgehog, which further contribute to tumor growth, invasiveness, and metastatic potential [18]. The tumor microenvironment also plays a critical role in PDAC biology. Dense desmoplastic stroma, hypoxia, and nutrient-deprived niches create selective pressures that shape tumor evolution and heterogeneity. Interactions between tumor cells, stromal fibroblasts, immune cells, and the extracellular matrix contribute to tumor progression and influence the molecular landscape of PDAC [19].

Together, these genetic, epigenetic, and microenvironmental factors define the molecular basis of pancreatic cancer development and provide the framework for understanding its aggressive biology and poor prognosis.

2.2. Gemcitabine and chemoresistance in pancreatic cancer

2.2.1. Mechanism of action of gemcitabine

Gemcitabine is a pyrimidine nucleoside analog that has been the cornerstone of pancreatic cancer chemotherapy for over two decades. This cytotoxic agent exerts its antitumor activity through multiple mechanisms following intracellular conversion to its active triphosphate form. To exert its antitumor activity, gemcitabine requires conversion to its active triphosphate form through sequential phosphorylation steps [20]. The drug first enters cells via nucleoside transporters, particularly human equilibrative nucleoside transporter 1 (hENT1), which facilitates the cellular uptake necessary for therapeutic efficacy [21].

Once inside the cell, gemcitabine undergoes phosphorylation by deoxycytidine kinase (DCK), the rate-limiting enzyme in gemcitabine activation, to form gemcitabine monophosphate (dFdCMP) [13]. This critical first phosphorylation step determines the overall effectiveness and even modest reductions in its expression can profoundly impair drug efficacy—positioning DCK as a critical vulnerability node in PDAC therapy. Subsequent phosphorylation by other cellular kinases produces gemcitabine diphosphate (dFdCDP) and ultimately gemcitabine triphosphate (dFdCTP), the active metabolite responsible for the drug's cytotoxic effects (Fig. 2.2) [13].

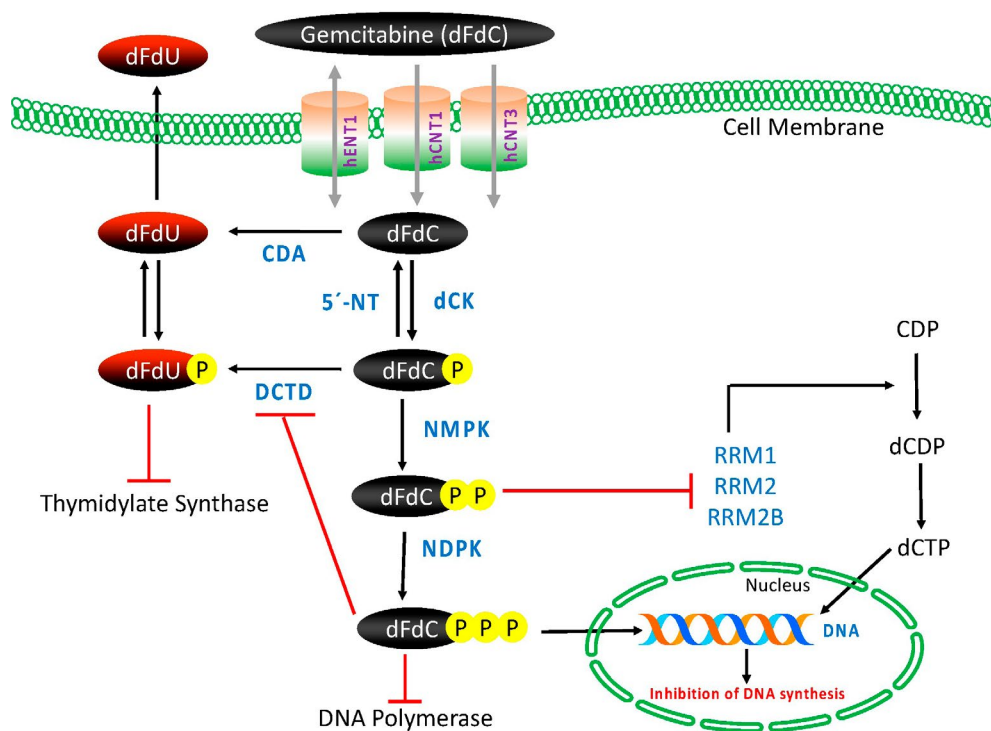


Fig. 2.2. Gemcitabine activation transport, intracellular activation/deactivation and mechanism of action

CDA: cytidine deaminase, dCK: deoxycytidine kinase, DCTD: deoxycytidylate deaminase, dFdC: 2',2'-difluorodeoxycytidine, dFdU: 2',2'-difluorodeoxyuridine, hENTs and hCNTs: human nucleoside transporters, NDPK: nucleoside diphosphate kinase, NMPK: nucleoside monophosphate kinase, RR(M1/M2), ribonucleotide reductase, 5'-NT: 5'-nucleotidase [13].

Gemcitabine triphosphate exerts its cytotoxic effects through incorporation into DNA, leading to chain termination and inhibition of DNA synthesis. The drug also inhibits ribonucleotide reductase, an enzyme essential for DNA synthesis, creating a dual mechanism of action that enhances its therapeutic potential [21]. This stress stimulates ELAVL1, a posttranscriptional mRNA regulator, to shuttle from the nucleus to the cytoplasm, where it stabilizes its target mRNAs, thus promoting the translation of various proteins including enzymes such as DCK [13].

2.2.2. Gemcitabine resistance

Beyond classical mechanisms, emerging evidence points to the role of post-transcriptional regulation in chemoresistance. The expression of protein phosphatase 32 (PP32, ANP32A) is low in poorly differentiated pancreatic

cancers and is linked to the levels of ELAVL1, a predictive marker for gemcitabine response [22]. Furthermore, epigenetic mechanisms have been shown to be a key determinant in the maintenance of subpopulations of cancer cells with high-level resistance to cytotoxic therapy [23]. Treatment with histone deacetylase inhibitors can synergistically enhance gemcitabine-induced inhibition of cell proliferation and apoptosis, suggesting that epigenetic modulation may overcome some resistance mechanisms [23]. However DCK remains one of the most promising targets since it represents a critical bottleneck in gemcitabine metabolism and therapeutic efficacy. It is usually inactive in gemcitabine-resistant cells [13,24]. There is evidence that a lack of cytoplasmic ELAVL1 protein is involved in gemcitabine resistance through a reduction in DCK protein [25].

The regulation of DCK expression occurs at multiple levels, including transcriptional, post-transcriptional, and post-translational mechanisms. The RNA-binding protein ELAVL1 plays a crucial role in DCK regulation by stabilizing DCK mRNA and enhancing protein expression. The mRNA encoding the DCK enzyme is a key target of ELAVL1, and reduced association of ELAVL1 with DCK mRNA causes a significant reduction in DCK protein levels [25]. This interaction demonstrates the importance of post-transcriptional regulation in determining chemosensitivity.

DCK deficiency has been identified as the primary mechanism of gemcitabine resistance in multiple studies. Using CRISPR knockout library screening, researchers found that DCK deficiency is the primary mechanism of gemcitabine resistance, with loss of DCK expression resulting in complete resistance to the drug [26]. Among gemcitabine sensitivity-related genes examined in pancreatic cancer, DCK alone showed a significant correlation with gemcitabine efficacy, with eight of 12 patients in the effective group having high DCK expression, whereas 16 of 23 patients in the non-effective group had low DCK expression [27]. These findings highlight the critical importance of DCK expression in determining treatment response and patient outcomes.

2.2.3. Clinical implications of chemoresistance

The development of chemoresistance has profound clinical implications for pancreatic cancer patients, directly impacting treatment efficacy and patient survival. Despite gemcitabine being the standard first-line therapy for advanced pancreatic cancer, the primary clinical challenge arises from the eventual acquisition of resistance [28]. Drug resistance poses a significant challenge to the efficacy of chemotherapy, with patients developing resistance early in the treatment [28]. Clinical studies have demonstrated that

high cytoplasmic ELAVL1 (cELAVL1) expression in PDAC was associated with significantly better overall survival in patients receiving gemcitabine, versus those with low cELAVL1. Patients with low cELAVL1 had ~7-fold increased risk of mortality after gemcitabine compared with those with high cytoplasmic ELAVL1 levels, after adjustment for other factors [29]. In patients with pancreatobiliary-type tumors, a high ELAVL1 cytoplasmic/nuclear ratio was associated with significantly reduced five-year overall survival in patients receiving adjuvant gemcitabine but not in untreated patients [30]. These findings suggest that its expression patterns may serve as predictive biomarkers for treatment selection.

The heterogeneity of DCK expression within tumors adds another layer of complexity to treatment planning. Analysis of pancreatic cancer patient samples revealed a significant decrease in ELAVL1 and DCK expression in pancreatic tissue upon becoming cancerous [25]. The high decrease of DCK coincides with the decrease of ELAVL1 and could partially explain the commonly observed resistance to gemcitabine in PDAC [25].

Understanding these resistance mechanisms has important implications for developing combination therapies and identifying patients most likely to benefit from gemcitabine-based treatment.

Given the central role of gemcitabine in PDAC treatment and the importance of DCK in drug activation, understanding how DCK is regulated is critical. Emerging evidence suggests that post-transcriptional regulation via ELAVL1 and its modulation by AHR may represent a previously underexplored axis linking environmental sensing, RNA regulation, and chemoresistance

2.3. The Aryl Hydrocarbon Receptor: from environmental sensor to cancer regulator

2.3.1. AHR activation and ligands

The aryl hydrocarbon receptor is a ligand-activated transcription factor belonging to the basic helix-loop-helix Per-ARNT-Sim (bHLH-PAS) protein family. The AHR is a cytosolic transcription factor that broadly suppresses immune cell function and performs various biological functions upon ligand activation [31].

Upon ligand binding, AHR undergoes a conformational changes and binds to specific DNA sequences called xenobiotic response elements (XREs) or dioxin response elements (DREs) in the regulatory regions of target genes [32]. Beyond its canonical role in xenobiotic metabolism, AHR has emerged as a critical regulator of diverse physiological processes. AHR activity is important in angiogenesis, cardiomyocyte differentiation, oocyte maturation,

oculomotor nerve formation, and hematopoietic stem cell maintenance, immune cell function and DNA damage repair [33]. This pleiotropic nature of AHR function underscores its importance in both normal physiology and disease pathogenesis.

Ligands for the AHR are divided into exogenous or endogenous and have agonistic or antagonistic activity [31,33]. For example, AHR ligands found in cigarette smoke, promote pancreatic dysplasia and PDAC progression in mouse models of this disease [34]. The diversity of exogenous ligands reflects AHR's evolutionary role as an environmental sensor capable of detecting a wide range of potentially harmful xenobiotic compounds.

Endogenous AHR ligands include tryptophan metabolites produced through various metabolic pathways. Endogenous ligands are involved in key pathway of constitutive and adaptive tumor immune resistance, with the immunosuppressive effects of kynurenine in the tumor microenvironment predominantly mediated by AHR [35]. The expression of IDO1, which is responsible for metabolizing tryptophan to kynurenine, has been detected in pancreatic cancer cell lines, suggesting ongoing endogenous AHR activation [35].

Additional endogenous ligands include indole derivatives produced by gut microbiota from dietary tryptophan, such as indole-3-carbinol and indole-3-acetic acid. The diverse implications and unclear regulation mean that AHR could behave differently in live systems compared to isolated cell cultures [31]. This complexity necessitates careful consideration when targeting AHR therapeutically, as the ligand milieu may vary significantly between patients and affect treatment outcomes.

2.3.2. AHR in cancer development and progression

The role of AHR in cancer is complex and context-dependent, with evidence supporting both tumor-promoting and tumor-suppressive functions depending on the cancer type, cellular context, and specific ligands involved. AHR can exert both tumor-promoting and tumor-suppressive effects depending on factors such as the specific ligand, cell type, and tissue microenvironment.

Increased AHR expression has been linked to adverse clinical outcomes in terms of tumor grade, stage, lymph node status, and metastasis in various malignancies [34,36].

Several mechanisms contribute to AHR's pro-tumorigenic effects. AHR regulates genes involved in cell proliferation, with functional mapping showing that overexpression of AHR as well as ligand treatment stimulates cell proliferation in cancer cells [34,37]. AHR activation can also promote epithelial-mesenchymal transition (EMT), a process critical for metastasis. In

thyroid cancer, the IDO1-Kyn-AHR pathway plays a pivotal role in mediating an immunosuppressive microenvironment and favoring the acquisition of a mesenchymal phenotype that promotes invasiveness and metastasis [35,38].

Conversely, in certain contexts, AHR exhibits tumor-suppressive properties. Loss of AHR expression leads to increased tumorigenesis in different mouse models, and previous cell culture studies have shown that AHR activation leads to G0-G1 arrest and inhibition of proliferation [31,37,39]. Functional expression of AHR as a potential novel therapeutic target has been demonstrated in multiple myeloma [40], bladder carcinogenesis [41] and numerous other oncologies.

AHR has also been linked to the regulation of inflammatory mediators and epithelial-mesenchymal transition (EMT). For example, AHR activation can upregulate PTGS2 expression, contributing to a pro-inflammatory and pro-tumorigenic microenvironment [42]. Simultaneously, AHR promotes EMT by inducing transcriptional programs that downregulate epithelial markers and upregulate mesenchymal genes, facilitating invasion and metastasis in esophageal squamous cell carcinoma [43].

2.3.3. AHR expression and function in pancreatic cancer

Although AHR is well documented and has emerged as a significant player in disease pathogenesis and therapeutic resistance, its function, especially in PDAC remain ambiguous. AHR is commonly upregulated in PDAC and correlates with disease progression and therapeutic resistance [25,34], [44–47]. PDAC is among the cancer types where upregulation of AHR has been detected, both in patient samples and cell lines, and its activation has been associated with protumorigenic gene expression. Analysis of PDAC and neighboring next-to-cancer tissues revealed differential AHR expression patterns, with implications for chemotherapy response [25,44]. Increased AHR expression offers a potential therapeutic target for pancreatic cancer, as AHR levels are elevated in tumor tissue compared to normal pancreas [46]. The expression of AHR in PDAC is heterogeneous, with variation among different cell lines.

The functional consequences of AHR activation in pancreatic cancer are multifaceted. AHR also modulates the tumour microenvironment in PDAC, leading to increased tumour progression and worse patient outcomes by altering the function of tumour-associated macrophages. This effect is mediated by AHR activation in CD4⁺ T cells, leading to their polarization to IL22-producing TH22 cells and regulatory T cell accumulation, ultimately driving a blunted CD8⁺ T-cell effector response [31,34,35]. Analysis of human

pancreata from organ donors revealed that smokers present with increased regulatory T-cell accumulation compared with nonsmokers [34].

A critical point is the relationship between AHR and chemoresistance in pancreatic cancer. AHR hinders the shuttling of ELAVL1 from the nucleus to the cytoplasm with increased AHR expression leading to the sequestration of ELAVL1 in the nucleus, resulting in chemoresistance [25,44]. Even though this molecular axis shows a lot of promise in reversing PDAC chemoresistance, it still remains largely unexplored.

2.4. The ELAVL1 protein: structure, function, and role in cancer

2.4.1. Molecular structure and RNA-binding properties

ELAVL1 is a ubiquitously expressed member of the embryonic lethal abnormal vision (ELAV) family of RNA-binding proteins. It regulates the expression of many tumor-associated molecules through posttranscriptional regulatory mechanisms [22,30,48]. The protein contains RNA recognition motifs that enable binding to target mRNA and translocation between the nucleus and cytoplasm in response to various cellular signals [48,49], [50]. This binding generally results in mRNA stabilization and enhanced translation, although in some contexts, ELAVL1 can also regulate alternative splicing, mRNA localization, and microRNA function [48,49]. The versatility of ELAVL1's regulatory functions makes it a master regulator of gene expression at the post-transcriptional level.

2.4.2. ELAVL1 in oncogenesis and chemoresistance of PDAC

ELAVL1 regulates the expression of hundreds of target mRNAs involved in diverse cellular processes critical for cancer development and progression. Among the well-characterized targets are mRNAs encoding proteins involved in cell cycle regulation (cyclin A, cyclin B, cyclin D1, p21), apoptosis (Bcl-2, Bcl-xL, XIAP), angiogenesis (VEGF, HIF-1 α), inflammation (COX-2, TNF- α , IL-8), and metastasis (MMP-9, uPA) [51–55]. The breadth of ELAVL1's target repertoire underscores its role as a master regulator of cancer-relevant gene expression programs.

ELAVL1 has many known and unknown targets, including immune-related proteins such as IFNB1, COX-2, IL-8, and TGFB. It also participates in ferroptosis, regulates mRNAs such as FOS, CDKN1A, CCNA2, CCNB1, NOS2, VEGFA, TNF, BCL2, IL3, and HIF1A, and affects cellular response to oxidative stress, autophagy, proliferation, differentiation, apoptosis, and senescence [56,57]. ELAVL1 activates MAPK and JNK signaling pathways,

causing resistance to therapy in various cancer types [58–60], as well as immune system processes and developmental pathways [61,62].

In PDAC, ELAVL1 is associated with increased tumor stage [63] and its silencing significantly decreases cell proliferation and anchorage-independent growth, as well as impairs migration and invasion in pancreatic cancer cells [63,64] and at the same time increases the effectiveness of gemcitabine treatment, in vitro [65]. NanoString nCounter analyses demonstrated that ELAVL1 regulates core biological processes, highlighting that ELAVL1 inhibition likely thwarts PDAC viability through post-transcriptional regulation of diverse signaling pathways including cell cycle, apoptosis, and DNA repair [64].

This creates a complex regulatory network where ELAVL1 simultaneously promotes both gemcitabine sensitivity (through DCK stabilization) and potential resistance mechanisms (through stabilization of survival factor mRNAs). This dual function of ELAVL1 in both promoting cell survival through cytoprotective mechanisms and enhancing chemosensitivity through DCK regulation creates a complex therapeutic challenge.

2.5. Epithelial-mesenchymal transition (EMT) in pancreatic cancer

2.5.1. EMT as a hallmark in oncogenesis

Epithelial–mesenchymal transition (EMT) is a dynamic and reversible biological process through which epithelial cells lose their polarity and cell–cell adhesion properties while acquiring mesenchymal characteristics, including enhanced migratory capacity, invasiveness, and resistance to apoptosis [66]. EMT plays a fundamental role in embryonic development and tissue repair but is aberrantly reactivated in cancer, where it contributes to tumor progression and metastasis [67]. In PDAC, EMT occurs with varying degrees of phenotypic plasticity contributing to intratumoral heterogeneity and treatment failure [68], [69], [70].

At the molecular level, EMT is characterized by the downregulation of epithelial markers such as E-cadherin (CDH1) and the upregulation of mesenchymal markers including N-cadherin (CDH2), vimentin, and fibronectin [66]. This phenotypic shift is orchestrated by key EMT-inducing transcription factors (EMT-TFs), such as SNAIL, SLUG, ZEB1/2, and TWIST, which repress epithelial gene expression and activate mesenchymal programs [71]. These transcription factors are regulated by multiple signaling pathways frequently dysregulated in cancer, including TGF- β , Wnt/ β -catenin, Notch, Hedgehog, and MAPK pathways [72].

Importantly, EMT is no longer considered a binary process but rather a spectrum of intermediate or “hybrid” epithelial/mesenchymal states. These partial EMT states are increasingly recognized as highly relevant in cancer biology, as they allow tumor cells to retain proliferative capacity while acquiring invasive properties [68].

2.5.2. AHR activation and EMT promotion

Given the emerging role of AHR in EMT and tumor aggressiveness, it is plausible that AHR simultaneously regulates both chemoresistance (via ELAVL1/DCK) and metastatic potential (via EMT), suggesting a dual role in PDAC progression. AHR has emerged as a critical regulator of EMT in multiple cancer types, with emerging evidence suggesting a pro-EMT role [73]. In thyroid cancer, the IDO1-Kynurenine-AHR pathway plays a pivotal role in mediating an immunosuppressive microenvironment and favoring acquisition of a mesenchymal phenotype that promotes invasiveness and metastasis [74]. Similar mechanistic pathways are likely operative in PDAC, where AHR activation drives EMT through multiple interconnected signaling cascades.

AHR regulates genes involved in cell proliferation and EMT. Specifically, AHR can promote epithelial-mesenchymal transition through multiple mechanisms including upregulation of PTGS2 (COX-2), contributing to a pro-inflammatory and pro-tumorigenic microenvironment [75]. Additionally, AHR promotes EMT by inducing transcriptional programs that downregulate epithelial markers and upregulate mesenchymal genes, facilitating invasion and metastasis in various cancer contexts [72].

However, the role of AHR in cancer and EMT regulation is complex and context-dependent. In certain cellular contexts, AHR exhibits tumor-suppressive properties, with loss of AHR expression leading to increased tumorigenesis and enhanced EMT in different mouse models [76]. In lung cancer, cytoplasmic AHR acts as an EMT suppressor via non-genomic pathways, with AHR overexpression accelerating mesenchymal vimentin degradation and preventing cell invasion in vitro and in vivo [77]. This paradoxical role highlights the importance of understanding tissue-specific and ligand-dependent AHR functions in cancer progression.

2.6. AHR and ELAVL1 research gaps in chemoresistance, EMT and cytoprotection

Recent studies suggest that the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor, may play a previously underappreciated role in modulating ELAVL1 function. Specifically, AHR overexpression has been

implicated in the nuclear retention of ELAVL1, thereby limiting its ability to stabilize target mRNAs such as DCK [78]. AHR has been independently associated with tumor progression, immune modulation, and epithelial–mesenchymal transition (EMT), all of which are processes linked to aggressive tumor behavior and therapeutic resistance [31,66,74,76]. However, the relationships between AHR, ELAVL1, cytoprotective pathways, EMT markers, and clinicopathological outcomes have not been systematically evaluated in PDAC (Fig 2.3).

