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EXPRESSION OF PROTEASE GENES IN IRE1 KNOCKDOWN U87 GLIOMA CELLS UPON GLUTAMINE DEPRIVATION

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Introduction. Proteases are an important part of the complex regulatory cascades in cells and play an extremely important role in the dynamic mechanisms of metabolism regulation in different pathological conditions. Living systems maintain a balance between proteases and their inhibitors and disturbing of this equilibrium leads to the development of many diseases, including malignant tumors. In this regard, the study of the role of key proteases and processes that they control is important for understanding the molecular mechanisms of cancer development. IRE1, the most evolutionarily conserved signaling pathway of the endoplasmic reticulum stress, is highly implicated in sustaining the proliferation of glioma cells and subsequent tumor growth, which is decreased by the inhibition of IRE1. Glutamine is an important factor of glioma development and a more aggressive behaviour. To explore the effect of glutamine deprivation on gene expressions in glioma cells in relation to the functional activity of IRE1 signaling, we studied the expression level of ubiquitin specific peptidase (USP) and cathepsin (CTS) genes, during glutamine deprivation in wild type U87 glioma cells (control cells) and cells with inhibited IRE1.

Methods. The following methods were used in this work: RNA extraction, electrophoresis in agarose and polyacrylamide gels, reverse transcription method, real-time qPCR and statistical analysis of results in Excel programs.

Results and Discussion. IIt was shown that the exposure of control glioma cells (transfected by empty vector) upon glutamine deprivation led to suppression of *USP1* (-13%), *CTSC* (-26%) and *CTSK* (-15%) gene expressions and upregulation of *CTSD* (+64%) and *CTSO* (+18%) mRNA. At the same time, glutamine deprivation did not significantly change the expression level of *USP4*, *USP10* and *USP14* genes in control glioma cells. Inhibition of IRE1 signaling enzyme function in U87 glioma cells increases the effect of glutamine deprivation on the expression of *USP1* gene (-32%), decreases *CTSD* gene expression (+38%) and introduces sensitivity of *USP4* and *USP14* genes to this condition.

Therefore, the inhibition of IRE1 signaling enzyme in U87 glioma cells modifies the effect of glutamine deficiency on the expression of most studied genes encoding cathepsins and ubiquitin specific peptidases: inducing the effect of glutamine deficiency on the *USP4* and *USP14* genes expression, decreasing – on the expression of *CTSD* gene, and amplifying – on the *USP1* gene expression.

Conclusions. Glutamine deprivation affects the expression level of most studied genes encoding cathepsins and ubiquitin specific peptidases in gene specific manner in relation to the functional activity of IRE1 signaling enzyme, a central mediator of endoplasmic reticulum stress, which control cell proliferation and tumor growth, and these changes in gene expressions possibly contribute to suppression of glioma cell proliferation.

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