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Regulation of the expression of TP53 and its associated proteins under hypoxia in glioma cells is ERN1 dependent

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Endoplasmic reticulum stress and hypoxia are necessary components of malignant tumors growth and suppression of ERN1 (from endoplasmic reticulum to nuclei-1) signalling pathway, which is linked to the apoptosis and cell death processes, significantly decreases proliferative processes.

The endoplasmic reticulum is a key organelle in the cellular response to hypoxia, ischemia, and some chemicals which activate a complex set of signaling pathways named the unfolded protein response. This adaptive response is activated upon the accumulation of misfolded proteins in the endoplasmic reticulum and is mediated by endoplasmic reticulum-resident sensor named ERN1.

Misfolded proteins in the endoplasmic reticulum lumen activate two distinct catalytic domains of ERN1, which display serine/threonine trans-autophosphorylation and endoribonuclease activities, respectively. ERN1-associated endoribonuclease activity is involved in the degradation of a specific subset of mRNA (RADD) and also initiates the cytosolic splicing of the pre-XBP1 (X-box binding protein 1) mRNA whose mature transcript encodes a transcription factor that stimulates the expression of unfolded protein response specific genes. It was shown that inhibition of the ERN1

enzyme leads to the inhibition of tumor vascularization and also to a significant decrease in the growth of tumor cells.

The aim of this work was to investigate the mechanisms of regulation of the expression of genes encoding TP53 and related to TP53 factors, namely: TP53 inhibitors - NME6, TOPORS and MDM2, TP53 activators - TP53BP1 and USP7, as well as its effectors - ZMAT3 and PERP in U87 glioma cells with suppressed function of the ERN1 enzyme under conditions of hypoxia.

The studies were carried out on U87 glioma cells and its subline from a dominant-negative construct based on the pcDNA3.1 vector containing cDNA of the sensor-signaling enzyme ERN1 without kinase and endoribonuclease domains (dnERN1). The expression level of TP53 mRNA and its activators was assessed by the data of the quantitative polymerase reaction in real time. The values of the studied genes mRNA expressions were normalized to the expression of beta-actin mRNA and represent as percent of control (100 %). Western blot analysis was also performed to confirm whether the mRNA expression level corresponded to the protein level. Using bioinformatic analysis microRNA binding sites were identified in the studied genes.

In this study, cells were cultured at a low oxygen level (3% oxygen, 5% carbon dioxide and 92% nitrogen) for 16 hours in an incubator at 37 °C.

Studies have shown that hypoxia has a different effect on the expression level of TP53 and its dependent genes while inhibiting the sensory - signaling enzyme ERN1. Expression of TP53, USP7 and ZMAT3 genes decreases under hypoxic conditions only in control glioma cells, but if the function of the ERN1 enzyme is excluded, these genes show clear resistance to hypoxia. Hypoxia condition is more meaningful to MDM2 and PERP genes, since their expression increase was observed in both cell types. Thus, we can say that the effect of hypoxia on the expression of the MDM2 and PERP genes depends on both the ERN1 activity and other factors.

It was found that hypoxia-induced changes in gene expression with the exclusion of the function of the sensory - signaling enzyme IRE1 lead to increased expression of the TP53BP1 gene and a decrease in the expression of TOPORS and NME6 genes.

Our data indicate that hypoxia exhibits a pro-tumor effect, and inhibition of the sensory-signaling enzyme ERN1 increases the expression of not only TP53 mRNA, but also its activators (TP53BP1 and USP7), which is consistent with a possible effect on suppressing tumor growth.

Thus, the cellular response to endoplasmic reticulum stress is an important mechanism by which tumor cells maintain the ability to continuously divide rapidly; therefore, the influence on the signaling pathways of the cellu-

lar response to endoplasmic reticulum stress and the identification of potential target genes can be used as a strategy for development of new anticancer drugs.