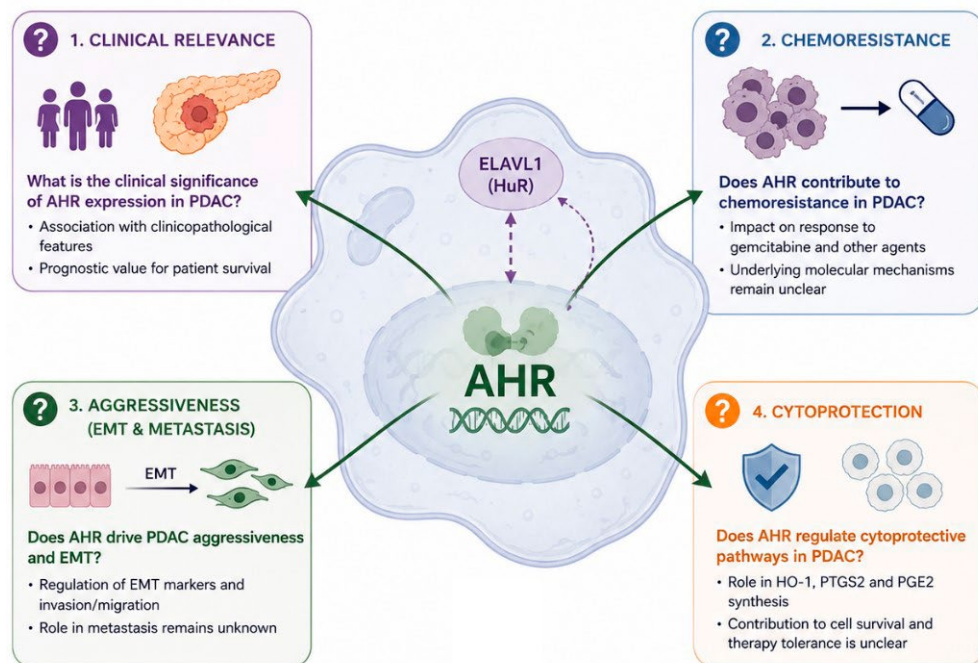


Fig. 2.3. Knowledge gaps addressed by the dissertation

The schematic summarizes the main research gaps investigated in this dissertation. AHR is presented as a central molecule potentially linking four clinically and biologically relevant aspects of pancreatic ductal adenocarcinoma: clinical relevance, chemoresistance, tumour aggressiveness and cytoprotection. (Illustration created using ChatGPT.com)

Multiple mechanisms of chemoresistance have been described, including alterations in drug transport, metabolism, and DNA repair pathways, these do not fully explain the heterogeneity of treatment response observed in clinical settings [13,28]. DCK has been consistently identified as a critical determinant of gemcitabine efficacy [24,26]. Emerging evidence highlights the importance of post-transcriptional regulation in this process, particularly through the RNA-binding protein ELAVL1 [25,29]. Notably, the subcellular

localization of ELAVL1 is a key determinant of its function [22], however it remains unclear whether AHR-mediated nuclear retention of ELAVL1 is sufficient to reduce DCK expression and functionally drive gemcitabine resistance in PDAC and if its modulation could reverse the chemoresistance of PDAC.

Furthermore, AHR has been independently associated with tumor progression, immune modulation, and EMT, all of which are processes linked to aggressive tumor behavior and therapeutic resistance [31,66,74,76]. Despite these observations, the integration of AHR signaling in EMT and PDAC aggressiveness has been lacking. It remains unclear whether AHR is directly linked to EMT and if its modulation could reduce PDAC aggressiveness through shifting the cell towards epithelial phenotype.

Lastly, AHR has been implicated in cell protection through upregulation of cytoprotective molecules and immune response. Despite recent advances, it is still unknown to what extent upregulation of AHR increases the cytoprotective molecules (HO-1, PTGS2) expression in PDAC and downstream synthesis of inflammatory molecules such as PGE2 and whether its inhibition could prove beneficial in terms of reducing cytoprotection and inflammation of PDAC.

Taken together, these gaps highlight a critical need to elucidate the interplay between AHR signaling, ELAVL1-mediated post-transcriptional regulation, and DCK-dependent gemcitabine metabolism in pancreatic cancer as well as AHR role in PDAC aggressiveness through EMT, cytoprotection and downstream synthesis of PGE2. A deeper understanding of these regulatory axis may not only provide novel insights into the mechanisms underlying chemoresistance and aggressiveness but also identify potential therapeutic targets and predictive biomarkers for improving treatment stratification in PDAC.

Based on these observations, this thesis hypothesizes that AHR promotes gemcitabine resistance in PDAC by inducing nuclear retention of ELAVL1, thereby reducing DCK expression, while simultaneously enhancing tumor aggressiveness through EMT and cytoprotective signaling pathways.

3. MATERIALS AND METHODS

3.1. Experimental setups and main readouts

3.1.1. Patient tissue analysis and clinical relevance of AHR

The analysis methodology of AHR associations with clinicopathology consisted of collection of patient tissue and data. The collected and snap-frozen tissue were later used for RNA and protein analysis. Western Blot, qRT-PCR, TNM classification and patient data was used to determine gene and protein expression and correlations (Fig. 3.1).

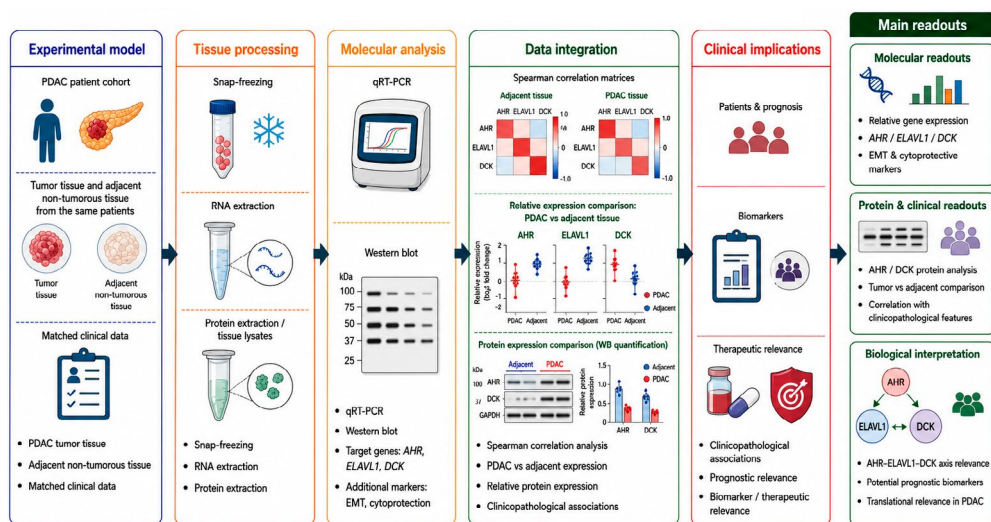


Fig. 3.1. Workflow of patient tissue collection and clinicopathological analysis

Illustration created using ChatGPT.com

3.1.2. Investigation of AHR-mediated chemoresistance through ELAVL1/DCK regulation

The analysis methodology of AHR implication in chemoresistance to gemcitabine consisted of in vitro study using BxPC-3 and Su.86.86 cell lines. The AHR in the cells was modulated using lipofectamine mediated siRNA transfection for gene silencing and inhibitor (BAY) together with activator (TAP) for protein activity modulation. Western Blot, qRT-PCR, RIP-IP, ICC, colony formation and metabolic activity assays were used to determine gene

and protein expression, molecule interplay and possible sensitization to gemcitabine (Fig. 3.2).

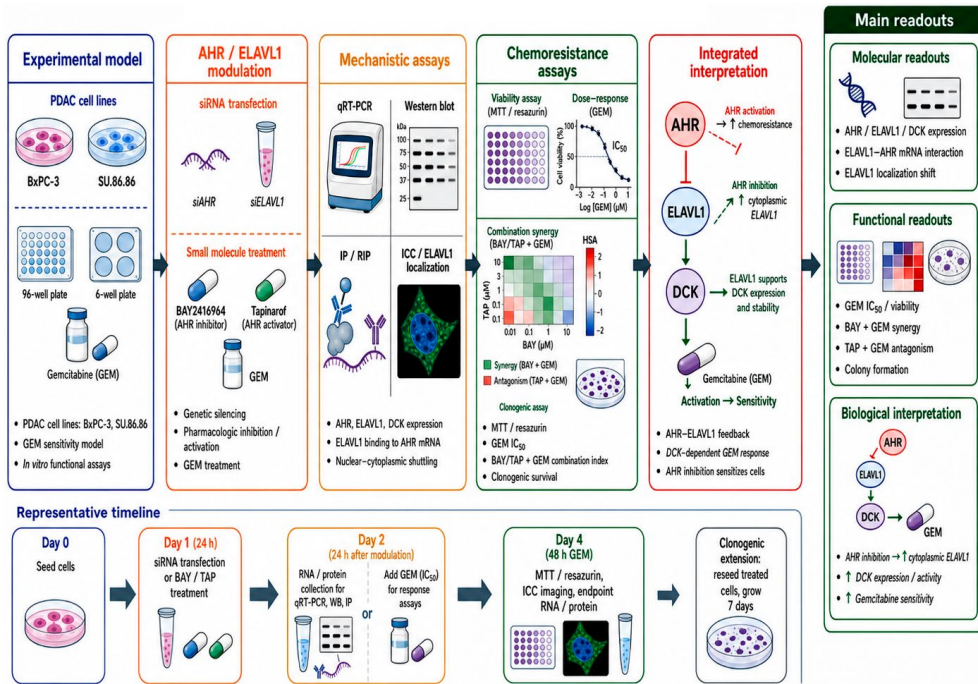


Fig. 3.2. Research workflow for investigating AHR-mediated gemcitabine chemoresistance through ELAVL1 regulation

Illustration created using ChatGPT.com

3.1.3. Investigation of AHR-mediated EMT-associated aggressiveness

The analysis methodology of AHR implication in cell aggressiveness through EMT regulation consisted of in vitro study using BxPC-3 and Su.86.86 cell lines. The AHR in the cells was modulated using lipofectamine mediated siRNA transfection for gene silencing and inhibitor (BAY) together with activator (TAP) for protein activity modulation. AHR CRISPR-KO cells were used as basis of DNA modulation. Western Blot, qRT-PCR, scratch assay, 3D migration and 3D basement membrane matrix based invasion assays were used to determine gene and protein expression and AHR modulation effects on cell aggressiveness (Fig. 3.3).

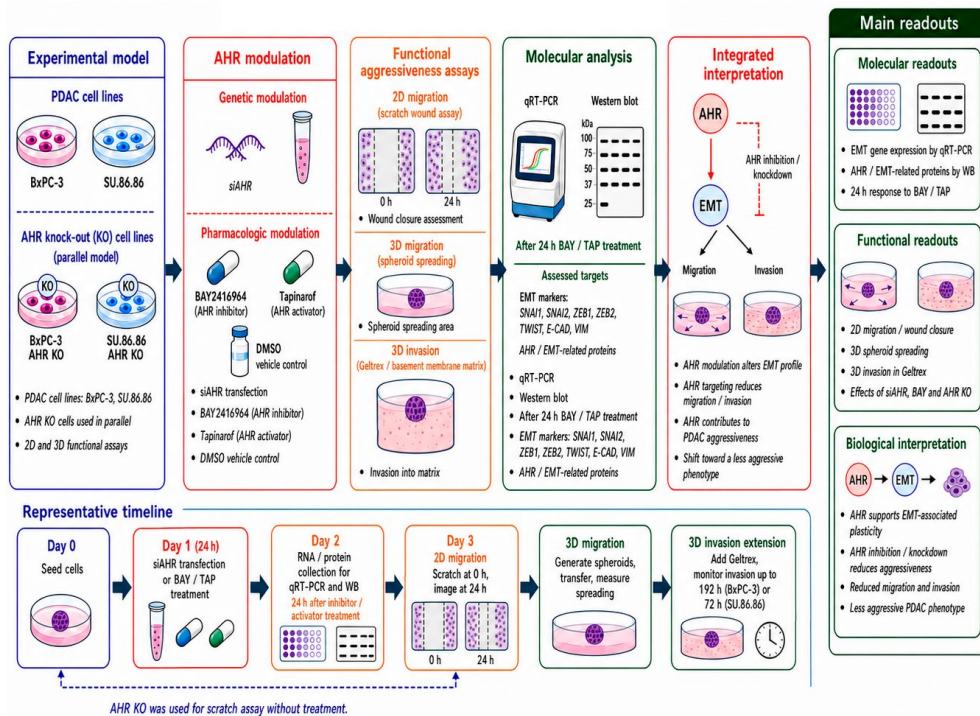


Fig. 3.3. Research workflow for investigating the role of AHR in PDAC cell aggressiveness through EMT regulation

Illustration created using ChatGPT.com

3.1.4. Investigation of AHR-mediated cytoprotective and inflammatory signalling

The analysis methodology of AHR implication in PDAC cytoprotection and inflammation through cytoprotective molecule regulation consisted of in vitro study using BxPC-3 and Su.86.86 cell lines. The AHR in the cells was modulated using lipofectamine mediated siRNA transfection for gene silencing and inhibitor (BAY) together with activator (TAP and TCDD) for protein activity modulation. Western Blot, qRT-PCR, ELISA assays were used to determine gene and protein expression and PGE2 synthesis as inflammatory output (Fig. 3.4).

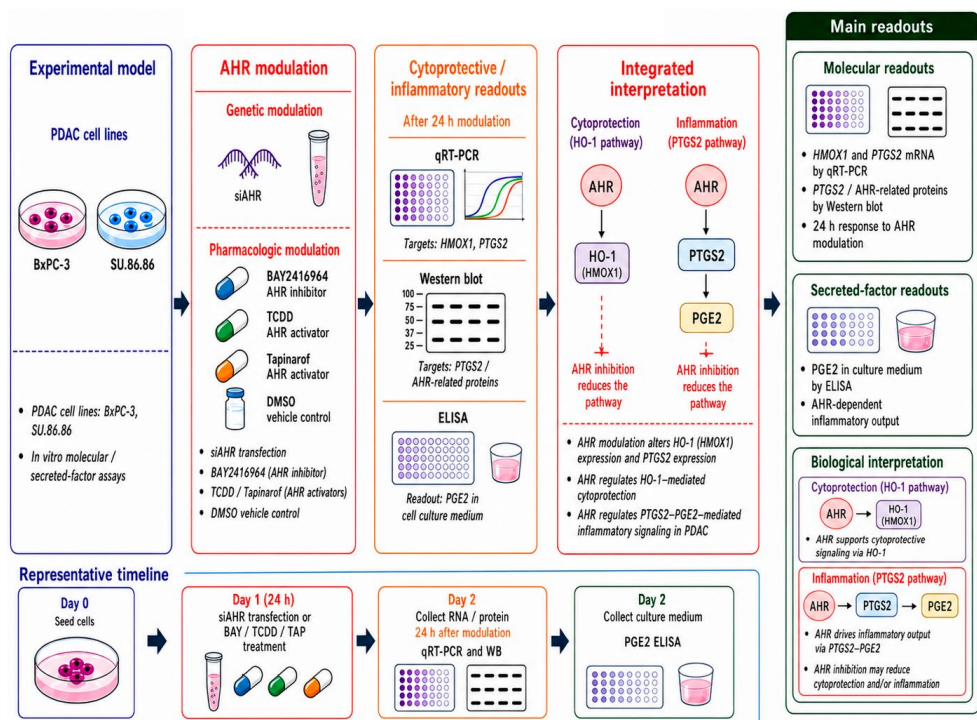


Fig. 3.4. Research workflow for investigating AHR-mediated cytoprotective and inflammatory signaling in PDAC cells

Illustration created using ChatGPT.com

3.2. PDAC cell line culture conditions

PDAC cell lines BxPC-3 (RRID:CVCL_0186), SU.86.86 (RRID:CVCL_3881) were obtained from the European Pancreas Center (Heidelberg, Germany). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO₂ and cultured in RPMI medium (Gibco, Life Technologies Limited, Paisley, UK) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin–streptomycin solution (P/S; Gibco). Cells were grown as adherent monolayers in 25 cm² or 75 cm² culture flasks. Depending on the proliferation rate of each cell line, cells were passaged every 3–7 days using 1× phosphate-buffered saline (PBS, Gibco) and 0.05% trypsin–EDTA solution (Gibco). Cell line validity was ensured by using cells that had undergone no more than 30 passages after thawing authenticated stocks obtained from the provider. Cell cultures were routinely examined under a microscope for bacterial contamination, and periodic testing for

mycoplasma contamination was performed using real-time polymerase chain reaction.

3.3. Gemcitabine treatment and IC₅₀ determination

Cells were seeded in 96-well plates at densities of $2\text{--}2.5 \times 10^3$ cells per well for BxPC-3 and $1\text{--}1.2 \times 10^3$ cells per well for SU.86.86. After seeding, cells were treated with increasing concentrations of gemcitabine (GEM; Fluorochem) ranging from 1 to 10,000 nM prepared by logarithmic dilution in order to determine the half-maximal inhibitory concentration (IC₅₀). Treated cells were incubated for 48 hours at 37 °C in a humidified incubator containing 5% CO₂. Cell metabolic activity was evaluated using the MTT assay as described in the section “Metabolic activity assays”. At least four independent experimental replicates were performed.

3.4. Metabolic activity assays

Cell viability was assessed using either the MTT assay (Invitrogen; 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) or the resazurin reduction assay (Sigma-Aldrich, Missouri, USA). For the MTT assay, MTT reagent was added to the cell culture medium at a final concentration of 0.5 mg/mL and the cells were incubated for 4 hours at 37 °C. Following incubation, the medium containing MTT was removed and the resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO; Carl Roth GmbH) with agitation in the spectrophotometer for 60 seconds. Absorbance was measured using a spectrophotometer (Tecan Sunrise Software v7.1) at a wavelength of 570 nm with a reference wavelength of 620 nm. Alternatively, for the resazurin reduction assay the culture medium was supplemented with 0.1% resazurin and cells were incubated for 3 to 4 hours at 37 °C. Absorbance was subsequently measured spectrophotometrically using 570 nm and 620 nm filters.

3.5. siRNA transfection

siELAVL1, siAHR, and negative control siRNA were obtained from Ambion (USA). Transfections were performed in 96-well plates, 6-well plates, or 25 cm² flasks using Lipofectamine 2000 (Gibco, Invitrogen) according to the manufacturer’s instructions with RPMI medium (Gibco, Invitrogen). All metabolic activity (MTT) experiments included two control groups consisting of untreated cells and cells transfected with negative control siRNA. Each experiment was performed with 3 independent replicates. The efficiency of

gene silencing was evaluated 24–72 hours after transfection using Western blot analysis and 24 hour period was chosen for optimal results.

3.6. AHR protein inhibition and activation

AHR inhibition or activation experiments were performed in 96-, 24-, or 6-well plates. BAY2416964 (BAY) (MedChemExpress, USA) dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA) was used for AHR inhibition. AHR activation was achieved using 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Cambridge Isotope Laboratories, USA) or tapinarof (TAP; HY-109044, MedChemExpress, USA), both dissolved in DMSO. The working concentrations of these compounds were determined by treating cells with increasing concentrations of each drug for 24 hours. The final concentrations were selected as the highest doses that did not affect cell proliferation or viability, as assessed by MTT and/or resazurin assays, but induced measurable changes in gene and protein expression according to qRT-PCR and Western blot analyses. The final concentrations used in experiments were 10 μ M for BAY and TAP and 10 nM for TCDD. DMSO was used as a vehicle control.

3.7. Generation of AHR Knock-out cell lines

The BxPC3 and SU.86.86 AHR KO cell lines were generated using CRISPR/Cas9. The following guide oligos were designed to express the sgRNA; for AHR we used forward primer 5'-CCTACGCCAGTCGCAAGCGG-3' and reverse primer 5'-CCGCTTGCGACTGGCGTAGG-3' that targeted exon 1 of AHR. The sgRNAs were cloned into the pSpCas9(BB)-2A-Puro (pX459) plasmid (Addgene, Watertown, MA, USA; plasmid #62988), containing *Streptococcus pyogenes* (Sp) Cas9 and puromycin genes. The pX459 AHR containing gRNA plasmid was transfected into BxPC3 or SU.86.86 cells, using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Three days after transfection, the cells were exposed to 1 μ g/mL puromycin for 5 days at 37°C and 5% CO₂. The puromycin containing media was exchanged with normal DMEM with 10% FBS medium and the cells were allowed to expand for 5 days. Some of the cells were used to confirm the efficiency of the gRNA at targeting AHR with a T7 endonuclease assay. The cells were diluted to 2 cells/well in a 1 x 96-well plate and colonies were expanded. For AHR ko cells, TCDD dependent increases in CYP1A1 mRNA were used to screen for AHR ko clones by reverse transcription-quantitative Polymerase Chain Reaction (RT-qPCR) as described below.

For T7 endonuclease assay and DNA sequencing, genomic DNA (gDNA) was isolated from wild-type, AHR KO cell lines. The AHR gene was ampli-

fied by PCR GC-RICH PCR System kit (Sigma-Aldrich) according to manufacturer's instructions. Genomic DNA was used in the following reaction conditions were set at a thermal cycler: 95°C for 3 minutes, 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, followed by 72°C for 45 seconds. The PCR primers used were; AHR forward primer 5'-CACTGTCCCGAGAG-GACG-3' and reverse primer 5'-GGGAATGGACCTAATCCCAG-3'. After PCR, the products were denatured and annealed using the following reaction settings: 95°C for 10 minutes, 85°C for 5 seconds, and then a decrease of the temperature of 2°C/second prior to 25°C for 5 seconds, and then a new decrease of 0.1°C/second until a hold on 4°C. Two µl of the T7 endonuclease I enzyme were added, and incubated for at 37°C for 40 min. The products were separated on 7% polyacrylamide gels. To confirm frameshift mutations in the gene edited cell lines, the genomic regions surrounding the gRNA recognition site in AHR and ELAVL1 were sequenced. PCR- amplicons from AHR and ELAVL1 genomic sequences flanking the gRNA binding sites were purified and ligated into PCR vector pCR2.1 (Invitrogen). Minipreps representing individual PCR products were sent for DNA sequencing at Eurofins Genomics. Insertions or deletions (indels) were detected, and frameshift mutations confirmed.

3.8. Drug combination index calculation

Cells were seeded in 96-well plates at densities of $2-2,5 \times 10^3$ cells per well for BxPC-3 cells and $1-1,2 \times 10^3$ cells per well for SU.86.86 cells and incubated for 24 hours. Cells were then treated with increasing concentrations ranging from 10 to 40 µM of BAY2416964 (CAS: 2242464-44-2; Princeton, USA) and tapinarof (CAS: 79338-84-4; Princeton, USA) for 24 hours at 37 °C in a 5% CO₂ incubator. After this incubation period, the culture medium was replaced and the cells were treated with varying concentrations of gemcitabine ranging from 1 to 1000 nM for an additional 48 hours. BAY and TAP were dissolved in DMSO (Carl Roth, Karlsruhe, Germany), and all drug dilutions were freshly prepared immediately before use. Cell viability was assessed using the resazurin reduction assay as described previously. Three independent experimental replicates were performed. The obtained data was analyzed using SynergyFinder+ software (University of Helsinki, Finland) [79]. Drug interaction effects were evaluated using the Highest Single Agent (HSA) mathematical model, and the median combination index was calculated.

3.9. Immunocytochemistry

Cells were seeded in 24-well plates at a density of 1×10^4 cells per well in 1000 μ l of medium. Cells were treated for 24 hours with 10 μ M BAY or TAP or transfected with siAHR or siELAVL1 followed by treatment with the IC50 concentration of gemcitabine for 48 hours. After treatment, cells were fixed using a mixture of 96% ethanol (Vilniaus degtinė, Vilnius, Lithuania) and 5% glacial acetic acid (Honeywell, North Carolina, USA). Permeabilization was performed using 0.5% Triton X-100 (Sigma-Aldrich, Missouri, USA) in phosphate-buffered saline. Cells were incubated with a primary mouse monoclonal ELAVL1 antibody (1:250; Thermo Scientific, Waltham, MA, USA; RRID:AB_2533394) followed by incubation with an Alexa Fluor 488 goat anti-mouse IgG secondary antibody (1:2000; Invitrogen, Carlsbad, CA, USA; RRID:AB_2534069). Cells were washed with PBS followed by nuclease-free water. Slides were mounted using ProLong Diamond Antifade Mountant containing 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen, Carlsbad, CA, USA) for nuclear staining and analyzed using an Olympus IX71 fluorescence microscope (Olympus Corporation, Tokyo, Japan). Three independent experiments were performed; however, one representative replicate is shown.

