LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

International Summer School Practice

Combatting of glioblastoma cancer cell resistance to anti-cancer drug by targeting c-Myc function

The aim of the project is to investigate the effects of the c-Myc targetting by PSMG3-AS1 and mir-143-3p on glioblastoma cell resistance to anticancer drug temozolomide (TMZ)

Summary

Glioblastoma (GBM) is the most common malignant solid brain tumor, the treatment of which is particularly complicated by the resistance of cancer cells to therapy, in which the anticancer drug temozolomide (TMZ) is widely used. Resistance to TMZ is determined by several factors. One of them is the signaling pathways regulated by c-Myc, which regulate many biological processes, such as the cell cycle, metabolism, cellular senescence, apoptosis, angiogenesis and immune response. The role of c-Myc in GBM resistance to TMZ is that this transcription factor promotes the survival of GBM cells under the stress caused by TMZ treatment. Recently published studies have demonstrated that TMZ increases the expression of the c-Myc-stabilizing long non-coding RNA PSMG3-AS1 in glioblastomas. It has also been shown that PSMG3-AS1 influences GBM resistance to TMZ, as PSMG3-AS1 inhibition significantly increased cell sensitivity to this drug, and PSMG3-AS1 overexpression in a TMZ-sensitive GBM cell line induced a TMZ-resistant phenotype. In addition to its c-Myc stabilizing function, PSMG3-AS1 can inactivate mir-143-3p, which acts as a suppressor of c-Myc function in GBM cells and sensitizes cancer cells to TMZ.

During the summer internship, the student will determine how inhibition of c-Myc function affects the response of glioblastoma cells to TMZ. The student will suppress c-Myc activity in the TMZ-resistant glioblastoma cell line U87TMZR in two directions: 1) by suppressing PSMG3-AS1 expression and 2) by artificially inducing miR-143-3p overexpression using the appropriate lentiviruses. C-Myc expression (inhibition) will be assessed by Western blot analysis, and changes in PSMG3-AS1 and miR-143-3p expressions in lentivirus-treated U87TMZR cells will be assessed by reverse transcriptase PCR. The effect of c-Myc inactivation on the response of U87TMZR cells to TMZ will be assessed by the the student will acquire the basics of working with plasmid DNA (propagation in bacteria, purification and use in the production of lentivirus particles), learn to cultivate eukaryotic cells, and master gene expression modulation and assessment methods.

Project objectives and means of implementation

1) Production of lentiviruses lenti-mir143 and lenti-sirPSMG, encoding, respectively, miR-143-3p and siRNA against PSMG3-AS1.

- Production of lentivirus particles lenti-mir143 and lenti-sirPSMG in HEK293 FT cell culture using pLKO/mir143 and pLKO/sirPSMG vectors.

2) Inhibition of C-Myc activity in TMZ resistant glioblastoma U87 cells (U87TMZR).

- Transduction of U87TMZR cells with lenti-mir143 and lenti-sirPSMG lentiviruses.
- Evaluation of changes in c-Myc expression in transduced U87TMZR cells by Western blotting.
- Evaluation of changes in PSMG3-AS1 and miR-143-3p expression in lentivirus-transduced U87TMZR cells by RT-PCR.

3) Evaluation of the effect of inhibition of c-Myc activity on TMZ resistance of glioblastoma cells.

- Evaluation of the response of U87TMZR cells to different concentrations of TMZ by MTT proliferation/cytotoxicity assay. U87TMZR cells transduced with lentiviruses lenti-mir143 and lenti-sirPSMG and transduced with control ("empty") lentivirus will be compared.

Expected results and perspectives

1. The effectiveness of a two-pronged attack against c-Myc in causing changes in the response of glioblastoma cells to TMZ will be determined.

2. The work performed will significantly add to the student's scientific competence, as they will gain experience working with plasmid DNA, learn to cultivate eukaryotic cells (grow, transfect and transduce them and assess their viability) and learn to assess the expression of the studied gene in cells.