MOLECULAR MECHANISMS OF INTERACTION OF HYPOXIA WITH ENDOPLASMIC RETICULUM STRESS SIGNALING PATHWAYS

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Background and aim. The aggressive phenotype of glioblastoma multiforme may be associated with hypoxia, which plays a key role in tumorigenesis through changes in gene expression controlled by the hypoxia-inducible factor 1 (HIF-1). The rapid growth of solid tumors generates microenvironmental changes in regards to processes relying on the activation of endoplasmic reticulum stress signaling pathways. It is well known that suppression of the function of IRE1/ERN1 (inositol requiring enzyme 1/endoplasmic reticulum to nucleus signaling 1) branch of endoplasmic reticulum stress has been demonstrated to result in significant anti-proliferative effect in glioma growth. IRE1 regulates the transcription of a large number of genes through transcription factor XBP1 (X-box binding protein 1). Thus, the goal of our study was to evaluate the effect of hypoxia on the expression of genes that play an essential role in tumorigenesis, namely *IDH2* (isocitrate dehydrogenase (NADP+), *NNT* (nicotinamide nucleotide transhydrogenase), *SLC1A1* (solute carrier family 1 member 1), high-affinity mitochondrial glutamate transporters that also play an essential role in transporting glutamate across plasma membranes, *SLC1A3* (solute carrier family 1 member 3), glutamate transporter, also known as excitatory amino acid transporters (EAATs), and widely distributed throughout the brain, *SLC1A4* (solute carrier family 1 member 4), and SLC25A12 (solute carrier family 25 member 12), which controls glutamate transporter and gluconeogenesis, as well as microRNA miR-182-5p, which have specific binding sites in mRNA SLC1A1, in response to inhibition of endoplasmic reticulum stress signaling mediated by IRE1 in U87 glioma cells; identify binding sites for XBP1 and HIF transcription factors in promoter region of a subset of genes by bioinformatics analysis.

Methods. The expression level of the genes was studied in control and IRE1 knockdown U87 glioma cells under hypoxia by quantitative polymerase chain reaction. Statistical analysis was performed according to two-tailed

Student’s t-test using Excel program and OriginPro 7.5. Besides, we used bioinformatics methods for identification of HIF- and XBP1-binding sites in the promoter region of cancer growth related genes.

Results and Discussion. It was shown that the expression level of *IDH2*, *NNT*, *SLC1A3*, and *SLC1A4* genes was up-regulated in IRE1 knockdown glioma cells in comparison with the control glioma cells. At the same time, inhibition of IRE1 endoribonuclease and protein kinase leads to down-regulation of *SLC1A1* gene expression. We also observed up-regulation of microRNA miR-182-5p, which has specific binding sites in mRNA SLC1A1, compared to the control glioma cells. Thus, there is negative correlation between decreased level of SLC1A1 mRNA expression and increased level of microRNA miR-182-5p indicating possible post-transcriptional regulation of this mRNA expression. Moreover, we have shown that hypoxia enhanced the expression of *NNT*, *SLC1A1*, *SLC1A3*, and *SLC1A4* genes, while the expression level of *IDH2* gene remained unchanged. Furthermore, the knockdown of IRE1 signaling enzyme function modified the response of all studied gene expressions to hypoxia. The expression level of *IDH2*, *NNT*, *SLC1A3*, and *SLC1A4* genes was up-regulated under hypoxia in IRE1 knockdown glioma cells, while expression level of *SLC1A1* gene was down-regulated. Besides, binding sites for HIF and XBP1 transcription factors were found in most studied genes and some of these binding sites can recognize both transcription factors. Such binding sites were identified in *SLC1A1*, *SLC1A3*, and *SLC25A12* gene promoters and shown that knockdown of IRE1 eliminates hypoxic regulation of these genes.

Conclusions. The expression of all studied genes depends on IRE1 signaling enzyme function in gene specific manner, because inhibition of IRE1 significantly affects their expression. Results of our investigation demonstrate that hypoxia may affect the expression of genes through binding sites for HIF and XBP1. Therefore, IRE1 can modify the effect of hypoxia on numerous gene expressions through induction of XBP1 and interaction of HIF and XBP1 transcription factor in promoter. This regulation is possibly realized through binding sites with specificity to both transcription factor sites, which were identified in *SLC1A1*, *SLC1A3*, and *SLC25A12* gene promoters. The regulation of gene expression can be both transcriptional and post-transcriptional.

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