3.10. Clonogenic assay

After treatment with siAHR, siELAVL1, siControl, BAY, TAP, DMSO or no treatment as described in the section “Immunocytochemistry,” cells were detached using trypsin/EDTA and seeded in 6-well plates at a density of 600 cells per well. Cells were allowed to grow for seven days to allow colony formation. Colonies were then fixed with 96% ethanol and stained with crystal violet (Sigma-Aldrich, Missouri, USA). Excess stain was removed by washing the wells with water, and the plates were allowed to dry at room temperature. Colonies were counted manually in a blinded manner by an investigator not involved in the study using an Olympus IX71 phase-contrast microscope. No less than three independent experimental replicates were performed.

3.11. 2D cell migration assay

2D Cell migration was evaluated using a scratch wound assay. Cells were cultured and treated as described in the “clonogenic assay” section. A total of 2×10^5 cells were seeded in 24-well plates. Additionally, untreated cells with AHR gene knockout generated using CRISPR-Cas technology were used, with vehicle-treated knockout cells serving as controls. After 24 hours of incubation, a scratch was created using a 200 μ l pipette tip, and the medium was replaced with fresh medium without fetal bovine serum. The wound

area was photographed immediately after scratching and after 24 hours using an Olympus IX71 phase-contrast microscope. At least three independent experimental replicates were performed.

3.12. 3D cell migration and invasion assay

3D cell migration was evaluated by treating cells with BAY or DMSO. After treatment $1-2 \times 10^3$ were seeded in ultra low attachment U bottom 96-well plates (Nunc, ThermoFisher Scientific) to generate spheroids. 24 hours after seeding the spheroids were transferred to a flat-bottom 96-well plate and allowed to spread out for 24 hours after which the covered area was measured using Olympus IX71 phase-contrast microscope. 3D cell invasiveness was evaluated by introducing Basement membrane matrix (Geltrex, Gibco) and allowing it to solidify. The invasion of the spheroids into the basement membrane was measured using Olympus IX71 phase-contrast microscope for up to 192 hours for BxPC-3 cell line or up to 72 hours for Su.86.86 cell line. At least three independent experimental replicates were performed.

3.13. Immunoprecipitation assay

PDAC cell lines were cultured in 150 cm² flasks until reaching approximately 80–90% confluence. Cells were lysed using the Magna RIP kit (Millipore, USA). Immunoprecipitation was performed using an ELAVL1 antibody (Ls-C7451, LsBio), while a GAPDH antibody (AM4300, Invitrogen) served as a negative control. Samples for Western blot and qRT-PCR analysis were collected both before and after immunoprecipitation.

3.14. Enzyme-linked immunosorbent assay for PGE₂

Cells were treated for 24 hours with DMSO, TCDD (10 nM), or BAY (10 μ M) in 6-well plates. Culture media were subsequently collected, and PGE₂ concentrations were measured using a PGE₂ ELISA kit (Enzo Life Sciences, USA) according to the manufacturer's protocol. Absorbance was measured using a Multiskan Ascent microplate photometer (Thermo Fisher Scientific, USA).

3.15. RNA extraction and quantitative real-time polymerase chain reaction

Cells were seeded in 6-well plates at a density of 1×10^5 cells per well in 1500 μ l of medium and treated as with siControl, siAHR, siELAVL1,

BAY, TAP or DMSO. Total RNA was extracted using the PureLink RNA Mini Kit (Invitrogen, USA) according to the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Complementary DNA was synthesized from 1 µg of RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative real-time PCR was performed in 20 µl reaction mixtures containing 2 µl of cDNA template, 1× PCR master mix (Applied Biosystems), and gene-specific primers. The following TaqMan assays were used: ELAVL1 (Hs00171309_m1), AHR (Hs00169233_m1), DCK (Hs01040725_m1), PTGS2 (Hs00153133_m1), HO-1 (Hs01110250_m1), SNAIL (Hs00195591_m1), SNAIL2 (Hs00950344_m1), ZEB1 (Hs01566410_m1), ZEB2 (Hs03832582_s1), TWIST (Hs01675818_s1), E-CAD (Hs01023895_m1), VIM (Hs0095811_m1) and GAPDH (Hs02786624) as the housekeeping gene. Amplification was performed using an ABI 7500 Fast Real-Time PCR system.

3.16. Tissue sample collection and RNA extraction

PDAC tissue and adjacent non-tumorous tissue samples were obtained from 55 patients who underwent partial duodenopancreatectomy (Whipple procedure) for pancreatic carcinoma. The study cohort included 31 male and 24 female patients, with a median age of 64 years at the time of surgery. The presence of cancerous tissue in the collected samples was confirmed histologically. Tumor TNM stage was assigned by pathologists at the Lithuanian University of Health Sciences Kauno Klinikos according to standard clinical criteria. Tumour tissue and adjacent non-tumorous tissue were collected from the same patients, with the latter serving as reference controls. Tissue samples were snap-frozen in liquid nitrogen immediately after surgical removal and stored at -80 °C until further use. Ethical approval was obtained from the Kaunas Regional Biomedical Research Ethics Committee, Lithuania (No. BE-2-31). Written informed consent for the use of surgical specimens for research purposes was obtained from all patients or their legal representatives. Total RNA extraction from frozen tissue samples was performed using the same kit described for cell lines.

3.17. Western blot analysis

Cells were seeded and treated as described in the “RNA extraction and quantitative real-time polymerase chain reaction” section. Cells or tissue were lysed using RIPA lysis buffer (Abcam, Cambridge, UK) supplemented with protease inhibitors (Roche, Basel, Switzerland). Tissue samples were

homogenized using ceramic bead-based homogenization, while cell lysates were subjected to sonication. Lysates were centrifuged at $10,000 \times g$ for 10 minutes at 4 °C, and the resulting supernatants were collected. Protein concentrations were determined using a BCA protein assay kit (Thermo Scientific, Waltham, MA, USA). Protein samples (25–50 µg) were heated at 97 °C for 5 minutes before separation by 4–12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transfer to polyvinylidene fluoride membranes (Merck Millipore, USA). Membranes were blocked with 5% skim milk blocking buffer (Sigma-Aldrich, Missouri, USA) for 60 minutes at room temperature and incubated with primary antibodies for 1.5 hours at room temperature or overnight at 4 °C. The following primary antibodies were used: anti-HuR (Thermo Fisher Scientific; 390600), anti-AHR (Thermo Fisher Scientific; MA1514), anti-GAPDH (Invitrogen; AM4300), anti-PTGS2 (LsBio; LS-C339544), and anti-DCK (Invitrogen; MA5-25502). Membranes were washed with TBST and incubated with horseradish peroxidase-conjugated secondary antibodies. Signals were detected using chemiluminescence substrates and visualized using a Bio-Rad documentation system. Three independent biological replicates were performed, and one representative result is shown.

3.18. Data analysis

Data were analysed using GraphPad Prism software version 6.01 (GraphPad Software Inc., La Jolla, CA, USA). All in vitro experiments were performed in at least three independent biological replicates unless stated otherwise. Data distribution was not assumed to be normal; therefore, non-parametric statistical tests were used. Results are presented as mean $\pm$ standard deviation for in vitro experiments and as median with interquartile range for patient tissue analyses, unless indicated otherwise. Comparisons between two independent groups were performed using the Mann–Whitney U test. Comparisons between paired tumour and adjacent non-tumorous tissue samples were performed using paired non-parametric analysis where applicable. Correlations between gene expression levels were assessed using Spearman's rank correlation coefficient (r). Patients were stratified according to AHR expression status as described in the Results section. Drug interaction effects were analysed using SynergyFinder+ software and the Highest Single Agent model, with results reported as median synergy scores. Statistical significance was defined as $p < 0.05$, and all tests were two-sided.

4. RESULTS

4.1. Clinical relevance of AHR expression in PDAC tissues

The results of patient tissue gene expression revealed a strong correlation between *AHR* and *ELAVL1* ($r = 0.757$), *AHR* and *DCK* ($r = 0.657$), *ELAVL1* and *DCK* ($r = 0.701$) (Fig. 4.1).

	AHR	ELAVL1	DCK	ZEB1	ZEB2	SNAI1	SNAI2	TWIST	E-CAD	VIM	PTGS2	HO-1
AHR		*	*	*		*	*		*	*	*	*
ELAVL1	0,757		*	*		*	*		*	*	*	*
DCK	0,657	0,701		*		*	*		*	*	*	*
ZEB1	0,727	0,701	0,651			*	*			*	*	*
ZEB2	0,084	-0,121	-0,238	0,166				*				
SNAI1	0,760	0,703	0,537	0,851	0,114		*	0,131	0,113	0,582	0,635	0,714
SNAI2	0,767	0,701	0,597	0,884	0,166	0,908		0,156	0,223	0,507	0,600	0,714
TWIST	-0,040	-0,254	-0,280	0,076	0,705	0,131	0,156		-0,028	0,109	-0,302	0,214
E-CAD	0,396	0,350	0,358	0,231	0,177	0,113	0,223	-0,028		0,327	0,365	
VIM	0,580	0,408	0,387	0,590	0,274	0,582	0,507	0,109	0,327		0,553	
PTGS2	0,703	0,693	0,669	0,628	-0,184	0,635	0,600	-0,302	0,365	0,553		*
HO-1	0,929	0,901	0,786	0,685	-0,250	0,714	0,714	0,214			0,757	

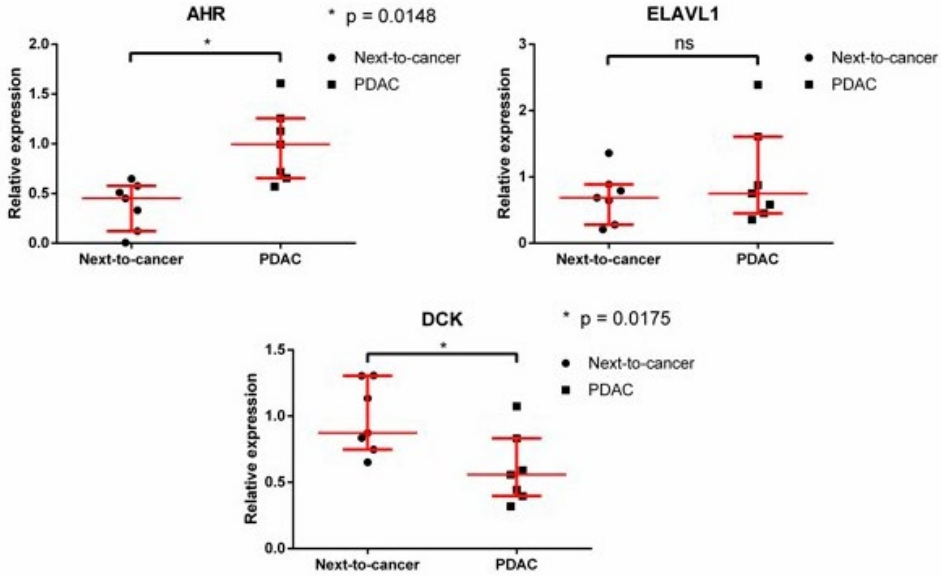
Fig. 4.1. Spearman correlation matrix of *AHR*, *ELAVL1*, *DCK*, EMT-related and cytoprotective gene expression in PDAC tissues

The heatmap presents pairwise Spearman correlation coefficients between the investigated markers in PDAC tissue samples. Green shading indicates positive correlations, whereas yellow shading indicates weak or negative correlations. Bold values marked with an asterisk (*) indicate statistically significant correlations ($p < 0.05$).

The correlation was subsequently tested using protein analysis. A comparison of the protein expression levels in next-to-cancer pancreas and PDAC tissues from the same patients revealed increased AHR protein expression and decreased DCK protein expression. Although patient mRNA data revealed a positive correlation between *AHR*, *ELAVL1* and *DCK* gene expression, protein analysis comparing cancer and adjacent healthy tissue revealed that increased AHR protein production leads to decreased DCK protein production, suggesting that there are other posttranscriptional

mechanisms involved, causing differences in gene expression and protein translation (Fig. 4.2 a,b).

a. Relative protein expression comparison of PDAC vs Next-to-cancer tissue



b. Membrane visualization of tissue samples

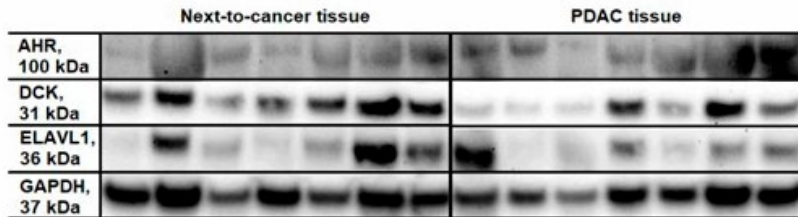


Fig. 4.2. Protein expression of AHR and DCK in paired PDAC and adjacent non-tumorous pancreatic tissues

Western blot analysis of paired patient tissue samples demonstrates increased AHR protein expression and decreased DCK protein expression in PDAC tissue compared with adjacent non-tumorous pancreatic tissue. Quantified relative protein expression is shown together with representative Western blot membrane images. a) Graphical representation of relative protein expression; median. b) Membrane blot images. N = 7 patients. * $p \leq 0.05$.

Additionally, cancer tissue analysis revealed strong correlations between AHR expression and expression of EMT transcription factors ZEB1 ($r = 0.727$), SNAI1 ($r = 0.760$) and SNAI2 ($r = 0.767$) as well weaker, but

positive correlations with classical EMT biomarkers E-CAD ($r = 0.396$) and Vimentin ($r = 0.580$) showing a possible role of AHR in maintaining a highly aggressive hybrid EMT form (Fig. 4.2). Additionally AHR showed a strong correlation with cytoprotective molecules PTGS2 ($r = 0.703$) and HO-1 ($r = 0.929$). To further investigate the role of AHR in regulation of EMT and PTGS2 expression, the cancer tissue was compared with adjacent healthy tissue of the same patient and the patient were divided into two groups – downregulated AHR and upregulated AHR (Fig. 4.3).

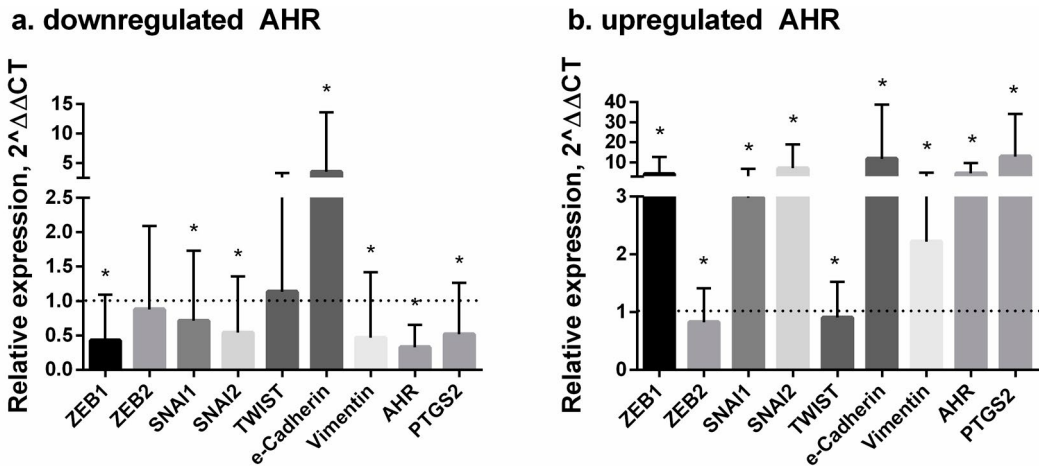


Fig. 4.3. EMT-related and cytoprotective gene expression in PDAC tissues stratified by AHR expression

Relative gene expression of EMT-associated markers and PTGS2 was compared between PDAC and paired adjacent non-tumorous pancreatic tissue after stratifying patients according to decreased or increased AHR expression in tumour tissue. a) Downregulated AHR patients. b) Upregulated AHR patients

The results showed that patients with decreased AHR levels in tumour tissue exhibited decreased ZEB1 (42%), SNAI1 (71%), SNAI2 (54%), Vimentin (46%), PTGS2 (52%) and increased E-Cadherine expression (3.5-fold) compared to adjacent healthy tissue. While patient with increased AHR expression exhibited increased ZEB1 (4.3-fold), SNAI1(3-fold), SNAI2(7.2 fold), E-Cadherine (11.8-fold), Vimentin (2.2-fold), PTGS2 (12.9-fold) expression and decreased ZEB2 (83%), TWIST (90%) expression. The results further elucidate the role of AHR in EMT regulation showing that AHR expression could be a promising biomarker for cancer aggressiveness and cytoprotection.

The further analysis of AHR expression relation with clinical data showed that T stage had no relation to AHR expression, however patients with higher

AHR expression presented with higher N stage of the disease. Patients in N0 group presented with only 3% (median) AHR expression compared to adjacent healthy tissue. Patients in N1 group presented with 70% (median) AHR expression compared to adjacent healthy tissue and patients in N2 group presented with 2.8-fold (median) increased AHR expression (Fig. 4.4).

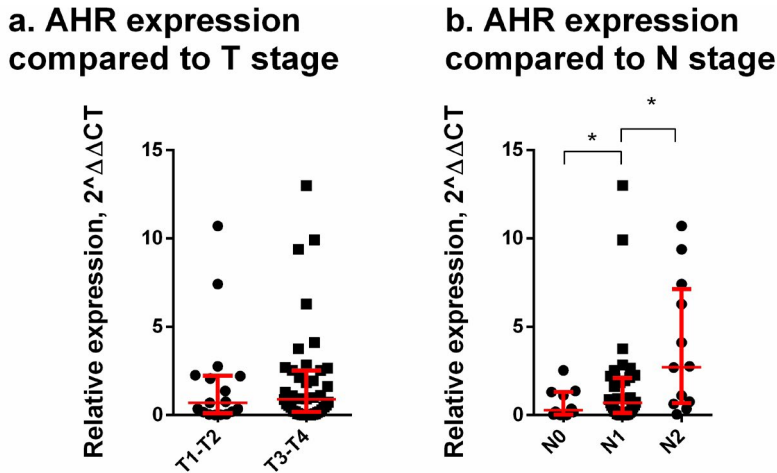


Fig. 4.4. Association of AHR expression with clinicopathological features in PDAC patients

a) relative AHR expression comparing T1-T2 and T3-T4 stages. b) relative AHR expression comparing N0, N1, N2 stages.

4.1.1. Tissue analysis section discussion

The results of the present study demonstrate that AHR expression is closely associated with multiple clinically relevant and biologically aggressive features of PDAC. The observed positive correlations between *AHR*, *ELAVL1* and *DCK* expression suggest the existence of a tightly interconnected regulatory network linking transcriptional and post-transcriptional mechanisms in PDAC biology. While previous studies have independently associated AHR and ELAVL1 with tumour progression and therapeutic resistance in pancreatic cancer, their simultaneous evaluation together with DCK, EMT-related markers and cytoprotective molecules in patient tissue has remained limited. The findings of this study therefore provide additional evidence supporting the role of AHR as a central regulator of PDAC aggressiveness and therapy response.

One of the most notable findings was the strong positive correlation between *AHR* and *ELAVL1* expression. Previous experimental studies

have suggested that AHR may influence ELAVL1 localisation and activity, particularly through modulation of its nuclear-cytoplasmic shuttling [78]. The current patient tissue data further support the existence of biologically relevant interplay between these molecules in PDAC. Interestingly, although mRNA analysis demonstrated positive correlations between *AHR*, *ELAVL1* and *DCK* expression, protein analysis revealed increased AHR protein expression together with decreased DCK protein expression in tumour tissue compared to adjacent healthy pancreatic tissue. This discrepancy suggests that transcriptional expression alone may not fully reflect the downstream functional consequences of AHR signalling. Instead, post-transcriptional regulatory mechanisms, potentially mediated through ELAVL1 localisation and activity, may contribute to altered DCK protein translation and subsequent chemoresistance. These findings are consistent with previous reports demonstrating that cytoplasmic localisation of ELAVL1 is required for DCK stabilisation and gemcitabine sensitivity [29].

The present study additionally demonstrated significant associations between *AHR* expression and EMT-related markers. Increased *AHR* expression positively correlated with transcription factors associated with EMT activation, including *ZEB1*, *SNAIL1* and *SNAIL2*, while also correlating with *vimentin* expression. These findings support previous evidence implicating AHR signalling in tumour progression and EMT regulation in multiple malignancies [47,73,74]. Importantly, simultaneous positive associations with both epithelial and mesenchymal markers may suggest that AHR contributes to maintenance of a partial or hybrid EMT phenotype rather than a complete epithelial-to-mesenchymal transition. Such intermediate EMT states are increasingly recognised as biologically important due to their association with increased tumour plasticity, metastatic potential and therapeutic resistance [66,68,70]. Although the present study was not specifically designed to characterise EMT states in detail, the observed expression patterns support the hypothesis that AHR contributes to aggressive and adaptable tumour phenotypes in PDAC.

Further supporting this interpretation, stratification of patient samples according to relative AHR expression revealed substantial differences in EMT-associated and cytoprotective gene expression. Tumours with increased *AHR* expression demonstrated increased expression of multiple EMT-related transcription factors together with markedly elevated *PTGS2* expression. *PTGS2* is a well-established mediator of inflammation, tumour progression and resistance to apoptosis, and its regulation by AHR has been previously described in other cancer models [78,80,81]. The strong increase in *PTGS2* expression observed in the current study suggests that AHR may contribute

not only to tumour invasiveness but also to maintenance of a pro-inflammatory and cytoprotective tumour microenvironment.

The association between AHR expression and clinicopathological parameters further highlights the potential clinical relevance of AHR signalling in PDAC. While no clear association was observed between AHR expression and primary tumour stage (T), increased *AHR* expression was associated with higher lymph node involvement. Lymphatic spread is a key indicator of aggressive disease and poor prognosis in PDAC, suggesting that elevated AHR expression may be linked to increased metastatic potential. These findings are in agreement with previous studies reporting protumourigenic roles of AHR in pancreatic cancer and other malignancies [45–47].

Taken together, the results of this section suggest that increased AHR expression is associated with aggressive tumour biology, EMT-related transcriptional reprogramming and cytoprotective signalling in PDAC. The observed associations between AHR, ELAVL1 and DCK further support the hypothesis that AHR may act as a central regulator linking tumour aggressiveness and chemoresistance through transcriptional and post-transcriptional mechanisms. Although additional mechanistic studies are required to fully elucidate the precise molecular pathways involved, these findings provide a strong rationale for further investigation of AHR as a potential prognostic biomarker and therapeutic target in pancreatic cancer.

4.2. Role of AHR in ELAVL1/DCK-mediated gemcitabine chemoresistance

4.2.1. Drug dose calculation

The metabolic activity of PDAC cells was determined by MTT assay. The cells were treated with LOG doses of GEM and IC50 doses were determined separately for both cell lines: Su.86.86 – 36.91 ± 1.40 nM, BxPC-3 – 26.67 ± 1.69 nM (Fig. 4.5 a,b).

a. viability graph by log concentrations

b. calculated IC50 concentrations in nM

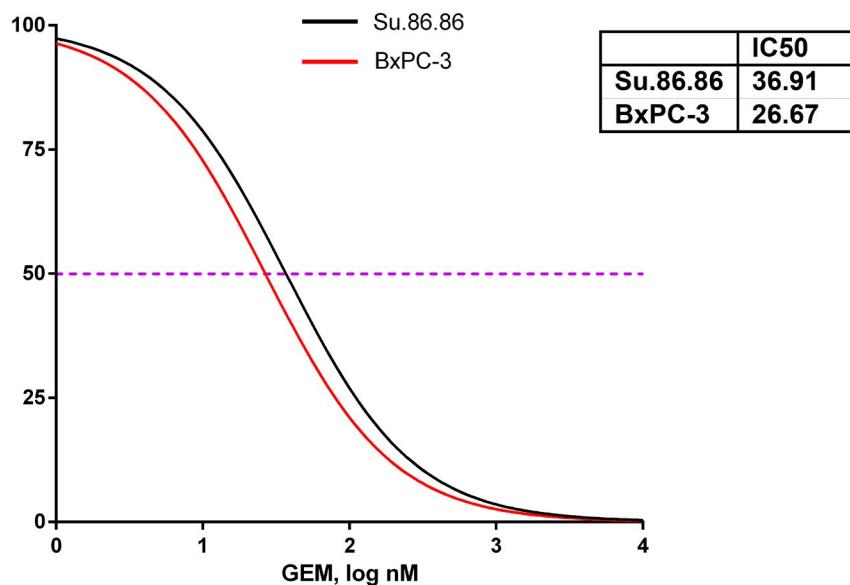


Fig. 4.5. Determination of gemcitabine IC50 concentrations in PDAC cell lines

a) log graph of IC50 calculation. b) table of numerical value of IC50 doses in nM.

The doses of BAY-2416964 (BAY)(AHR inhibitor) and tapinarof (TAP) (AHR activator) compounds used for the experiments were determined by treating the cells with increasing concentration of BAY and TAP after which cell metabolic activity was measured using resazurin reduction assay. The highest tested dose that did not affect cell metabolism was 10 μ M for both BxPC-3 and Su.86.86 pancreatic adenocarcinoma cell lines (Fig. 4.6a).

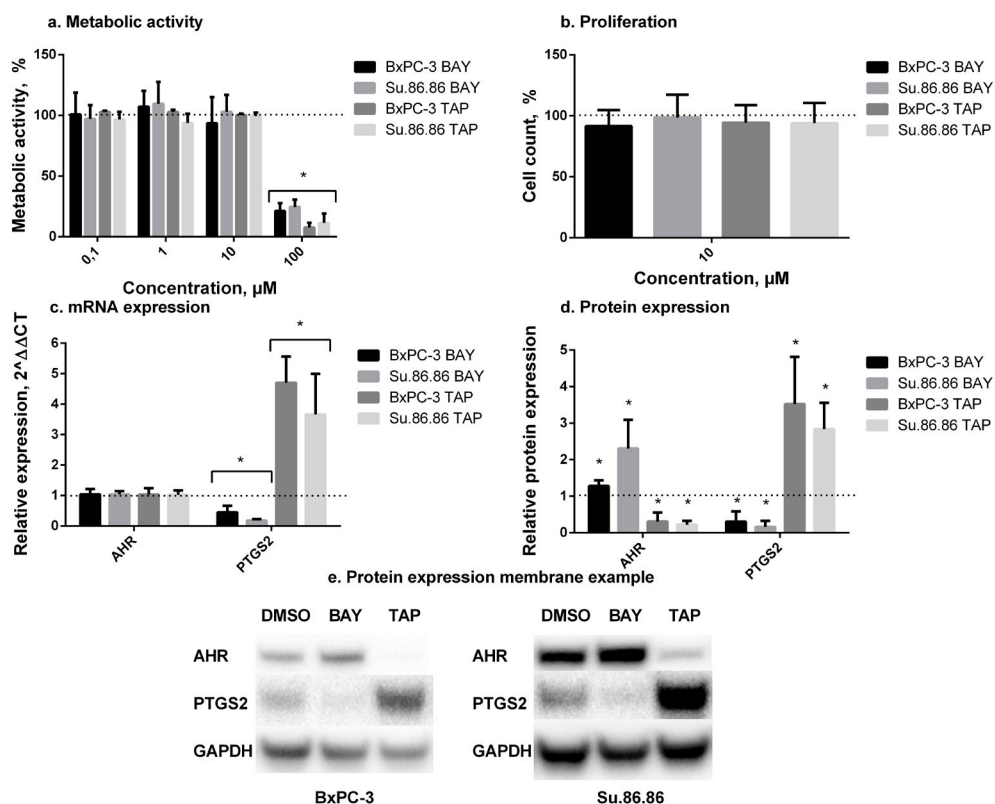


Fig. 4.6. BAY and TAP dose determination after 24 h treatment for PDAC cell lines

a) Effects on cell metabolic activity. b) Effects of 10 μM BAY or TAP on cell proliferation. c) Effects of 10 μM BAY or TAP on AHR and PTGS2 mRNA expression. d) Effects of 10 μM BAY and TAP on AHR and PTGS2 protein expression. e) Western blot membranes. N = 3 independent experiments (protein expression data are shown for 1 representative WB experiment). * $p \leq 0.05$ when comparing to vehicle control. Dotted line = vehicle control (a, b – 100%; c, d – 1).

While 10 μM concentration was shown to have no effect on cell metabolism, 100 μM TAP or BAY significantly decreased PDAC cell metabolism to 8–25% (Fig. 4.6 a). Additionally, 10 μM BAY or TAP had no effect on cell proliferation (Fig. 4.6 b). Although 10 μM BAY or TAP did not have adverse effects on cell metabolic activity, it did have a significant effect on AHR activity, as shown by prostaglandin-endoperoxide synthase-2 (PTGS2) which is regulated by AHR [81] (Fig. 4.6 c-e). BAY and TAP had no effect on AHR mRNA expression; however, BAY (AHR inhibitor) decreased PTGS2 mRNA expression to 45% in BxPC-3 and 19% in Su.86.86, whereas TAP (AHR activator) increased PTGS2 mRNA expression 4.7-fold in BxPC-3 and 3.7-

fold in Su.86.86. Similar PTGS2 changes were observed in terms of protein expression. BAY decreased PTGS2 expression to 31% in BxPC-3 and 16% in Su.86.86, whereas TAP increased PTGS2 expression 3.5-fold in BxPC-3 and 2.8-fold in Su.86.86 (Fig. 4.6 d, e). With respect to changes in AHR protein expression, AHR inhibition (BAY) increased AHR protein expression (BxPC-3 1.3-fold; Su.86.86 2.3-fold), whereas AHR activation (TAP) decreased AHR protein expression (BxPC-3 to 31%; Su.86.86 to 22%).

4.2.2. AHR and ELAVL1 expression interplay

To assess and confirm the relationship of AHR and ELAVL1 Human PDAC cell lines (BxPC-3, Su.86.86) were transfected with small interfering RNAs (siRNAs): siAHR or siELAVL1 for 24 hours. Western blot analysis revealed that the AHR and ELAVL1 genes were silenced almost completely after 24 hours in both lines. In BxPC-3, siAHR decreased AHR mRNA to 20% and the protein was not detected; in Su.86.86, AHR mRNA decreased to 29% and the protein decreased to 2% compared to non-treated control cells (Fig 4.7 a-f)

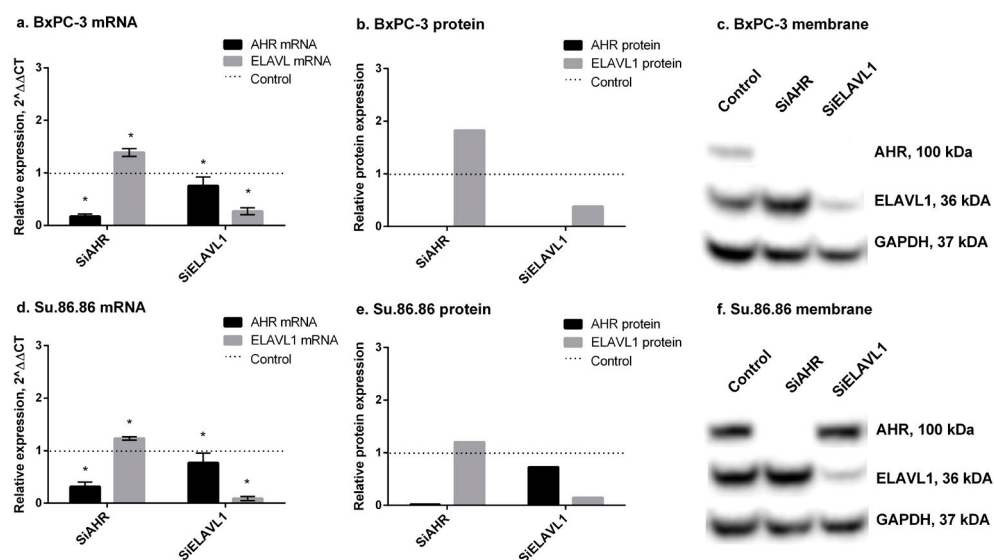


Fig. 4.7. AHR and ELAVL1 qRT-PCR and WB analysis

mRNA and protein expression of AHR and ELAVL1 genes and proteins, after AHR or ELAVL1 silencing by transfection. qRT-PCR N = 3, MEAN±SD. * p < 0.05. a) BxPC-3 qRT-PCR analysis b) BxPC-3 WB analysis c) membrane of BxPC-3 WB d) Su.86.86 qRT-PCR analysis. e) Su.86.86 WB analysis. f) membrane of Su.86.86 WB.

When silencing ELAVL1 in BxPC-3 cells, the ELAVL1 mRNA levels decreased to 31% and the ELAVL1 protein levels decreased to 38% compared to non-treated control cells. Similarly, in Su.86.86 cells, the ELAVL1 mRNA levels decreased to 9% and the ELAVL1 protein levels decreased to 15% compared to non-treated control cells. These results indicate successful silencing of both the target mRNA and the subsequent protein expression for ELAVL1 (Fig. 4.7 a-f).

Moreover, AHR silencing increased ELAVL1 mRNA in both cell lines (BxPC3 – 1.4-fold; Su.86.86 – 1.2-fold). The same increase was seen in protein levels (BxPC-3 ELAVL1 protein increased 1.83-fold and Su.86.86 1.2-fold).

ELAVL1 silencing caused a decrease in AHR mRNA (in BxPC-3 AHR mRNA decreased significantly to 76% and in Su.86.86 to 77%) as well as in protein (in BxPC-3 AHR protein was absent and in Su.86.86 decreased to 73%).

4.2.3. ELAVL1 protein mediated AHR mRNA stabilization

Since a relationship between AHR and ELAVL1 can be seen on mRNA and protein expression levels, the immunoprecipitation (IP) assay was used to determine a direct link between AHR mRNA and ELAVL1 protein. ELAVL1 is known to bind to various mRNAs including DCK [29] and HO-1 [82]. The AHR gene has 9 adenylate uridylate (AU)-rich Elements (AREs) (ATTTA) which is used as a binding motif for ELAVL1 protein [83]. The qRT-PCR results of IP showed that the ELAVL1 protein binds AHR mRNA (Fig. 4.8 a).

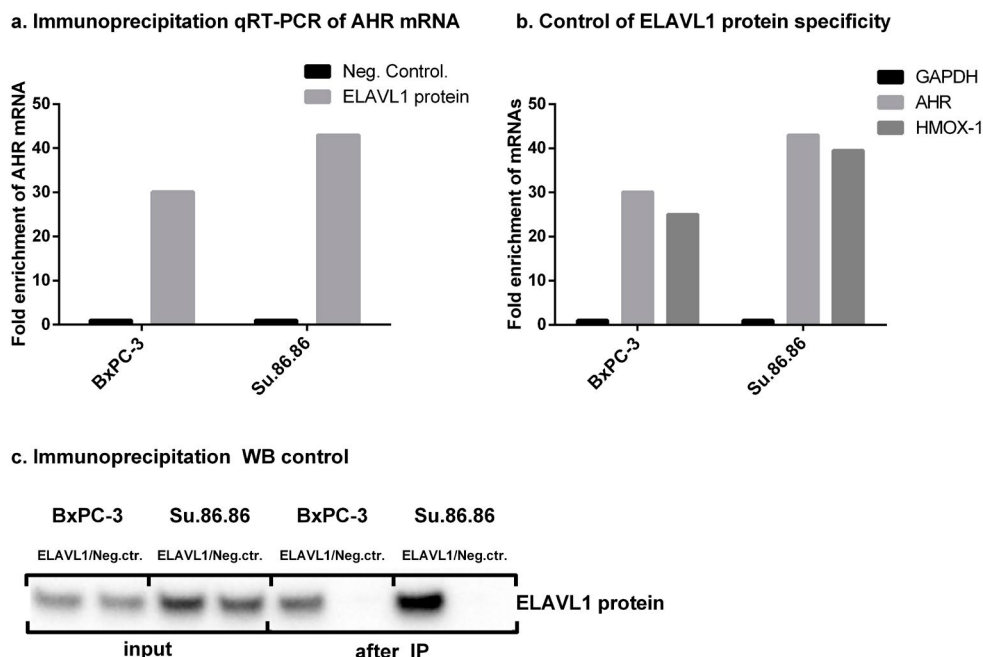


Fig. 4.8. Immunoprecipitation of ELAVL1 protein

IP of ELAVL1 protein showing the ability of the ELAVL1 protein to bind AHR mRNA. a) qRT-PCR assay showing fold change in AHR mRNA when compared to the negative control (GAPDH). b) qRT-PCR assay showing GAPDH as a negative target mRNA and AHR and HO-1 as positive mRNA targets for ELAVL1 protein. c) WB assay showing absence and presence of the ELAVL1 protein after the IP assay.

As a control for the assay, GAPDH mRNA was tested as a negative control, which ELAVL1 protein did not bind to, and HO-1 mRNA as a known target for a positive control (Fig. 4.8 b). AHR mRNA sequence analysis, together with our experimental data, indicate that the ELAVL1 protein binds AHR mRNA and thereby potentially modulates AHR translation. Western blot assay was used as a control of the IP assay (Fig. 4.8 c).

4.2.4. ELAVL1 cellular localization shift

Cellular localisation shifts in ELAVL1 and AHR protein after the silencing of ELAVL1 or AHR genes was elucidated by immunocytofluorescence (ICF) assay.

AHR mainly resides in the cytoplasm of both PDAC cell lines. The shift of AHR protein was not noticeable in either cell line when silencing ELAVL1 and/or treating with GEM (photos not included).

The ELAVL1 protein mainly resides in the nucleus of both PDAC cell lines (Fig. 4.9).

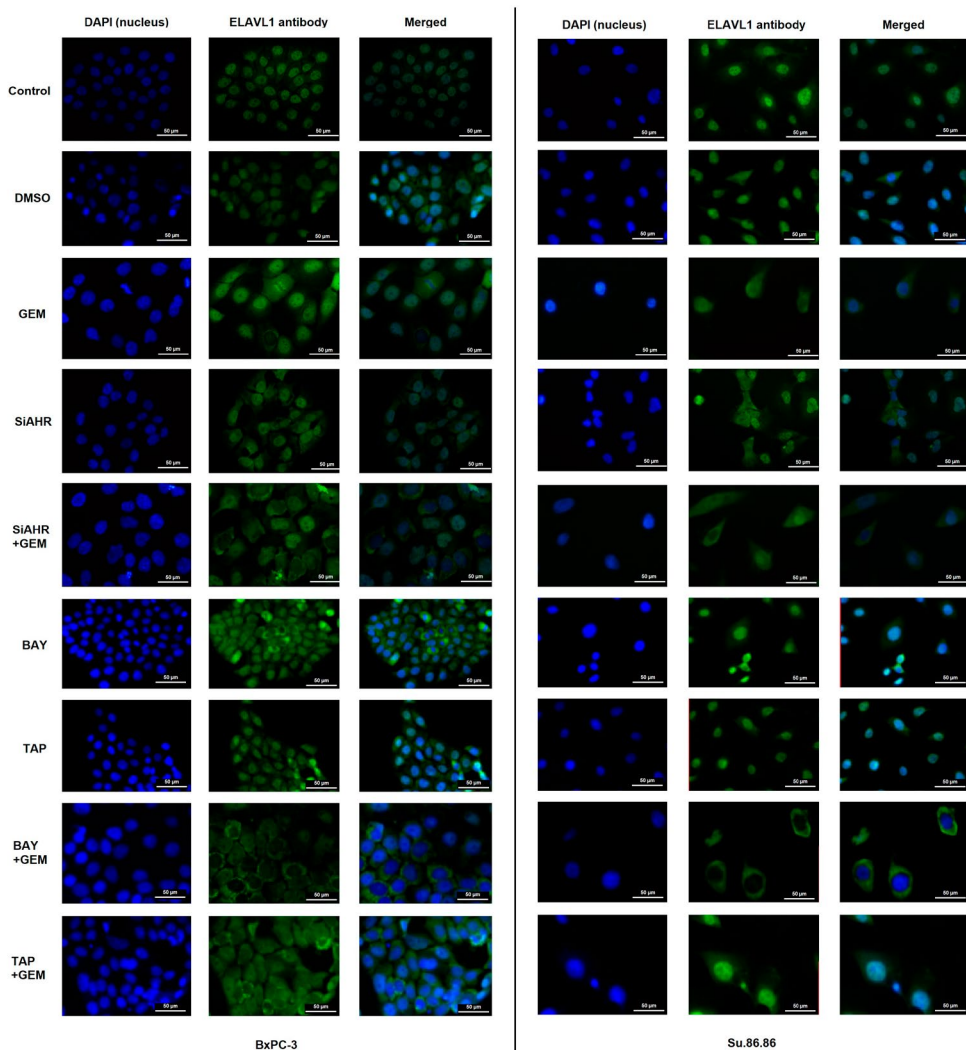


Fig. 4.9. *ELAVL1 subcellular localization after AHR modulation and gemcitabine treatment in PDAC cell lines*

Representative immunocytochemistry images showing ELAVL1 localization in BxPC-3 and Su.86.86 cells after AHR silencing or pharmacological modulation with BAY2416964 and tapinarof, with or without gemcitabine treatment.

When treating cells with GEM, the concentration of cytoplasmic ELAVL1 increases, showing that GEM acts as a signal for ELAVL1 to shift its localisation. When silencing AHR, the concentration of cytoplasmic ELAVL1

increases, showing that AHR can be involved in sequestering ELAVL1 in the nucleus. The joint effect of AHR silencing and GEM treatment increased cytoplasmic ELAVL1 concentrations even more than separately, indicating that the silencing of AHR could be beneficial to ELAVL1 translocation in response to GEM treatment.

These results were seconded by using AHR inhibitor and activator. ICF assay revealed treatment of BxPC-3 cells with BAY caused ELAVL1 translocation in a small number of cells, however TAP treatment had no effect on ELAVL1 localisation. This effect was not observed in the Su.86.86 cell line as BAY or TAP treatment had little to no effect on ELAVL1 localisation. Compared with treatment with GEM alone, treatment with BAY prior to GEM caused ELAVL1 translocation to the cytoplasm in higher ratio of observed cells, whereas TAP treatment had the opposite effect. These results indicate that AHR activity can increase or decrease ELAVL1 nuclear-cytoplasmic shuttling.

4.2.5. AHR modulation effect on GEM related gene expression

The findings of AHR-ELAVL1 interplay suggest a mutual influence and potential regulatory relationship between AHR and ELAVL1. As ELAVL1 primarily exerts its activity in the cytoplasm, it was important to investigate the mRNA and protein expression levels of its downstream targets related to gemcitabine resistance, specifically DCK – the rate limiting or bottleneck enzyme of gemcitabine activity.

SiAHR increased DCK mRNA (1.6-fold) and protein slightly (1.1-fold) in the BxPC-3 cell line, however it did not change DCK mRNA and even decreased protein levels (to 56%) in Su.86.86 cell line.

When silencing of ELAVL1 DCK mRNA did not change in either cell line; however, the DCK protein decreased in both (BxPC-3 – 65%; Su.86.86 – 57%) (Fig 4.10).

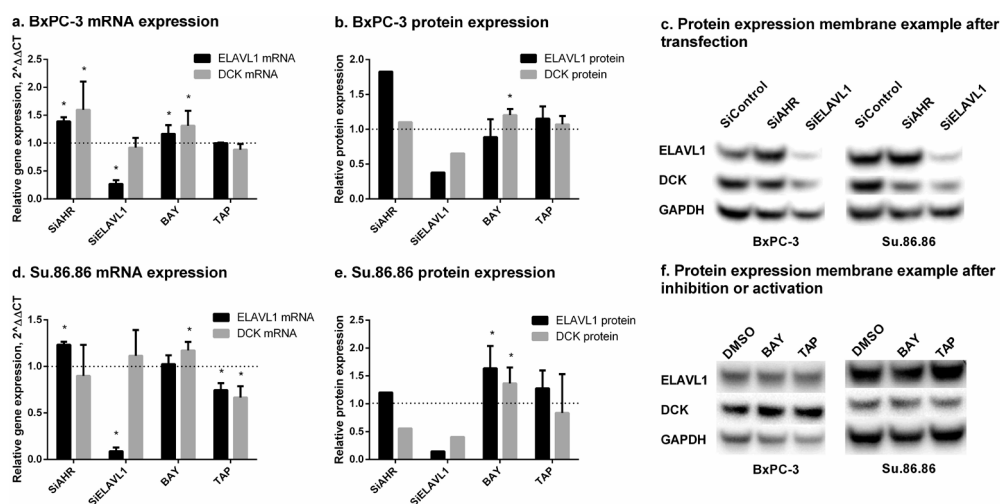


Fig. 4.10. AHR and ELAVL1 modulation effect on GEM related genes and proteins

a. BxPC-3 mRNA expression. b. BxPC-3 protein expression. c. Example membranes after AHR and ELAVL1 transfection. d. Su.86.86 mRNA expression. e. Su.86.86 protein expression. f. Example membranes after AHR modulation. N = 3. *p < 0.05.

These results show that ELAVL1 pathway genes and proteins somewhat correspond to changes in AHR and ELAVL1 expression levels; however, it is more noticeable for protein level and differs between cell lines.

The results of the effects of BAY and TAP on GEM-related gene and protein expression (ELAVL1; DCK) revealed that BAY increased ELAVL1 mRNA expression 1.17-fold but did not change protein expression in the BxPC-3 cell line. As for Su.86.86, ELAVL1 mRNA expression was not affected, whereas ELAVL1 protein expression increased 1.6-fold (Fig. 4.10). Treating cells with TAP did not affect ELAVL1 mRNA or protein expression. In Su.86.86, TAP decreased ELAVL1 mRNA expression to 75% but did not change protein expression. Although ELAVL1 mRNA and protein expression somewhat differed between the cell lines, DCK expression changes were similar between the mRNA and protein levels in each of the cell lines. BAY increased DCK mRNA expression (BxPC-3 – 1.31-fold; Su.86.86 – 1.17-fold) and protein expression (BxPC-3 – 1.2-fold; Su.86.86 – 1.36-fold). On the other hand, TAP did not change DCK mRNA expression in BxPC-3 and decreased its expression in Su.86.86 to 66%. TAP treatment did not change protein expression in either cell line. Although the results were ambiguous when comparing inhibitors with mRNA silencing and the levels of changes were different between the cell lines, the results showed that inhibition of

AHR can lead to increased DCK expression both on mRNA and protein levels (Fig. 4.10)

4.2.6. AHR modulation effect on response to GEM treatment

To determine the effect of AHR or ELAVL1 gene silencing on cell chemoresistance, AHR or ELAVL1 genes were silenced for 24-hours by transfection with siAHR or siELAVL1. After silencing, the cells were treated with an IC₅₀ dose of GEM for 48-hours. The metabolic activity of all groups was compared to the control group (100%) of non-treated cells for GEM alone or siControl for transfected cells. GEM alone significantly reduced cell viability to 57% for the BxPC-3 cell line and 48.4% for the Su.86.86 cell line. There was no significant effect on BxPC-3 or Su.86.86 cell viability after the silencing of AHR or ELAVL1. However, the silencing of AHR together with GEM significantly decreased the viability of both cell lines when compared to GEM alone (BxPC-3 to 25.8% and Su.86.86 to 23.1% cell viability). In BxPC-3, the silencing of ELAVL1 together with GEM did not have a significant effect on cell viability when compared to GEM alone; however, in Su.86.86, the combined effect of siELAVL1 and GEM when compared to GEM alone significantly decreased cell viability to 23.1% (Fig. 4.11 a, b).

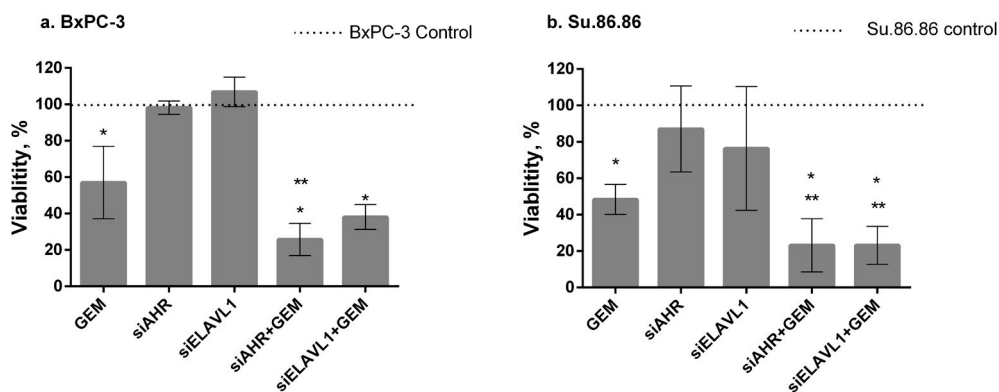


Fig. 4.11. Cell viability analysis by MTT assay

PDAC cell viability after silencing of AHR or ELAVL1 for 24 hours and treatment with or without IC₅₀ GEM for 48 hours. a) BxPC-3 cells. b) Su.86.86 cells. N ≥ 4, MEAN±SD
*p < 0.05 when comparing to respective control. **p < 0.01 when comparing to GEM alone

The effect was further investigated by using varying doses of BAY or TAP and GEM and calculating combination index after measuring metabolic activity. The metabolism inhibition effect of BAY and GEM was dose-dependent both as monotherapies and in combination (Fig. 4.12 a, b).

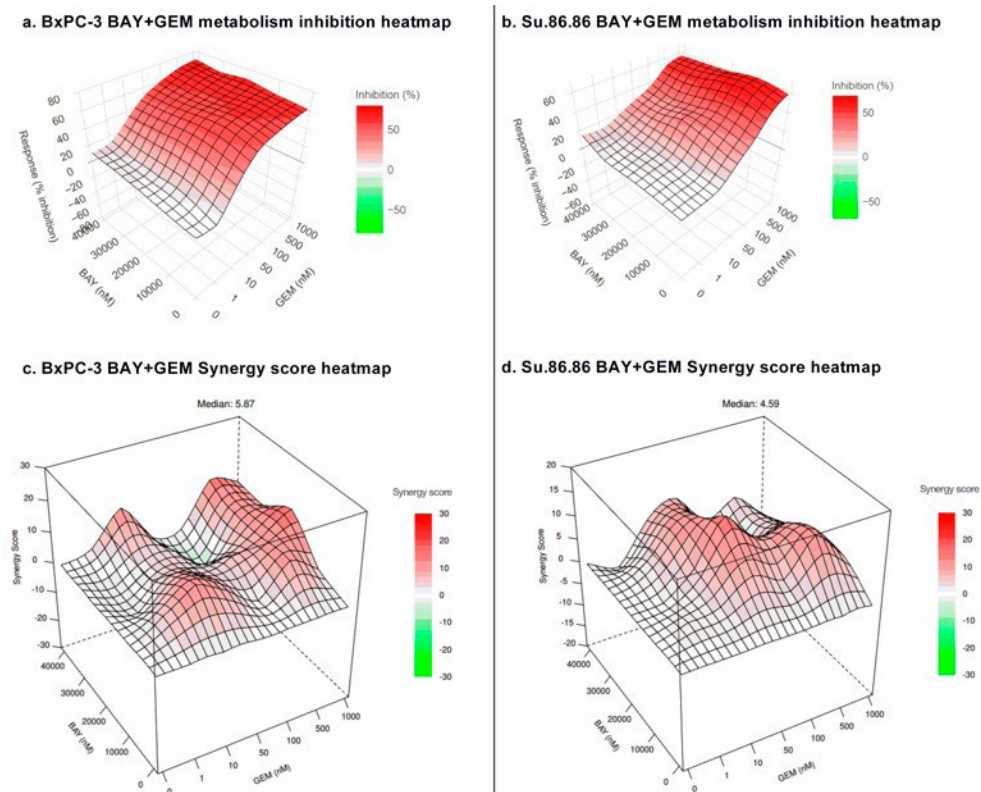


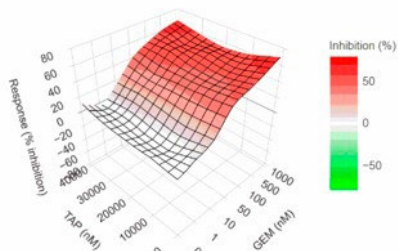
Fig. 4.12. Metabolism inhibition and HSA synergy score calculation heatmaps

a) Metabolism inhibition of BAY + GEM in the BxPC-3 cell line. b) Su.86.86 cell line metabolism inhibition of BAY + GEM. c) BxPC-3 cell line synergy of BAY + GEM. d) Su.86.86 cell line synergy of BAY + GEM. N = 3 independent experiments.

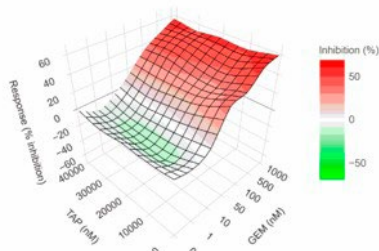
The combination score median from the HSA synergy model revealed that BAY synergizes with GEM and increases its effectiveness (median BxPC-3: 5.87; median Su.86.86: 4.59) (Fig. 4.12 c, d).

The cell viability inhibition effects of TAP and GEM were similar in terms of their dose–response effects on BxPC-3 cells (Fig. 4.13 a). However, in Su.86.86, cell viability was increased by TAP monotherapy as well as in combination with low doses of GEM (Fig. 4.13 b). Additionally, TAP had an antagonistic effect on the GEM response (BxPC-3 median –5.52; Su.86.86 median –9.42) (Fig. 4.13 c, d).

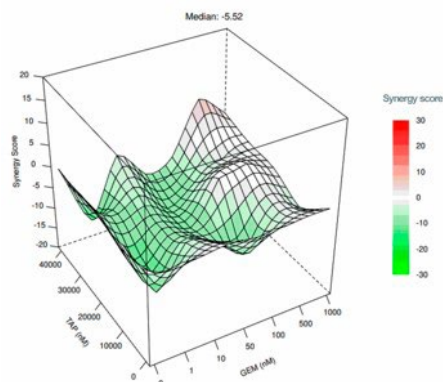
a. BxPC-3 TAP+GEM metabolism inhibition heatmap



b. Su.86.86 TAP+GEM metabolism inhibition heatmap



c. BxPC-3 TAP+GEM Synergy score heatmap



d. Su.86.86 TAP+GEM Synergy score heatmap

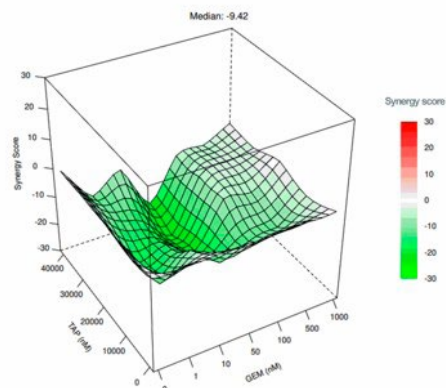


Fig. 4.13. Metabolism inhibition and HSA synergy score calculation heatmaps

a) cell viability inhibition of TAP + GEM in the BxPC-3 cell line. b) Su.86.86 cell line metabolism inhibition of TAP + GEM. c) BxPC-3 cell line synergy of TAP + GEM. d) Su.86.86 cell line synergy of TAP + GEM. N = 3 independent experiments.

Notably, not all the tested dose combinations showed strong synergistic effects (BAY) or antagonistic effects (TAP), and the overall median of BAY+GEM or TAP+GEM were not universal between the cell lines and were not very pronounced. Nonetheless, inhibition of AHR activity increased GEM effectiveness, whereas activation of AHR activity decreased GEM effectiveness.

4.2.7. Long term survival analysis using clonogenic assay

The ability of cell lines to form colonies was measured by clonogenicity assay (Fig. 4.14).

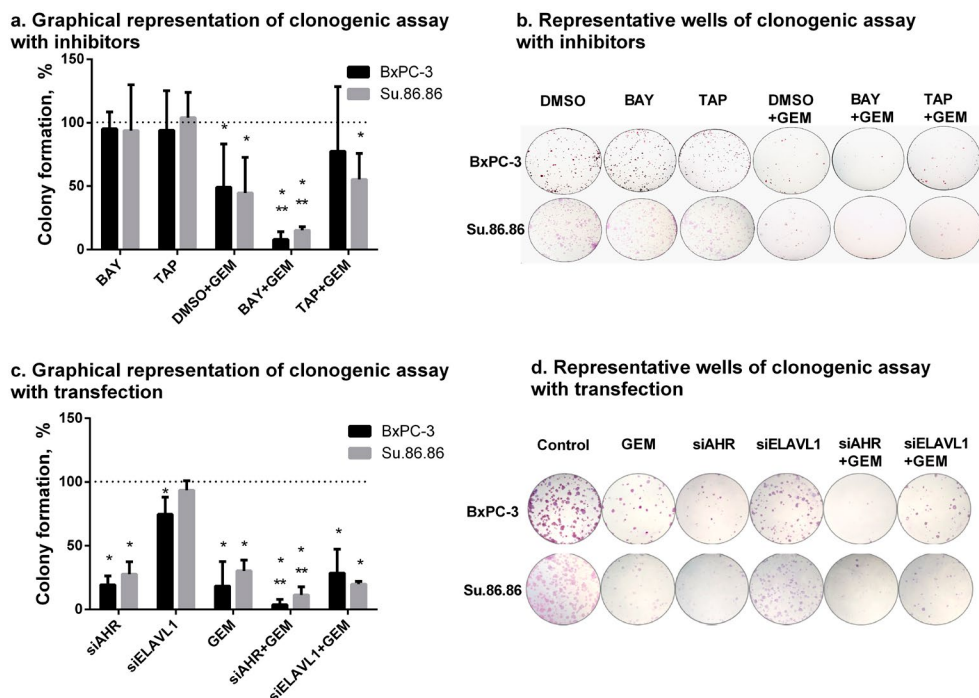


Fig. 4.14. PDAC cell colony formation. Colony formation after using siAHR, siELAVL1, BAY or TAP and/or exposure to GEM

Cells for clonogenic assay were seeded after treatment and grown for 168 hours after seeding. a) Graphical representation of experiments using AHR inhibitor and activator. b) Inhibitor/activator experiment colony formation example photos. c) Graphical representation of experiments using siAHR and siELAVL1 transfection. d) Transfection experiment colony formation example photos.. $N \geq 4$, $MEAN \pm SD$ * $p < 0.05$ when comparing to respective control. ** $p < 0.05$ when comparing to GEM or GEM+DMSO

The effect of gemcitabine was more detrimental to long-term than short-term cell survival. IC₅₀ doses of GEM significantly decreased cell colony formation to 18.3% compared to non-treated control in the BxPC-3 cell line and to 30.3% in the Su.86.86 cell line. The silencing of AHR also had a more detrimental effect on long-term than short-term survival. Both cell lines had a significant decrease in colony formation (19.3% formation in the BxPC-3 cell line and 27.7% formation in the Su.86.86 cell line compared to the non-treated control). The silencing of ELAVL1 had a significant effect on BxPC-3 colony formation (colony formation decreased to 74.7%) but had no effect on Su.86.86 cell line colony formation. The silencing of AHR significantly increased the effect of GEM (BxPC-3 to 3.7% and Su.86.86 to 11.3%); however, due to severe effects on both GEM and siAHR, it is unclear whether

the effect is due to increased sensitivity to GEM or the additive effect of siAHR and GEM. The silencing of ELAVL1 did not significantly change the effects of GEM on either cell line (Fig 4.14 a,b). AHR activity modulation and GEM treatment effect on cell colony formation showed different results to that of mRNA silencing. While BAY or TAP alone did not have a significant effect on the colony formation of either of the PDAC cell lines, the compounds did change the cell response to GEM. GEM+DMSO (vehicle control) significantly decreased cell colony formation to 49% for BxPC-3 and 45% for Su.86.86 (Fig. 4.14 c,d). When cells were treated with BAY prior to GEM treatment, cell colony formation decreased to 8% for BxPC-3 cells and 15% for Su.86.86 cells, which was a statistically significant decrease compared with that of GEM+DMSO. Treating cells with TAP prior to GEM treatment did not yield a statistically significant difference in results compared to GEM+DMSO. In comparison to vehicle control, BxPC-3 cell line colony formation decreased only to 78%, which was not statistically different, whereas Su.86.86 colony formation decreased to 55% (10% weaker than that of GEM).

4.2.8. Chemoresistance section discussion

One of the most important determinants of gemcitabine sensitivity is DCK, the rate-limiting enzyme responsible for intracellular activation of gemcitabine through phosphorylation [24,28]. Reduced DCK expression has consistently been associated with impaired gemcitabine activation and resistance in pancreatic cancer. Previous studies have additionally demonstrated that ELAVL1 stabilizes DCK mRNA and enhances gemcitabine sensitivity, particularly when ELAVL1 is localized in the cytoplasm [29,84,85]. The findings of the present study further extend this regulatory model by identifying AHR as a novel upstream regulator of ELAVL1 localization and DCK-associated chemoresistance.

The current study demonstrated a reciprocal relationship between AHR and ELAVL1, suggesting the existence of a previously undescribed feedback regulatory mechanism. Silencing of AHR increased cytoplasmic localization of ELAVL1, particularly following gemcitabine exposure, whereas pharmacological activation of AHR reduced ELAVL1 cytoplasmic translocation. These findings suggest that AHR activity may contribute to sequestration of ELAVL1 within the nucleus, thereby limiting its post-transcriptional stabilizing activity on DCK mRNA. Importantly, RIP-IP analysis further demonstrated that ELAVL1 directly binds AHR mRNA, indicating that ELAVL1 simultaneously regulates AHR expression while also being functionally regulated by AHR activity. Together, these findings

suggest the existence of a dynamic negative feedback interaction between AHR and ELAVL1.

The biological significance of this relationship appears highly context-dependent. ELAVL1 regulates multiple transcripts involved in cell survival, inflammation, angiogenesis and metastasis, including BCL-2, VEGF, HIF-1 α , PTGS2 and MMP-9 [48,52,54]. Consequently, increased cytoplasmic localization of ELAVL1 may simultaneously exert both pro-survival and chemosensitizing effects depending on the dominant downstream signaling pathways involved. Within the context of gemcitabine treatment, however, increased cytoplasmic ELAVL1 appears beneficial due to enhanced stabilization of DCK mRNA and improved gemcitabine activation [29,84].

The effects of AHR modulation on DCK expression further support the proposed AHR–ELAVL1–DCK regulatory axis. AHR silencing or pharmacological inhibition generally increased DCK expression and enhanced sensitivity to gemcitabine, whereas AHR activation showed opposite tendencies. Although the magnitude and consistency of responses differed between BxPC-3 and Su.86.86 cell lines, the overall direction of the observed effects suggests that AHR activity contributes to maintenance of a chemoresistant phenotype. The variability observed between cell lines likely reflects the well-recognized molecular heterogeneity of PDAC, including differences in KRAS signaling, differentiation status and baseline EMT-associated phenotypes [68,69,86].

The therapeutic relevance of targeting AHR signaling is additionally supported by previous studies demonstrating improved antitumor efficacy following inhibition of upstream kynurenine pathway signaling and AHR activation [35,87,88]. Together with the findings of the present study, these observations suggest that pharmacological inhibition of AHR may represent a promising strategy for overcoming gemcitabine resistance in PDAC. Importantly, the current study strengthens this concept by demonstrating consistent effects using multiple experimental approaches, including siRNA-mediated gene silencing, pharmacological modulation and patient tissue analysis.

Taken together, the findings of this section support the hypothesis that AHR contributes to gemcitabine resistance in PDAC through modulation of ELAVL1 localization and downstream DCK expression. The identified AHR–ELAVL1 feedback interaction introduces an additional layer of post-transcriptional regulation influencing chemotherapy response and highlights AHR as a potential therapeutic target for sensitizing PDAC cells to gemcitabine treatment.

4.3. Role of AHR in EMT-associated PDAC cell aggressiveness

4.3.1. AHR modulation effect on cancer aggressiveness related genes

The results of AHR silencing showed a decrease in PTGS2 (down to 40% in BxPC-3 and 27% in Su.86.86). For EMT transcription factors, SNAI2 mRNA expression was decreased in both cells lines (down to 56% in BxPC-3 cell line and 61% in Su.86.86 cell line) as well as ZEB2 which was decreased to 70% in BxPC-3 while Su.86.86 cells showed no effect. Epithelial biomarkers E-CAD mRNA expression was increased in BxPC-3 cell line (1.3-fold) and decreased in Su.86.86 cell line (down to 62%). Mesenchymal markers VIM mRNA was decreased in both cell lines (down to 89% in BxPC-3 and 39% in Su.86.86 cell line). HO-1 mRNA was affected only in BxPC-3 cell line and increased 3.1-fold (Fig. 4.15).

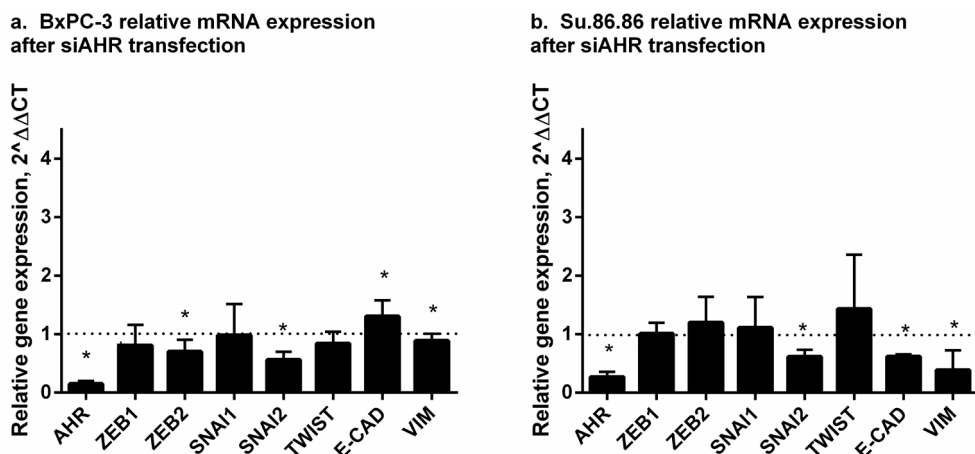


Fig 4.15. Effect of AHR silencing on EMT-related gene expression in PDAC cell lines

Relative mRNA expression of EMT-associated markers was assessed in BxPC-3 and Su.86.86 cells following siRNA-mediated AHR silencing. a) BxPC-3 cell line. B) Su.86.86 cell line. N = 3. *- p < 0.05. Dotted line – control cells normalized to 1.

4.3.2. AHR modulation effect on PDAC cell migration and invasiveness

The results of 2D scratch assay after AHR modulation showed decreased migration in majority of the test groups. Migration was decreased by silencing AHR (BxPC-3 by 73%; Su.86.86 by 54%) (Fig. 4.16 a), inhibiting (BxPC-3

by 36%; Su.86.86 – no change) (Fig. 4.16 b) and knocking-out (BxPC-3 by 51%; Su.86.86 – KO not tested) (Fig. 4.16 c).

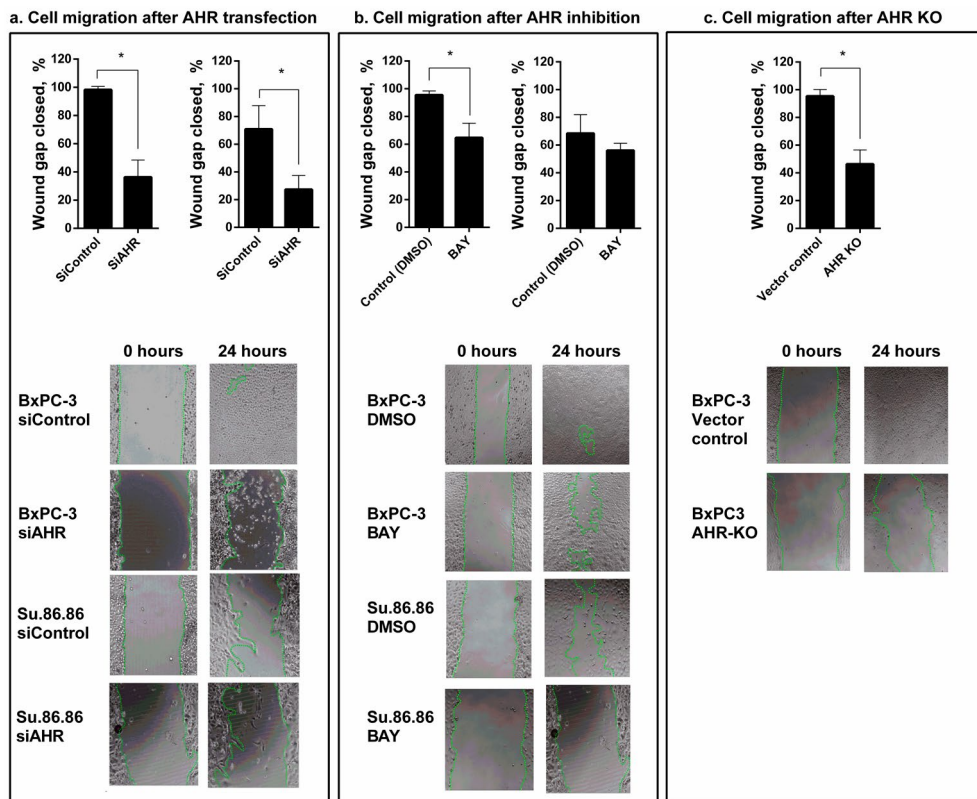


Fig. 4.16. Effect of AHR modulation on 2D migration in PDAC cell lines

Representative images and quantitative analysis of scratch wound migration in BxPC-3 and Su.86.86 cells following AHR silencing, pharmacological AHR inhibition and CRISPR/Cas9-mediated AHR knockout. a) Cell migration after AHR transfection. b) Cell migration after AHR inhibition. c) Cell migration after AHR KO. N = 3, *p < 0.05.

To further investigate the effect of AHR inhibition has on migration and invasiveness of PDAC cell lines, 3D sheroid method was used. The results showed that inhibition of AHR protein decreased cell migration in 3D setting – BxPC-3 to 65%, Su.86.86 to 69%, compared to vehicle control (Fig. 4.17 a).

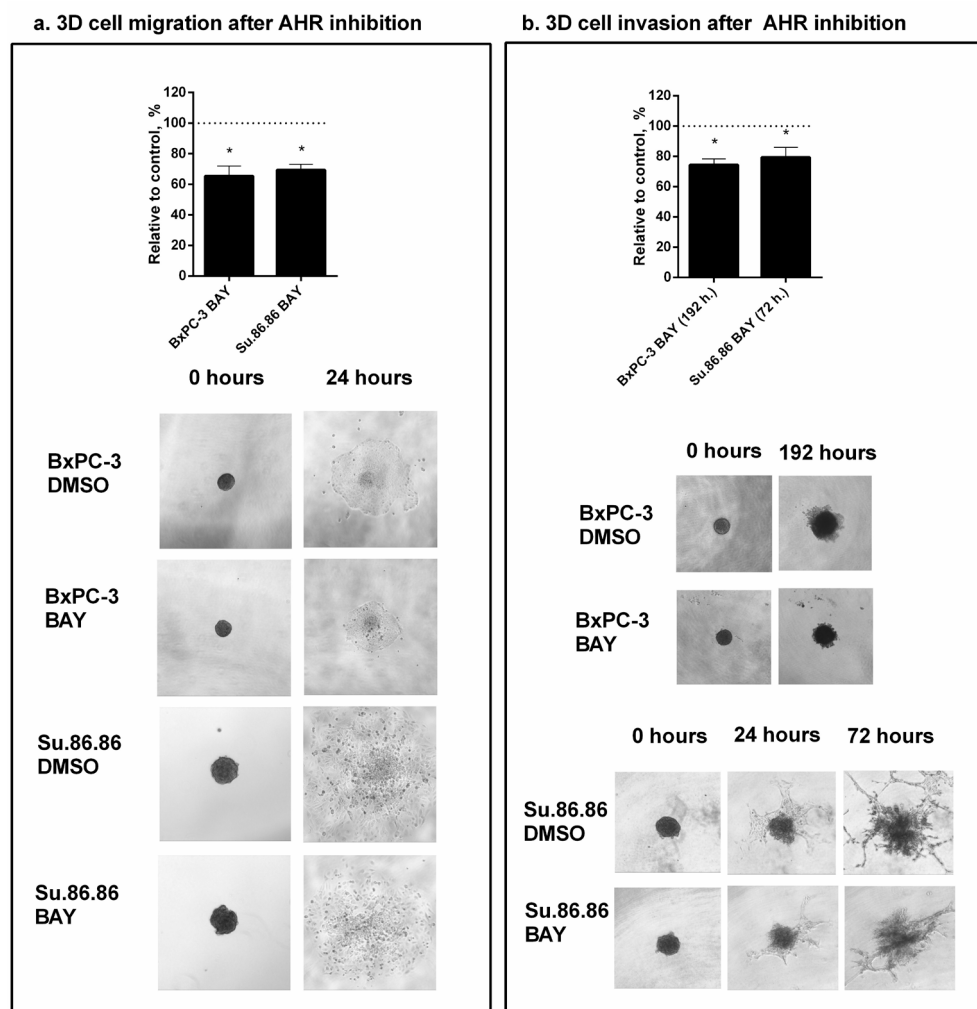


Fig. 4.17. Effect of AHR inhibition on 3D migration and invasion of PDAC spheroids.

Representative images and quantitative analysis of 3D spheroid migration and basement membrane matrix-based invasion in BxPC-3 and Su.86.86 cells following pharmacological AHR inhibition. a) 3D cell spheroid migration after AHR inhibition. b) 3D cell spheroid invasion after AHR inhibition. N = 3, *p < 0.05.

A weaker, but still statistically significant effect was also seen when investigating cell invasiveness. BxPC-3 cell line exhibited a much slower invasion into the basement membrane and after 192 hours post BAY treatment the invasiveness was decreased to 74% compared to vehicle control. Su.86.86 exhibited a much more robust invasion which was already visible after 24

hours. After 72 hours post BAY treatment, Su.86.86 cell line invasiveness was decreased to 79% compared to vehicle control (Fig. 4.17 b).

The results of migration and invasion analysis revealed that targeting AHR decreases PDAC cell line migration and invasion capabilities in both 2D and 3D setting in the majority of investigated experiment groups.

4.3.3. EMT section discussion

Tumour aggressiveness and metastatic progression remain among the major factors contributing to poor prognosis in PDAC. One of the key biological processes associated with increased tumour invasiveness, metastatic dissemination and therapeutic resistance is epithelial–mesenchymal transition (EMT). EMT is a highly dynamic and reversible process during which epithelial tumour cells acquire mesenchymal characteristics, enabling enhanced migration, invasion and adaptation to hostile microenvironmental conditions [66]. Increasing evidence suggests that EMT in PDAC rarely occurs as a complete transition but rather as a spectrum of intermediate or hybrid phenotypic states associated with increased tumour plasticity and survival capacity [68,69,89].

The findings of the present study suggest that AHR contributes to the regulation of EMT-associated aggressiveness in PDAC. Increased AHR expression in patient tissues positively correlated with multiple EMT-related transcription factors, including ZEB1, SNAIL and SNAIL2, together with increased vimentin expression. The functional significance of these findings was further supported by in vitro experiments. AHR modulation altered migration and invasion capacity of PDAC cell lines, while AHR silencing and pharmacological inhibition generally reduced aggressive cellular behavior. These observations support the concept that AHR signaling contributes to PDAC cell plasticity and invasive potential. Importantly, the effects differed between BxPC-3 and Su.86.86 cell lines, further highlighting the biological heterogeneity of PDAC and suggesting that the consequences of AHR signaling may depend on the molecular background and differentiation status of individual tumour subtypes.

The role of AHR in tumour progression has been increasingly recognized in multiple malignancies. Beyond its classical role as an environmental sensor and regulator of xenobiotic metabolism, AHR has emerged as an important mediator of cellular adaptation, inflammatory signaling and tumour progression [31,47]. Recent studies have additionally demonstrated that AHR signaling may promote protumorigenic immune responses and facilitate tumour progression in pancreatic cancer [34]. The present findings extend these observations by suggesting that AHR may additionally contribute to

tumour aggressiveness through regulation of EMT-associated pathways and cellular motility.

The precise molecular mechanisms linking AHR signaling and EMT regulation remain incompletely understood and are likely context-dependent. AHR signaling interacts with numerous pathways involved in EMT regulation, including TGF- β , NF- κ B, hypoxia-associated signaling and inflammatory mediators [47,81]. Additionally, several EMT-associated transcription factors contain regulatory regions potentially influenced by AHR-mediated transcriptional activity. The current study suggests that AHR may function not necessarily as a direct initiator of EMT, but rather as a regulator of EMT-associated cellular plasticity and adaptive behaviour.

An important observation of the present study was that inhibition of AHR reduced migration and invasion without uniformly reversing all EMT-associated molecular markers. This finding further supports the concept that EMT represents a continuum rather than a binary biological process. It is therefore possible that AHR primarily contributes to maintenance of invasive and adaptive phenotypes rather than complete epithelial-to-mesenchymal conversion. Such partial EMT states have been associated with enhanced metastatic efficiency, increased resistance to therapy and increased tumour-initiating capacity [68,70,90]

The relationship between AHR and ELAVL1 identified in the current study may additionally contribute to regulation of tumour aggressiveness. ELAVL1 stabilizes numerous transcripts involved in migration, invasion and survival, including MMP-9, VEGF and inflammatory cytokines. Consequently, AHR-mediated regulation of ELAVL1 localization may indirectly influence multiple pathways associated with EMT and metastatic progression. The interplay between AHR signaling, ELAVL1 activity and EMT-associated transcriptional programs therefore likely represents a highly interconnected regulatory network contributing to aggressive PDAC biology.

Taken together, the findings of this section suggest that AHR contributes to PDAC aggressiveness through modulation of EMT-associated pathways, migration and invasion. Rather than acting solely as a classical transcription factor involved in xenobiotic signaling, AHR appears to function as a broader regulator of tumour plasticity and adaptive cellular behaviour. These findings further support the potential relevance of AHR as a therapeutic target in PDAC, particularly in the context of limiting tumour invasiveness, metastatic progression and therapy-associated adaptation.

4.4. Role of AHR in cytoprotective and inflammatory signalling in PDAC cells

The results of AHR silencing showed a decrease in PTGS2 (down to 40% in BxPC-3 and 27% in Su.86.86. HO-1 mRNA was affected only in BxPC-3 cell line and increased 3.1-fold (Fig. 4.18).

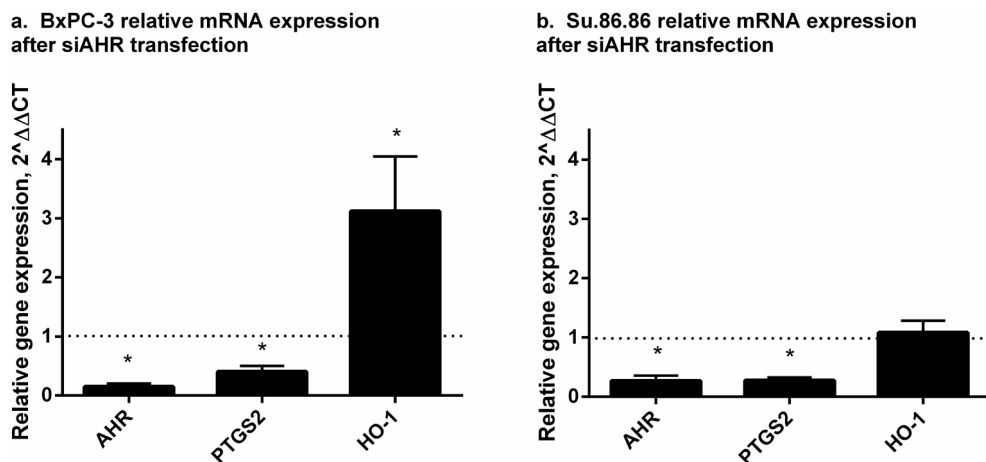


Fig. 4.18. Effect of AHR silencing on cytoprotective gene expression in PDAC cell lines

Relative mRNA expression of cytoprotective markers was assessed in BxPC-3 and Su.86.86 cells following siRNA-mediated AHR silencing. a) BxPC-3 cell line. b) Su.86.86 cell line. N = 3. *- p < 0.05. Dotted line – control cells normalized to 1.

Since both of the cell lines showed a decrease in PTGS2 expression after AHR silencing (Fig. 4.18), downstream analysis of PGE2 synthesis was conducted using modulation of AHR protein using 10 μ M BAY (AHR inhibitor) or 10 nM TCDD (AHR activator) (Fig. 4.18).

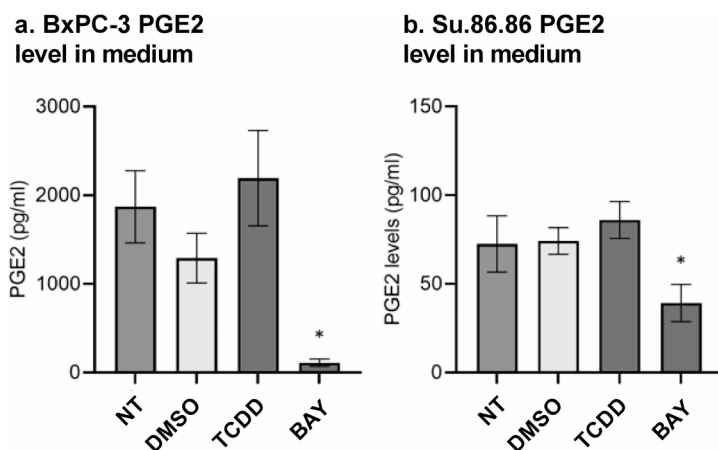


Fig. 4.19. *Effect of AHR activity modulation on PGE2 production in PDAC cell lines*

PGE2 concentrations were measured in the culture medium of BxPC-3 and Su.86.86 cells following pharmacological modulation of AHR activity. a) PGE2 levels in BxPC-3 medium. b) PGE2 levels in BxPC-3 medium. N = 3. *p < 0.05 compared to non treated control (NT). DMSO – vehicle control; TCDD – AHR activator; BAY – AHR inhibitor.

In both cell lines, the cells secreted PGE2 to medium without any treatment (NT). The detected concentration was lower in SU.86.86 cells (72 pg/mL) and higher in BxPC-3 cells (1870 pg/mL) (Fig. 4.19). To confirm if the regulatory effect of AHR on PTGS2 expression can be detected also downstream on the effector molecule, PGE2 concentrations were measured after TCDD (10 nM) or BAY (10 μ M) treatment for 24 h in cell media. The results demonstrate that although additional activation of AHR by TCDD did not lead to increased secretion of PGE2, the inhibition of AHR significantly decreased PGE2 levels in the media. In SU.86.86 the decrease was from 72 to 39 pg/mL and an even more drastic reduction of PGE2 levels was observed in BxPC-3 cells – from 1870 to 112 pg/mL (Fig. 4.19). In the current study, we did not focus on assessing the physiological effect resulting from the changes of PGE2 levels. However, PGE2 also acts in an autocrine manner through EP2 and EP4 receptors which are also expressed in both cell lines (data not shown). Thus, it is reasonable to hypothesise that AHR, through regulating PTGS2-PGE2, could influence PDAC cell migration and metastasis [80].

4.4.1. Cytoprotection section discussion

PDAC is characterized by a highly hostile tumour microenvironment involving chronic inflammation, oxidative stress, hypoxia and continuous exposure to cytotoxic stimuli. To survive under these conditions, PDAC

cells activate multiple cytoprotective and adaptive signaling pathways that promote resistance to apoptosis, metabolic adaptation and therapy tolerance. Increasing evidence suggests that AHR signaling plays an important role in coordinating many of these adaptive cellular responses through regulation of inflammatory mediators, oxidative stress responses and immune-associated signaling pathways [10,17,91].

The findings of the present study demonstrate that AHR modulation significantly influences the expression of cytoprotective and inflammatory molecules in PDAC cells. Increased AHR activity promoted expression of PTGS2 and HO-1 together with increased PGE2 synthesis, whereas inhibition or silencing of AHR reduced expression of these molecules. These findings suggest that AHR contributes to maintenance of a cytoprotective and pro-inflammatory phenotype that may support tumour survival and resistance to stress-associated damage.

PTGS2 represents one of the key mediators of inflammation-associated tumour progression. Increased PTGS2 expression has previously been associated with enhanced proliferation, angiogenesis, resistance to apoptosis and metastatic progression in multiple malignancies, including PDAC. Its downstream product, PGE2, additionally contributes to immune suppression, tumour-promoting inflammation and cellular survival signaling [80,92]. The increased PTGS2 expression and PGE2 production observed following AHR activation therefore suggest that AHR signaling may contribute to creation of a tumour-supportive inflammatory microenvironment. Previous studies have similarly demonstrated interactions between AHR signaling and inflammatory pathways in cancer progression [31,81,93].

The observed increase in HO-1 expression further supports the cytoprotective role of AHR signaling in PDAC. HO-1 is an important stress-response enzyme involved in protection against oxidative damage, ferroptosis and apoptosis. Increased HO-1 activity has previously been associated with tumour survival, resistance to oxidative stress and chemoresistance in pancreatic cancer and other malignancies. The ability of AHR signaling to increase HO-1 expression therefore suggests that AHR may enhance the adaptive capacity of PDAC cells under conditions of oxidative and therapeutic stress.

Importantly, the cytoprotective functions of AHR signaling likely extend beyond regulation of individual downstream molecules. AHR has increasingly been recognized as a central integrator of environmental, metabolic and inflammatory signals. Through interactions with NF- κ B, hypoxia-associated pathways and tryptophan metabolism, AHR signaling may coordinate broad adaptive transcriptional programs that support tumour survival and immune evasion [87,94,95]. Recent studies have demonstrated that kynurenine

pathway-mediated activation of AHR contributes to immunosuppressive tumour microenvironments and tumour progression [35,87,96]. The findings of the present study suggest that AHR may additionally contribute to PDAC progression by promoting intracellular cytoprotective mechanisms.

The interplay between AHR and ELAVL1 identified in the current study may further increase the complexity of cytoprotective regulation. ELAVL1 stabilizes numerous mRNAs encoding inflammatory and survival-associated proteins, including COX-2/PTGS2, VEGF, TNF- α and HIF-1 α . Consequently, modulation of ELAVL1 localization by AHR may indirectly influence multiple cytoprotective and inflammatory pathways simultaneously. This interconnected regulatory network may partially explain the broad adaptive advantages associated with increased AHR signaling in PDAC cells.

An important observation of the present study was that inhibition of AHR consistently reduced expression of cytoprotective mediators despite differences between cell lines in the magnitude of response. These findings suggest that although the downstream consequences of AHR signaling may vary depending on tumour context, the overall contribution of AHR to adaptive survival signaling remains conserved. Given the extreme resistance of PDAC to chemotherapy, oxidative stress and immune-mediated elimination, such adaptive signaling mechanisms likely play a major role in disease progression and therapeutic failure.

Taken together, the findings of this section suggest that AHR contributes to PDAC cytoprotection and inflammatory adaptation through regulation of PTGS2, HO-1 and PGE2-associated signaling pathways. Beyond its classical role in xenobiotic sensing, AHR appears to function as a broader regulator of tumour survival and adaptive stress responses. These findings further support the hypothesis that AHR represents a central regulator linking chemoresistance, tumour aggressiveness and cytoprotective signaling in PDAC and may therefore represent a promising therapeutic target for overcoming tumour adaptation and therapy resistance.

5. DISCUSSION

5.1. Main findings of the study

The present dissertation demonstrates that AHR is associated with aggressive PDAC biology and may contribute functionally to gemcitabine resistance, EMT-associated cell plasticity and cytoprotective inflammatory signaling. By integrating patient tissue analysis with in vitro modulation experiments, this study identifies the AHR–ELAVL1–DCK axis as a potential regulatory mechanism linking post-transcriptional control with therapeutic response. The findings of the present study identify AHR as a central regulator linking chemoresistance, EMT-associated aggressiveness and cytoprotective signaling through interconnected transcriptional and post-transcriptional mechanisms involving ELAVL1 (Fig. 5.1).

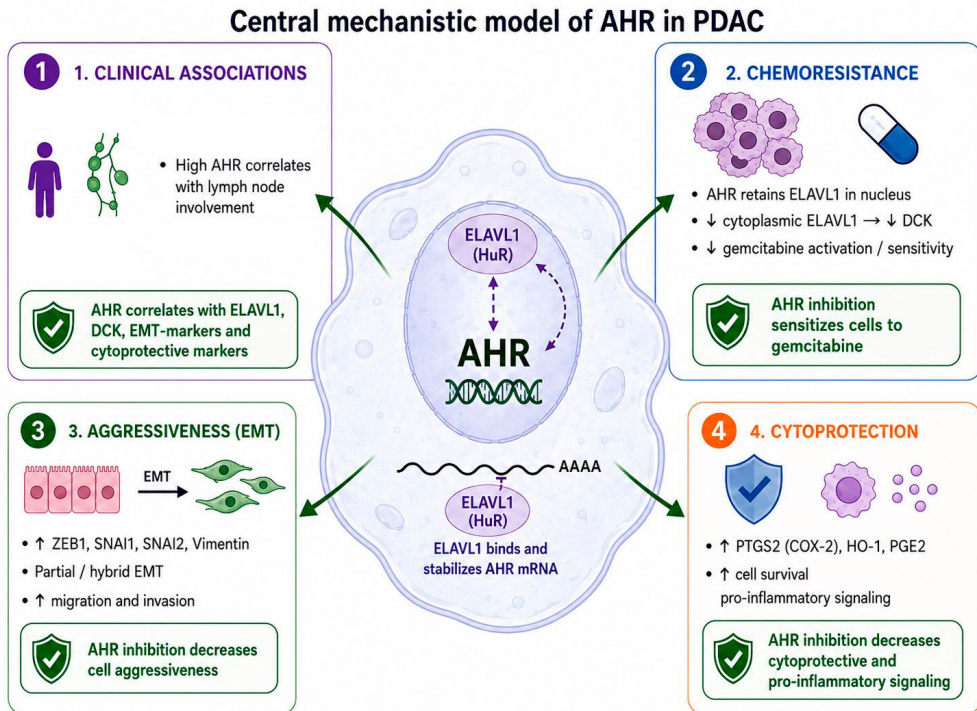


Fig. 5.1. Central mechanistic model of AHR-mediated chemoresistance, aggressiveness and cytoprotective signaling in PDAC

High AHR expression is associated with ELAVL1, DCK, EMT-related markers, cytoprotective markers and lymph node involvement (1). Mechanistically, AHR promotes nuclear retention of ELAVL1, reducing cytoplasmic ELAVL1-dependent DCK regulation and thereby limiting gemcitabine activation (2). AHR also contributes to EMT-associated aggressive-

ness, migration and invasion (3), as well as PTGS2/HO-1/PGE2-mediated cytoprotective and pro-inflammatory signalling (4). Inhibition of AHR sensitizes PDAC cells to gemcitabine, decreases cell aggressiveness and reduces cytoprotective inflammatory signaling. (Illustration created using ChatGPT.com)

5.2. Clinical relevance of AHR expression in PDAC

The first major finding of this dissertation was the identification of clinically relevant associations between AHR expression and aggressive PDAC characteristics. Increased AHR expression was associated with lymph node involvement and increased expression of EMT-related and cytoprotective molecules. These findings support the growing evidence that AHR functions beyond its classical role as an environmental sensor and xenobiotic-response regulator [45–47,97]. Instead, AHR appears to participate in multiple adaptive tumour-associated processes promoting tumour progression and therapy resistance.

A particularly important finding of this dissertation was the identification of a previously undescribed functional relationship between AHR and ELAVL1. ELAVL1 has long been recognized as an important RNA-binding protein regulating mRNA stability and translation of numerous cancer-associated transcripts. However, the interaction between AHR and ELAVL1 in PDAC has remained poorly understood. The findings of the present study demonstrated that ELAVL1 binds AHR mRNA, while AHR activity simultaneously regulates ELAVL1 nuclear-cytoplasmic localization. These observations suggest the existence of a dynamic feedback regulatory loop between the two molecules.

5.3. AHR in ELAVL1/DCK-mediated chemoresistance

The biological significance of this relationship became particularly evident in the context of gemcitabine resistance. Multiple mechanisms contributing to chemoresistance in PC have been extensively described. These include recurrent genetic alterations such as mutations in *KRAS*, *TP53*, and *MUC4* [86,98–101], as well as dysregulation of microRNAs, including miR-21, miR-181b, miR-106b, and miR-29a [58,102–105]. In addition, aberrant expression of proteins such as heat shock protein 27 and ribonucleotide reductase subunit M1 has been linked to decreased drug sensitivity [106–108]. While these mechanisms provide important insights, they do not fully explain the rapid and robust resistance observed clinically, suggesting that additional regulatory layers—particularly post-transcriptional control—play

a crucial role. We showed that one of these post-transcriptional regulators could be ELAVL1 working through a novel AHR-ELAVL1-DCK pathway.

Increased cytoplasmic localization of ELAVL1 has been associated with improved survival in PC patients treated with gemcitabine or 5-fluorouracil [29,84,85,109,110], highlighting its importance as both a functional regulator and a potential prognostic biomarker. The current study demonstrated that inhibition or silencing of AHR increased cytoplasmic localization of ELAVL1 and generally increased DCK expression together with gemcitabine sensitivity. In contrast, increased AHR activity reduced ELAVL1 cytoplasmic translocation and promoted a more chemoresistant phenotype. These findings support the hypothesis that AHR contributes to gemcitabine resistance by functionally limiting ELAVL1-mediated stabilization of DCK mRNA. Since DCK is the rate-limiting enzyme responsible for gemcitabine activation, even modest alterations in DCK expression may substantially influence treatment response.

5.4. AHR in EMT-associated tumour aggressiveness

Importantly, the findings of this dissertation suggest that AHR-mediated regulation of PDAC biology extends beyond chemoresistance alone. Increased AHR expression was associated with increased expression of EMT-associated transcription factors, while AHR inhibition reduced migration and invasion in vitro. These findings support the concept that AHR contributes to tumour aggressiveness and cellular plasticity. Notably, the observed expression patterns suggest that AHR may not necessarily induce a complete epithelial-to-mesenchymal transition, but rather support maintenance of partial or hybrid EMT phenotypes associated with increased metastatic potential and therapy resistance. Such intermediate EMT states are increasingly recognized as biologically important drivers of tumour adaptation and progression.

5.5. AHR in cytoprotective and inflammatory signalling

The present study additionally demonstrated that AHR contributes to cytoprotective and inflammatory signaling in PDAC. Activation of AHR increased PTGS2, HO-1 and PGE2-associated signaling, whereas AHR inhibition reduced expression of these cytoprotective mediators. These findings suggest that AHR signaling supports tumour survival under conditions of oxidative, inflammatory and therapeutic stress. Since ELAVL1 additionally regulates numerous inflammatory and survival-associated transcripts, the identified AHR-ELAVL1 interaction likely represents part of

a broader adaptive signaling network coordinating tumour survival, invasion and resistance.

5.6. Clinical implications

An important strength of the present study is the integration of patient tissue analysis with multiple in vitro experimental approaches. The use of patient samples, siRNA-mediated gene silencing, pharmacological modulation, RIP-IP analysis and functional assays allowed evaluation of both clinical relevance and mechanistic interactions. Importantly, the observed findings were generally consistent across multiple experimental systems, strengthening the biological significance of the proposed AHR regulatory axis.

The previous observations of this study are consistent with previous studies of other research teams showing that AHR contributes to cancer cell proliferation, invasion, and immune evasion in multiple tumor types [36,111–113]. Furthermore, AHR has been identified as a therapeutic target in a variety of malignancies, including breast, kidney, gastrointestinal cancers, lymphoma, and leukemia [114–118], reinforcing its relevance as a central regulator of cancer biology.

An important consideration arising from our findings is the heterogeneity of AHR expression among patients. Not all PDAC tumors exhibit elevated AHR levels, suggesting that therapeutic strategies targeting AHR must be carefully tailored. Increased AHR expression has been associated with enhanced tumor aggressiveness and migration [36,111,112], as well as immune resistance [88]. Therefore, patients with high AHR activity may represent a subset that would benefit most from AHR-targeted therapies. This highlights the importance of incorporating biomarker-driven approaches and precision medicine strategies into future clinical applications.

Beyond chemoresistance, both AHR and ELAVL1 are involved in a wide range of physiological and pathological processes. AHR signaling is highly responsive to environmental factors, including dietary components, gut microbiota-derived metabolites, and xenobiotics [94,119,120]. It also plays a central role in immune regulation and inflammation [61,111,112]. Similarly, ELAVL1 regulates numerous mRNAs involved in immune responses, cell cycle control, and stress adaptation. The broad functional scope of these molecules suggests that modulation of the AHR–ELAVL1 axis could have far-reaching effects beyond tumor cells, particularly in the tumor microenvironment.

These considerations also underscore the limitations of in vitro models. Both AHR and ELAVL1 are deeply integrated into immune signaling networks, which are absent in cell culture systems. As a result, the full impact

of AHR modulation may not be fully captured *in vitro*. Future studies using *in vivo* models and patient-derived systems will be essential to better understand the systemic effects of targeting this pathway.

Taken together, this dissertation identifies AHR as a central regulator of PDAC adaptation and progression through interconnected regulation of chemoresistance, EMT-associated aggressiveness and cytoprotective signaling. The identified AHR–ELAVL1 regulatory axis provides novel mechanistic insight into PDAC biology and represents a promising direction for future translational and therapeutic research.

5.7. Limitations of the study

First, functional experiments were performed using only two pancreatic cancer cell lines. While these models are widely used, they do not fully capture the genetic, molecular and phenotypic heterogeneity of PDAC. In particular, differences in baseline AHR activity, ELAVL1 localization, DCK expression and epithelial–mesenchymal transition (EMT) status across PDAC subtypes may influence the generalizability of the observed effects.

Second, only a limited number of AHR modulators were evaluated. Although both genetic and pharmacological approaches were employed, siRNA-based strategies primarily serve as mechanistic tools and may not fully reflect clinically achievable modulation of AHR activity. In addition, pharmacological inhibitors and activators may produce concentration-dependent or off-target effects. Although control experiments were included, further validation using additional AHR-targeting compounds and complementary genetic approaches would strengthen these findings.

Third, the size of the patient cohort, particularly for protein-level analyses, was relatively limited. This restricts the statistical power of the study and may limit the robustness and generalizability of the observed associations between AHR, ELAVL1, DCK expression and clinical outcomes. Furthermore, the patient tissue analyses are primarily associative and therefore do not prove direct causality in human tumours. Validation in larger, independent cohorts will be necessary to confirm the clinical relevance of these findings.

Fourth, the study lacks *in vivo* validation. The tumour microenvironment, including stromal components, immune cells, extracellular matrix and metabolic factors, plays a critical role in regulating AHR signalling and ELAVL1 function. In particular, AHR is known to interact with immune pathways and respond to endogenous ligands such as kynurenine, which are not fully recapitulated in conventional *in vitro* systems. As a result, the impact of the AHR–ELAVL1 axis on tumour progression, EMT plasticity and chemoresistance may differ *in vivo*.

Fifth, ELAVL1 localization was assessed mainly by immunocytochemistry. Although this approach provided important visual evidence of nuclear-cytoplasmic redistribution, it remains partly qualitative. Future studies using nuclear-cytoplasmic fractionation, automated image analysis or live-cell imaging would allow more precise quantification of ELAVL1 shuttling after AHR modulation and gemcitabine treatment.

Finally, while the study identifies a novel AHR–ELAVL1–DCK regulatory axis, the precise molecular mechanisms underlying this interaction remain only partially characterized. In particular, the direct transcriptional targets of AHR in this context, as well as the full spectrum of ELAVL1-regulated mRNAs contributing to chemoresistance, EMT-associated plasticity and cytoprotective signalling, require further investigation.

5.8. Future research

The findings of this dissertation open several important directions for future research. First, the clinical relevance of AHR, ELAVL1 and DCK should be validated in larger and independent PDAC patient cohorts. Although the present study demonstrated associations between increased AHR expression, lymph node involvement, EMT-related marker expression and cytoprotective signalling, confirmation in larger cohorts would strengthen the prognostic and predictive value of these observations. Future studies should include both mRNA and protein-level analyses, preferably using standardized immunohistochemical scoring of AHR, cytoplasmic and nuclear ELAVL1, DCK, PTGS2 and HO-1. Importantly, these markers should be evaluated in relation to treatment response, especially in patients receiving gemcitabine-based chemotherapy, in order to determine whether the AHR–ELAVL1–DCK axis may serve as a clinically useful biomarker for treatment stratification.

Second, future studies should further characterize the molecular mechanisms underlying the interaction between AHR and ELAVL1. The present findings suggest that ELAVL1 binds AHR mRNA, while AHR modulation affects ELAVL1 subcellular localization and DCK expression. However, the precise regulatory events responsible for this feedback loop remain incompletely understood. Further investigation using RNA immunoprecipitation sequencing, CLIP-seq, transcriptomics and proteomics could identify the full spectrum of ELAVL1-regulated transcripts affected by AHR activity. In parallel, chromatin immunoprecipitation-based approaches could help determine whether AHR directly regulates genes involved in ELAVL1 trafficking, RNA metabolism, gemcitabine activation or cytoprotective signalling.

Third, future research should move beyond conventional two-dimensional cell culture models. Patient-derived organoids, spheroids, co-culture systems

and patient-derived xenografts would better reproduce the heterogeneity and complexity of PDAC. Such models would allow evaluation of whether AHR inhibition restores gemcitabine sensitivity across different PDAC subtypes and whether this effect depends on baseline AHR expression, ELAVL1 localization or DCK status. In vivo studies are particularly important because AHR is closely linked to immune regulation, inflammation, metabolism and ligand availability, all of which cannot be fully reproduced in isolated tumour cell cultures.

Fourth, additional studies should investigate the role of AHR in EMT plasticity and metastatic progression. The present study suggests that AHR may support a partial or hybrid EMT phenotype rather than inducing a complete epithelial-to-mesenchymal transition. This should be explored using single-cell RNA sequencing, spatial transcriptomics and lineage-tracing approaches in order to determine how AHR activity influences tumour cell states, invasion, lymphatic dissemination and metastatic colonization. Since increased AHR expression was associated with lymph node involvement, future studies should also examine whether AHR-driven EMT plasticity directly contributes to regional metastasis in PDAC.

Fifth, future work should further evaluate the cytoprotective and inflammatory consequences of AHR signalling. The regulation of PTGS2, HO-1 and PGE2 suggests that AHR contributes to adaptive tumour survival under inflammatory, oxidative and therapeutic stress. However, it remains unclear whether these effects are primarily tumour-cell intrinsic or whether they also involve stromal and immune components of the tumour microenvironment. Future studies should therefore assess AHR signalling in pancreatic stellate cells, fibroblasts, macrophages, T cells and other components of the PDAC microenvironment. Particular attention should be given to endogenous AHR ligands, including kynurenine pathway metabolites, as these may influence both tumour aggressiveness and immune suppression.

Finally, future translational studies should assess the therapeutic potential of AHR-targeted strategies. AHR inhibition should be tested in combination with gemcitabine and other clinically relevant treatment regimens to determine whether targeting AHR can simultaneously enhance chemosensitivity, reduce EMT-associated aggressiveness and suppress cytoprotective inflammatory signalling. Because AHR expression appears heterogeneous among PDAC patients, such approaches should be biomarker-driven rather than applied universally. Patients with high AHR activity, reduced cytoplasmic ELAVL1 localization, low DCK expression or elevated PTGS2/HO-1 signalling may represent the subgroup most likely to benefit from AHR-directed therapy.

Taken together, future research should aim to validate the AHR–ELAVL1–DCK axis in clinically relevant models, define its molecular regulation in

greater detail and determine whether AHR inhibition can be translated into a biomarker-guided therapeutic strategy. Such studies may help clarify whether AHR represents not only a marker of aggressive PDAC biology, but also a practical therapeutic target for overcoming chemoresistance, EMT-associated progression and cytoprotective adaptation.

CONCLUSIONS

1. AHR expression is associated with clinically and biologically relevant features of pancreatic ductal adenocarcinoma. Increased AHR expression correlated with the increased expression of *ELAVL1*, *DCK*, EMT markers, *PTGS2*, and *HO-1*, as well as lymph node invasion, indicating its association with tumour aggressiveness.
2. AHR and ELAVL1 form a functionally interconnected regulatory system that determines the response to gemcitabine treatment. Inhibition of AHR promoted the cytoplasmic localization of ELAVL1, modulated DCK expression, and increased pancreatic cancer cell sensitivity to gemcitabine, confirming the importance of the AHR–ELAVL1–DCK axis in chemoresistance.
3. AHR promotes EMT-driven aggressiveness of pancreatic cancer cells. Increased AHR expression was associated with increase in EMT marker expression, while AHR inhibition reduced cell migration, invasion, and EMT-associated mesenchymal phenotypic characteristics.
4. AHR regulates cytoprotective and inflammatory signalling pathways in pancreatic cancer cells. Inhibiting AHR reduced PTGS2 and HO-1 expression as well as prostaglandin E₂ production, indicating its role in tumour survival and treatment resistance mechanisms.

SANTRAUKA

1. ĮVADAS

1.1. Tyrimo aktualumas bei naujumas

Šio tyrimo aktualumą lemia bloga kasos lataų adenokarcinomos (PDAC) prognozė, kurią daugiausia nulemia ankstyvas metastazavimas ir ryškus atsparumas gemcitabino pagrindu taikomai chemoterapijai. Aprašyta daug molekulinų mechanizmų, tačiau vis dar trūksta bendro supratimo, kaip transkripciniai, potranskripciniai, EMT ir citoprotekciniai uždegiminiai procesai yra tarpusavyje susiję.

Šio darbo naujumas pirmiausia grindžiamas AHR kaip centrinio reguliacinio mazgo PDAC biologijoje identifikavimu. Skirtingai nei ankstesni tyrimai, kuriuose AHR buvo nagrinėjamas daugiausia kaip transkripcijos faktorius ar uždegiminės signalizacijos tarpininkas (mediatorius), šiame darbe jis analizuojamas kaip jungiamoji grandis, jungianti chemoresistenciją, EMT plastiškumą ir citoprotekcinį atsaką viename funkciname tinkle.

Ypač svarbus naujumo aspektas yra AHR–ELAVL1–DCK reguliacinės ašies identifikavimas. Parodyta, kad AHR veikia ELAVL1 lokalizaciją ląstelėje, o ELAVL1 kartu prisijungia prie AHR mRNR ir sudaro dvikryptį grįžtamąjį ryšį tarp transkripcinės ir potranskripcinės reguliacijos. Šis mechanizmas yra naujas PDAC kontekste ir leidžia paaiškinti, kaip AHR netiesiogiai mažina DCK raišką bei prisideda prie gemcitabino aktyvacijos sutrikdymo.

Kitas esminis naujumo aspektas – AHR funkcijos EMT ir naviko plastiškumo reguliacijai įrodymas. Šis tyrimas rodo, kad AHR raiška yra susijusi su EMT transkripcijos veiksniais bei padidėjusiais ląstelių migracijos ir invazijos gebėjimais – taip atskleidžiamas jo indėlis į agresyvius, hibridinius EMT fenotipus, kurie yra svarbūs metastazavimui ir terapiniam atsparumui.

Taip pat naujai pabrėžiamas AHR vaidmuo citoprotekciniais ir uždegiminiams procesams. Nustatyta, kad AHR aktyvacija skatina PTGS2, HO-1 ir PGE2 signalizaciją, o jo slopinimas šiuos procesus mažina. Tai rodo, kad AHR dalyvauja naviko adaptacijos prie oksidacinio, uždegiminio ir chemoterapinio streso, kuris yra esminis PDAC atsparumo veiksnys, procesuose.

Galiausiai tyrimo klinikinė reikšmė sustiprinama ryšiais tarp AHR raiškos, limfmazgių pažeidimo ir blogesnio išgyvenamumo, taip pat jo sąsajomis su EMT ir citoprotekciniais žymenimis. Tai leidžia AHR–ELAVL1–DCK ašį vertinti kaip potencialų prognozinį ir terapinį biologinį žymenį, ypač pacientų, gydomų gemcitabinu, stratifikacijai.

Apibendrinant šis tyrimas pateikia naują koncepcinį požiūrį į PDAC biologiją – AHR yra ne pavienis reguliatorius, o centrinis jungiamasis (integra-

cinis) veiksnys, jungiantis chemorezistenciją, EMT plastiškumą ir citoprotekcinį uždegiminį atsaką.

Tyrimo tikslas

Ištirti arilo angliavandenilių receptoriaus klinikinę reikšmę ir funkcinį vaidmenį kasos latakų adenokarcinomai, ypatingą dėmesį skirti jo dalyvavimui ELAVL1/DCK nulemtam atsparumo gemcitabinui, EMT sukulto naviko agresyvumo bei citoprotekcinio PTGS2/HO-1/PGE2 signalinių kelių procesuose.

Tyrimo uždaviniai

1. Įvertinti AHR raiškos ryšį su klinikiniais patologiniais PDAC požymiais.
2. Ištirti AHR ir ELAVL1 sąveiką bei jos reikšmę DCK raiškai ir jautrumui gemcitabinui.
3. Nustatyti AHR vaidmenį EMT procesams bei navikinių ląstelių migracijos ir invazijos procesuose.
4. Įvertinti AHR įtaką citoprotekcinio ir uždegiminių molekulių PTGS2, HO-1 bei PGE2 reguliacijai.

2. LITERATŪROS APŽVALGA

Kasos latakų adenokarcinoma yra viena agresyviausių onkologinių žmogaus ligų, nulemiančių itin didelį mirtingumą ir blogą ilgalaikę prognozę. Pasiekta reikšminga diagnostikos, chirurginio gydymo ir sisteminės terapijos pažangos, tačiau daugumos pacientų diagnozuojama vėlyvų stadijų liga, kai radikalus gydymas tampa nebeįmanomas. Penkerių metų išgyvenamumas išlieka vienas mažiausių tarp visų onkologinių ligų, o prognozes dar labiau blogina ankstyvas metastazavimas, ryškus naviko biologinis agresyvumas ir atsparumas gydymui. Šios priežastys lemia būtinybę geriau suprasti molekulinis mechanizmus, atsakingus už naviko progresavimą, invazyvumą ir terapinį atsparumą.

Gemcitabinas ilgą laiką buvo pagrindinis vaistas gydant kasos vėžį, tačiau klinikinį jo veiksmingumą riboja įgimtas arba gydymo metu atsirandantis atsparumas. Vienas svarbiausių veiksnių, lemiančių gemcitabino aktyvumą ląstelėje, yra DCK, kuri aktyvina vaistą fosforilinimo būdu. Sumažėjusi DCK raiška yra siejama su silpnesniu atsaku į gydymą ir mažesniu chemoterapijos veiksmingumu. Todėl molekulinis mechanizmas, reguliuojančių DCK raišką ir funkciją, identifikavimas yra svarbus siekiant pagerinti gydymo rezultatus.

Pastaraisiais metais daug dėmesio sulaukė RNR surišantis baltymas ELAVL1, reguliuojantis daugelio genų mRNR stabilumą ir transliaciją. Nu-

statyta, kad citoplazminė ELAVL1 lokalizacija gali skatinti DCK raišką ir didinti navikinių ląstelių jautrumą gemcitabinui. Be to, ELAVL1 dalyvauja reguliuojant daugelį su naviko progresavimu susijusių procesų, įskaitant proliferaciją, uždegimą, angiogenezę ir atsparumą gydymui. Nors sukaupta žinių apie ELAVL1 svarbą kasos vėžiui, tačiau jo sąsajos su kitais naviko progresavimą reguliuojančiais signaliniais keliais iki šiol nėra iki galo išaiškintos.

Vis daugiau duomenų rodo, kad reikšmingą įtaką navikų biologijai turi AHR. Tradiciškai AHR buvo laikomas transkripcijos faktoriumi, atsakingu už organizmo atsaką į aplinkos toksinus ir ksenobiotikus, tačiau šiuolaikiniai tyrimai atskleidė daug platesnę biologinę jo reikšmę. Nustatyta, kad AHR dalyvauja ląstelių diferenciacijos, proliferacijos, uždegiminių reakcijų, imuninio atsako, metabolizmo ir naviko progresavimo reguliacijos procesuose. Įvairių navikų padidėjęs AHR aktyvumas siejamas su blogesne prognoze, didesniu invazyvumu, metastazavimu ir atsparumu gydymui. Tačiau duomenys apie AHR įtaką kasos latakų adenokarcinomai išlieka fragmentiški ir neretai prieštaringi.

Kitas svarbus naviko progresavimo mechanizmas yra EMT, kurios metu epitelinės ląstelės praranda savo diferenciacijos požymius ir įgyja mezenchiminų ląstelių savybių. EMT skatina migraciją, invaziją, metastazavimą ir atsparumą gydymui. Šio proceso metu keičiasi įvairių transkripcinių veiksnių ir struktūrinių baltymų sintezė, o navikinės ląstelės įgauna didesnę plastiškumą bei gebą prisitaikyti prie nepalankių aplinkos sąlygų. Pastaraisiais metais nustatyta duomenų, leidžiančių manyti, kad AHR gali dalyvauti EMT reguliacijos procesuose, tačiau jo įtaka kasos vėžio ląstelių plastiškumui ir agresyvumui išlieka nepakankamai ištirta.

Ne mažiau svarbūs yra citoprotekciniai ir prouždegiminiai signaliniai keliai, kurie leidžia navikinėms ląstelėms išgyventi nepalankiomis sąlygomis. Tarp jų reikšmingą vaidmenį atlieka PTGS2, HO-1 ir PGE2. Šios molekulės dalyvauja uždegimo reguliacijos, oksidacinio streso kontrolės, angiogenezės ir navikinių ląstelių išgyvenimo procesuose. Padidėjusi jų raiška dažnai siejama su blogesne ligos prognoze ir atsparumu gydymui. Literatūroje aprašytos galimos AHR sąsajos su šiais signaliniais keliais, tačiau jų reikšmė kasos latakų adenokarcinomai iki šiol nebuvo išsamiai ištirta.

Atsižvelgiant į ribotas žinias apie AHR įtaką kasos vėžiui, šiame darbe buvo siekiama kompleksiškai ištirti AHR įtaką naviko biologijai, vertinant jo sąsajas su klinikiniais ligos rodikliais, jautrumu gemcitabinui, ELAVL1 ir DCK reguliacija, EMT procesais bei citoprotekciniais signaliniais keliais. Tyrimo metu identifikuota nauja AHR–ELAVL1–DCK reguliacinė ašis, kuri gali būti svarbi kasos latakų adenokarcinomos progresijai ir atsparumo gydymui vystymuisi.

3. PAGRINDINIAI TYRIMO METODAI

3.1. PDAC pacientų audinių analizė ir klinikinių požymių koreliacija

Pacientų audinių tyrimas atliktas naudojant 55 pacientų, kuriems atlikta pankreatikoduodenektomija (*Whipple* operacija), navikinių ir gretimų ne navikinių audinių mėginius. Audiniai buvo užšaldyti skystajame azote ir saugomi –80 °C temperatūroje. Iš jų buvo išskirta RNR ir baltymai tolesnei analizei.

AHR raiška buvo vertinama naudojant qRT-PCR ir *Western blot* metodus. Gauti molekuliniai duomenys buvo susieti su klinikiniais patologiniais parametrais bei pacientų išgyvenamumo duomenimis. Statistinei analizei naudoti koreliaciniai ir išgyvenamumo modeliai, leidę įvertinti AHR biologinę ir prognozinę reikšmę PDAC.

3.2. AHR vaidmuo chemorezistencijai per ELAVL1 reguliaciją

Chemorezistencijos mechanizmai buvo tirti *in vitro* naudojant PDAC ląstelių linijas BxPC-3 ir SU.86.86. AHR funkcija buvo moduluojama dviem būdais: siRNA pagrindu atliktu genų nutildymu (siAHR, siELAVL1) ir farmakologine moduliacija naudojant AHR inhibitorius (BAY2416964) bei agonistus (*tapinarof*, TCDD).

Ląstelių atsakas į gemcitabiną buvo vertinamas MTT ir resazurino testais, kolonijų formavimosi bei metabolinio aktyvumo analizėmis. ELAVL1–AHR sąveika analizuota RIP-IP metodu, o baltymų vieta ląstelėje – imunocitochemijos būdu. Šie metodai leido įvertinti AHR įtaką chemoterapiniam jautrumui ir ELAVL1 tarpininkaujamam potranskripciniam reguliavimui.

3.3. AHR vaidmuo ląstelių agresyvumo ir EMT reguliacijos procesuose

AHR įtaka ląstelių migracijai ir invazijai buvo vertinta naudojant tą pačią PDAC ląstelių sistemą, papildomai įtraukiant CRISPR/Cas9 pagrindu sukurtas AHR *inaktyvinimo* (“*Knock-Out*”; KO) linijas.

Ląstelių agresyvumas buvo analizuojamas naudojant *scratch* (žaizdos uždarymo) testą, trimatės (3D) migracijos modelius bei invazijos per pamatinę membraną (Geltrex) sistemas. EMT procesai buvo vertinami analizuojant su EMT susijusių genų (SNAI1, ZEB1, VIM, E-CAD) raišką qRT-PCR ir *Western blot* metodais. Šie eksperimentai leido nustatyti AHR reikšmę PDAC migracijai bei invazijai.

3.4. AHR vaidmuo citoprotekcijai ir uždegiminiams procesams

AHR įtaka uždegiminiams ir citoprotekciniais signalams tirta analizuojant PGE2 gamybą bei su uždegimu susijusių genų raišką. Ląstelės apdorotos AHR inhibitoriais ir agonistais, įskaitant TCDD ir BAY2416964.

PGE2 koncentracija ląstelių terpėje buvo matuojama ELISA metodu, o PTGS2, HO-1 ir kitų uždegimo žymenų raiška – qRT-PCR bei *Western blot* analizėmis. Šie tyrimai leido įvertinti AHR įtaką PDAC ląstelių citoprotekcijai ir uždegiminei mikroaplinkai.

3.5. PDAC ląstelių kultūros sąlygos

Naudotos PDAC ląstelių linijos BxPC-3 ir SU.86.86 buvo kultivuojamos RPMI terpėje, papildytoje 10 proc. FBS ir 1 proc. penicilino–streptomicino. Ląstelės augintos 37 °C temperatūroje, 5 proc. CO₂ sąlygomis laikantis standartinių aseptinių procedūrų. Persėjimų skaičius neviršijo 30, siekiant užtikrinti genetinį stabilumą. Periodiškai tikrintas mikoplazmų užterštumas.

3.6. Eksperimentiniai metodai

Gemcitabino IC₅₀ nustatytas atliekant dozių atsako eksperimentus (1–10 000 nM) 48 val. laikotarpiu. Ląstelių gyvybingumas vertintas MTT ir resazurino testais.

Genų funkcijai moduluoti naudota siRNA transfekcija (*Lipofectamine* 2000), o AHR aktyvumas reguliuotas farmakologiniais inhibitoriais ir agonistais (BAY2416964, TAP, TCDD). CRISPR/Cas9 sistema naudota AHR KO linijoms generuoti, redagavimas patvirtintas T7 endonukleazės testu ir sekoskaita.

Vaistų sinergija tarp gemcitabino ir AHR moduliatorių vertinta naudojant *SynergyFinder*+ programą (HSA modelis).

Ląstelių proliferacija ir kolonijų formavimasis analizuoti ląstelių kolonijų formavimo gebos (*clonogenic assay*) metodu. Migracija vertinta įbrėžimo (*scratch*) testu, o trimatė (3D) migracija ir invazija – sferoidų formavimo bei *Geltrex* matrikso modeliuose.

RIP-IP metodas naudotas ELAVL1–RNR sąveikoms nustatyti, o PGE2 kiekis – ELISA analize. Genų raiška nustatyta qRT-PCR, naudojant *TaqMan* zondus.

Baltymų sintezė analizuota *Western blot* metodu, naudojant specifinius antikūnus (AHR, ELAVL1, PTGS2, DCK ir kt.).

Statistinė analizė atlikta *GraphPad Prism* programa. Naudoti Mann–Whitney ir Spearmano testai, statistinis reikšmingumas laikytas $p < 0,05$.

4. PAGRINDINIAI REZULTATAI

4.1. AHR raiškos klinikinė reikšmė PDAC audiniams

Pacientų kasos lataų adenokarcinomos (PDAC) audinių analizė atskleidė reikšmingus ryšius tarp AHR, ELAVL1 ir DCK raiškos. Genų raiškos koreliacine analize nustatytas stiprus teigiamasis ryšys tarp AHR ir ELAVL1 ($r = 0,757$), AHR ir DCK ($r = 0,657$) bei ELAVL1 ir DCK ($r = 0,701$) – tai rodo glaudų šių molekulių funkcinį tarpusavio ryšį PDAC biologijoje.

Baltymų lygmens analizė, atlikta naudojant suporuotus navikinio ir greito sveiko audinio mėginius, parodė aiškų skirtumą tarp transkripcinių ir baltymų translacijos duomenų. PDAC audiniuose nustatytas padidėjęs AHR baltymo kiekis, o DCK baltymo raiška buvo sumažėjusi. Šis neatitikimas tarp mRNR koreliacijų ir baltymų sintezės rodo, kad AHR reguliuojamas tinklas PDAC nėra vien transkripcinis, bet reikšmingai priklauso nuo potranskripcinių mechanizmų.

Šie duomenys leidžia manyti, kad AHR gali prisidėti prie DCK translacijos slopinimo, neatsižvelgiant į teigiamas mRNR koreliacijas. Tai ypač svarbu, nes DCK yra ribojantis gemcitabino aktyvacijos fermentas, kurio sumažėjusi raiška yra vienas pagrindinių chemorezistencijos veiksniai PDAC.

Papildomai atlikta EMT žymenų analizė parodė, kad AHR raiška labai koreliuoja su EMT transkripcijos faktoriais ZEB1 ($r = 0,727$), SNAI1 ($r = 0,760$) ir SNAI2 ($r = 0,767$). Taip pat nustatytos teigiamos koreliacijos su vimentinu ($r = 0,580$) ir net epiteliniu žymeniu E-kadherinu ($r = 0,396$).

Be EMT, AHR labai koreliavo su citoprotekciniais ir uždegimo žymenimis PTGS2 ($r = 0,703$) ir HO-1 ($r = 0,929$) – tai rodo AHR įtaką ne tik ląstelių plastiškumui, bet ir uždegiminės mikroaplinkos reguliacijai.

Pacientų stratifikacija pagal AHR raišką parodė aiškius molekulinis skirtumus. Navikų, kurių sumažėjusi AHR raiška, buvo reikšmingai sumažėjęs EMT ir uždegimo genų aktyvumas (ZEB1, SNAI1, SNAI2, VIM, PTGS2) bei padidėjusi E-kadherino raiška. Taip pat padidėjusios AHR raiškos grupėje buvo stebimas stipri EMT aktyvacija, padidėjusi PTGS2 raiška ir sumažėjusi ZEB2 bei TWIST raiška, rodanti skirtingus EMT reguliacijos režimus.

Klinikinė analizė parodė, kad AHR raiška nėra susijusi su pirminiu T stadijos dydžiu, tačiau reikšmingai koreliuoja su limfmazgių invazija (N stadija). Pacientai, kuriems nustatyta N2 stadija, turėjo 2,8 karto padidėjusią AHR raišką, o N0 grupėje AHR raiška buvo daug mažesnė.

Apibendrinant pacientų audinių analizė rodo, kad AHR yra glaudžiai susijęs su PDAC agresyvumu, EMT aktyvacija ir citoprotekcija.

4.2. AHR vaidmuo ELAVL1/DCK reguliuojamai gemcitabine chemorezistencijai

Atlikus gemcitabino IC₅₀ skaičiavimą, nustatyta, kad BxPC-3 ląstelių IC₅₀ yra $26,67 \pm 1,69$ nM, o SU.86.86 – $36,91 \pm 1,40$ nM – tai atspindi skirtingą jautrumą chemoterapijai tarp PDAC linijų.

AHR farmakologinė moduliacija (BAY2416964 – inhibitorius; tapinarof ir TCDD – aktyviekliai) parodė, kad 10 μ M koncentracija neturi įtakos ląstelių gyvybingumui, tačiau reikšmingai keičia AHR signalinį aktyvumą, vertintą per PTGS2 raišką. AHR inhibicija sumažino PTGS2 raišką, o aktyvacija ją padidino iki 3–5 kartų.

siRNA eksperimentais nustatytas veiksmingas AHR ir ELAVL1 nutildymas. Įdomu, kad AHR slopinimas padidino ELAVL1 raišką, o ELAVL1 slopinimas sumažino AHR raišką – tai rodo abipusę reguliacinį ryšį.

RIP-IP analizė patvirtino, kad ELAVL1 tiesiogiai jungiasi prie AHR mRNR, vadinasi, ELAVL1 gali reguliuoti AHR transkripto stabilumą. AHR mRNR turi elementų, būdingų ELAVL1 jungimosi vietoms, todėl AHR gali būti tiesioginis ELAVL1 potranskripcinio reguliavimo taikiny.

Ląstelių lokalizacijos tyrimai parodė, kad ELAVL1 daugiausia yra branduolyje, tačiau gemcitabino gydymas skatina jo translokaciją į citoplazmą. AHR slopinimas dar labiau padidino šį procesą, ypač po gemcitabino poveikio, o AHR aktyvacija mažino ELAVL1 citoplazminę lokalizaciją.

Funkciškai AHR slopinimas padidino DCK mRNR ir baltymo raišką (ypač BxPC-3), o ELAVL1 slopinimas sumažino DCK baltymo kiekį abiejose ląstelių linijose. Tai patvirtina, kad DCK reguliacija yra labiau potranskripcinė nei transkripcinė.

Gemcitabino jautrumo eksperimentais nustatyta, kad AHR slopinimas reikšmingai didina chemoterapijos veiksmingumą, sumažindamas ląstelių gyvybingumą iki ~25 proc., palyginti su ~50 proc. vien gemcitabino poveikiu.

Sinergijos analizė (HSA modelis) parodė, kad BAY + gemcitabinas veikia sinergistiškai, o tapinarofas – antagonistišškai. Tai rodo, kad AHR inhibicija sustiprina chemoterapijos atsaką.

Kolonijų formavimo analizė patvirtino ilgalaikį AHR poveikį. AHR slopinimas kartu su gemcitabinu beveik visiškai panaikino kolonijų formavimą (iki 3,7–11 proc.) tai rodo stiprų ilgalaikį citotoksinį poveikį.

Apibendrinant AHR reguliuoja ELAVL1 lokalizaciją ir DCK raišką – taip formuojama centrinė chemorezistencijos ašis PDAC ląstelėse.

4.3. AHR vaidmuo su EMT susijusios PDAC agresijos aktyvumui

AHR slopinimas reikšmingai sumažino EMT transkripcijos veiksmų raišką, įskaitant SNAI2, ZEB2 ir VIM, bei sumažino PTGS2 raišką. Kartu E-kadherino raiška padidėjo BxPC-3 ląstelėse, tačiau SU.86.86 linijoje pokyčiai buvo heterogeniški, rodantys skirtingus molekulinis mechanizmus.

Funkciniais tyrimais nustatyta, kad AHR slopinimas labai sumažina migraciją (iki 73 proc. BxPC-3 ir 54 proc. SU.86.86). AHR inhibitorius taip pat sumažino migraciją dvimačiame (2D) scratch modelyje, o CRISPR KO patvirtino šį poveikį.

Naudojant trimačius (3D) sferoidų modelius nustatyta, kad AHR slopinimas sumažina tiek migraciją, tiek invaziją per pamatinę membraną. Invazijos sumažėjimas siekė ~74–79 proc., atsižvelgiant į ląstelių liniją ir laiką.

Šie rezultatai rodo, kad AHR reguliuoja ne tik EMT žymenis, bet ląstelių migraciją bei invaziją.

4.4. AHR vaidmuo citoprotekciniais ir uždegiminiams procesams

AHR slopinimas sumažino PTGS2 raišką abiejose ląstelių linijose ir padidino HO-1 raišką BxPC-3 ląstelėse. Tai rodo AHR įtaką reguliuojant tiek uždegiminius, tiek oksidacinio streso atsako mechanizmus.

PGE2 analizė parodė, kad PDAC ląstelės išskiria PGE2, tačiau AHR slopinimas reikšmingai sumažina jo koncentraciją (ypač BxPC-3: nuo ~1870 pg/ml iki ~112 pg/ml). AHR aktyvacija PGE2 nepadidino, vadinausi, pagrindinis signalas jau yra maksimaliai aktyvus.

Šie duomenys rodo, kad AHR reguliuoja PTGS2–PGE2 ašį, kuri yra svarbi uždegimui, proliferacijai ir galimam metastazavimui.

HO-1 padidėjimas rodo AHR įtaką oksidacinio streso adaptacijai, tai svarbu PDAC išgyvenimui hipoksijos ir cheminėmis stresinėmis sąlygomis.

5. REZULTATŲ APTARIMAS

5.1. Pagrindiniai tyrimo atradimai

Disertacijoje aprašyta, kad AHR yra esminis PDAC biologijos reguliatorius, dalyvaujantis keliuose tarpusavyje susijusiuose patogenezės procesuose. Sujungiant pacientų audinių analizę su *in vitro* funkciniais modeliais, nustatyta, kad AHR prisideda prie gemcitabino chemorezistencijos, su EMT susijusio ląstelių plastiškumo bei citoprotekcinio ir uždegiminių signalinių kelių aktyvinimo.

Svarbiausias šio darbo rezultatas – identifikuota AHR–ELAVL1–DCK reguliacinė ašis, jungianti transkripcinius ir potranskripcinius mechanizmus

su terapiniu atsaku. Šis modelis rodo, kad AHR veikia ne tik kaip klasikinis ligandų aktyvinamas transkripcijos faktorius, bet kaip centrinis reguliatorius, koordinuojantis kelis vėžio progresavimo lygmenis: chemorezistenciją, EMT plastiškumą ir citoprotekcinę adaptaciją.

5.2. Klinikinė AHR reikšmė PDAC

Pacientų audinių analizė atskleidė, kad AHR raiška glaudžiai susijusi su agresyvia PDAC eiga. Padidėjusi AHR raiška buvo susijusi su limfmazgių metastazėmis, blogesniu išgyvenamumu ir padidėjusia EMT ir citoprotekcinę žymenų raiška. Šie duomenys patvirtina, kad AHR funkcija viršija klasikinę jo vaidmenį kaip ksenobiotikų sensorinio receptoriaus.

Ypač svarbus atradimas yra glaudus AHR ir ELAVL1 funkcinis ryšys. ELAVL1 jau anksčiau buvo žinomas kaip svarbus RNR surišantis baltymas, reguliuojantis mRNR stabilumą. Tačiau šiame darbe pirmą kartą parodyta, kad ELAVL1 ne tik jungiasi prie AHR mRNR, bet ir pats AHR aktyvumas reguliuoja ELAVL1 branduolio–citoplazmos translokaciją. Tai leidžia manyti, kad tarp šių dviejų molekulių egzistuoja dinaminė grįžtamojo ryšio kilpa, kuri gali lemti PDAC ląstelių adaptaciją.

5.3. AHR vaidmuo ELAVL1/DCK reguliuojamai chemorezistencijai

Gemcitabino chemorezistencija yra vienas svarbiausių PDAC klinikinių iššūkių. Tradiciškai ji aiškinama genetiniais pokyčiais (KRAS, TP53), mikroRNR pusiausvyros sutrikimu ir baltymų raiškos pokyčiais. Tačiau šie mechanizmai nepaaiškina visos klinikinės rezistencijos dinamikos, todėl būtini papildomi reguliaciniai sluoksniai.

Šio tyrimo rezultatai rodo, kad AHR–ELAVL1–DCK ašis yra vienas iš tokių potranskripcinių reguliacinių mechanizmų. DCK, kaip ribojantis gemcitabino aktyvacijos fermentas, pamatinis vaisto efektyvumo požymis. Net nedideli jo raiškos pokyčiai gali reikšmingai keisti gydymo atsaką.

Nustatyta, kad AHR slopinimas skatina ELAVL1 translokaciją į citoplazmą, kur jis stabilizuoja DCK mRNR – taip didinamas DCK baltymo kiekis ir gemcitabino jautrumas. Priešingai, AHR aktyvacija mažina ELAVL1 citoplazminį aktyvumą ir prisideda prie chemorezistencinio fenotipo.

Šie rezultatai patvirtina hipotezę, kad AHR veikia kaip netiesioginis DCK reguliatorius, per ELAVL1 ribodamas gemcitabino aktyvaciją.

5.4. AHR vaidmuo su EMT susijusiam naviko agresyvumui

Be chemorezistencijos, AHR dalyvauja PDAC ląstelių agresyvumo reguliacijos procesuose. Padidėjusi AHR raiška koreliavo su EMT transkripcijos

faktoriais (ZEB1, SNAI1, SNAI2) ir vimentinu, o funkciniuose modeliuose AHR slopinimas sumažino migraciją ir invaziją.

Svarbu pažymėti, kad AHR poveikis neatitinka klasikinio EMT modelio. Greičiau jis palaiko tarpinę, hibridinę EMT būseną, kuri pasižymi tuo pačiu metu epitelinių ir mezenchiminių savybių išlaikymu. Tokios būsenos yra susijusios su didesniu plastiškumu, metastazavimu ir terapiniu atsparumu.

Tai leidžia manyti, kad AHR veikia kaip EMT reguliatorius, o ne kaip vien tik induktorius. Jis palaiko adaptacinę ląstelių lankstumą, kuris yra esminis PDAC progresavimo veiksnys.

5.5. AHR citoprotekcinė ir uždegiminė funkcija

AHR taip pat reguliuoja citoprotekcinis ir uždegiminius signalinius kelius, įskaitant PTGS2, HO-1 ir PGE2 gamybą. Šie tarpininkai (mediatoriai) yra svarbūs ne tik uždegimo reguliavimui, bet ir naviko išgyvenimui oksidacinio streso bei gydymo sąlygomis.

AHR aktyvacija padidino PTGS2 ir PGE2 gamybą, o AHR slopinimas juos sumažino. Tai rodo, kad AHR prisideda prie pro-uždegiminės, naviką palaikančios mikroaplinkos formavimo. HO-1 indukcija papildomai rodo AHR vaidmenį oksidacinio streso adaptacijai.

ELAVL1 reguliuoja daugelį uždegiminių transkriptų, todėl AHR–ELAVL1 sąveika gali veikti kaip kompleksinis tinklas, koordinuojantis tiek vidinius ląstelinius, tiek mikroaplinkos adaptacinius procesus.

5.6. Klinikinės reikšmė

Vienas svarbiausių šio darbo aspektų yra klinikinių pacientų duomenų sąsaja su eksperimentiniais modeliais. Tiek audinių, tiek ląstelių AHR, ELAVL1 ir DCK analize nustatyti nuoseklūs rezultatai, patvirtinantys biologinę šios ašies reikšmę.

Pacientų AHR raiškos heterogeniškumas rodo, kad šis žymuo gali būti svarbus pacientų stratifikacijai. Pacientai, turintys didelę AHR raišką, galėtų būti laikomi didelės rizikos grupe, kuriai būdinga didesnė metastazavimo rizika ir blogesnis atsakas į gemcitabiną.

Be to, AHR ir ELAVL1 yra glaudžiai susiję su imuniniu atsaku, uždegimu ir metaboline adaptacija, todėl jų moduliacija gali turėti poveikį ne tik navikinėms, bet ir stromos bei imuninėms ląstelėms.

5.7. Tyrimo ribojimai

Šis tyrimas turi keletą apribojimų. Pirma, naudotos tik dvi PDAC ląstelių linijos, kurios neviseškai atspindi PDAC heterogeniškumą. Antra, AHR mo-

duliacija atlikta daugiausia *in vitro* sistemose, kurios negali visiškai atkurti mikroaplinkos ir imuninės sistemos įtakos.

Trečia, pacientų kohorta buvo riboto dydžio, todėl rezultatai turi būti validuoti didesnėse populiacijose. Ketvirta, trūksta *in vivo* modelių, kurie leistų įvertinti sisteminį AHR–ELAVL1 ašies poveikį.

Penkta, ELAVL1 lokalizacijos analizė buvo daugiausia kokybinė, todėl reikėtų tikslesnių kiekybinių metodų.

Galiausiai tikslūs molekuliniai mechanizmai, kaip AHR reguliuoja ELAVL1 ir DCK, dar nėra visiškai išaiškinti.

5.8. Ateities tyrimų kryptys

Ateities tyrimai turėtų pirmiausia validuoti AHR–ELAVL1–DCK ašį didesnėse pacientų kohortose, įskaitant gydymo atsako analizę. Tai leistų įvertinti šios ašies kaip biologinio žymens gebą gemcitabino terapijai.

Taip pat būtina detalizuoti molekulinis mechanizmus naudojant RNA-seq, CLIP-seq ir proteominius metodus, siekiant identifikuoti visą ELAVL1 taikinių spektrą AHR reguliacijos procesuose.

Svarbus žingsnis – pereiti prie sudėtingesnių modelių, tokių kaip organoidai, PDX modeliai ir kokultūros sistemos, kurios geriau atspindi PDAC biologiją.

Be to, reikėtų tirti AHR vaidmenį imuninei mikroaplinkai, ypač T ląstelių, makrofagų ir stromos ląstelių sąveikai, nes AHR yra glaudžiai susijęs su imunine reguliacija ir metabolitais (pvz., kynurenino keliu).

Galiausiai terapiniai tyrimai turėtų įvertinti AHR inhibitorių derinius su gemcitabinu, siekiant nustatyti jų poveikį klinikinėje praktikoje. Pacientų stratifikacija pagal AHR/ELAVL1/DCK/PTGS2 profilį galėtų tapti svarbia individualizuotosios terapijos strategija.

IŠVADOS

1. AHR raiška yra susijusi su kliniškai ir biologiškai reikšmingais kasos lataktų adenokarcinomos požymiais. Padidėjusi AHR raiška koreliuoja su ELAVL1, DCK, EMT žymenų, PTGS2 ir HO-1 raiška ir limfmazgių invazija rodant ryšį su vėžio agresyvumu.
2. AHR ir ELAVL1 sudaro funkciškai susijusią reguliacinę sistemą, lemiančią atsaką į gydymą gemcitabinu. AHR slopinimas skatino ELAVL1 lokalizaciją citoplazmoje, moduliavo DCK raišką ir didino kasos vėžio ląstelių jautrumą gemcitabinui parodant AHR–ELAVL1–DCK svarbą chemoterapijam atsparumui.

3. AHR skatina EMT lemiamą kasos vėžio ląstelių agresyvumą. AHR raiška buvo susijusi su EMT žymenų pokyčiais, o jos slopinimas mažino ląstelių migraciją, invaziją ir EMT būdingas fenotipines savybes.
4. AHR reguliuoja citoprotekcinis ir uždegiminiis signalinius kelius kasos vėžio ląstelėse. AHR slopinimas mažino PTGS2 ir HO-1 raišką bei prostaglandino E₂ gamybą parodant AHR vaidmenį vėžio išgyvenimo bei atsparumo mechanizmuose.

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LIST OF DISSERTATION PUBLICATIONS

1. **Stukas D**, Jasukaitiene A, Bartkeviciene A, Matthews J, Maimets T, Teino I, Jaudzems K, Gulbinas A, Dambrasukas Z. Targeting AHR Increases Pancreatic Cancer Cell Sensitivity to Gemcitabine through the ELAVL1-DCK Pathway. *Int J Mol Sci*. 2023 Aug 24;24(17):13155. doi: 10.3390/ijms241713155. PMID: 37685961; PMCID: PMC10487468.
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