New diagnostic and therapeutical possibilities in the treatment of glioblastoma multiforme

Új diagnosztikus és terápiás lehetőségek a glioblastoma multiforme kezelésében

Ph.D. Thesis
Ph.D Tézisek

Dr. Bellyeiné Dr. Pozsgai Éva

University of Pécs, Department of Clinical Chemistry and Biochemistry

Program leader/ Program vezető: Prof. Dr. Sümegi Balázs

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Introduction

Malignant gliomas, such as glioblastoma multiforme, are the most frequent type of primary brain tumors. The standard approach of treatment is multimodal: surgical resection is followed by adjuvant radiotherapy and chemotherapy, with the alkylating agent temozolomide. Malignant gliomas can be typified by diffuse infiltration of the brain and increased resistance to conventional cancer therapies. Despite notable advancements in oncology, however, the early diagnosis and successful treatment of malignant gliomas continues to present a great challenge.

Heat shock proteins (Hsp) are a ubiquitous group of proteins found in all living organisms, that play an important role in cytoprotection and cell survival. Small Hsps (sHsp) have a molecular weight ranging between 2-43 kDa. Like other Hsp, small stress proteins also act as molecular chaperones and have also been found to be expressed at higher levels in different malignancies. Previously, our working group identified and characterized a novel small heat shock protein, Hsp 16.2. Preliminary studies indicated that Hsp16.2 is expressed in neuroectodermal tumors. Therefore, we began to study the expression of Hsp 16.2 in different types of brain tumors. Our aim was to examine whether Hsp16.2 plays a part in the development of various types of brain tumors and whether the level of its expression correlates with the malignancy of the tumor.

Growth hormone-releasing hormone (GHRH) induces growth hormone (GH) secretion after binding to pituitary-type GHRH receptors (pGHRH-R) in the anterior pituitary. The insulin-like growth factor I (IGF-I) stimulated by GH, plays an important role in the mechanism of malignant transformation, metastasis and tumorigenesis in various cancers, including brain cancers. GHRH antagonists have been applied successfully for the treatment of different types of experimental tumors, including malignant gliomas. The effects of the GHRH antagonists' on cancer cell viability and cell signaling pathways have not yet been elucidated. In the present study, we investigated the mechanism of action of two new potent GHRH antagonists: JMR-132 and MIA-602. Our goal was to examine the signal transduction and cellular response of brain tumor cells to treatment with GHRH antagonists and to investigate the effectiveness of GHRH antagonist MIA-602 *in vivo.*

The inhibitory effects of GHRH antagonists on tumor growth, invasion and metastatic ability of various cancers *in vivo* have previously been investigated. In our *in vitro* study in three highly malignant cell lines including the glioblastoma cell line, DBTRG-05, it was our goal to demonstrate how MIA-602 affects the critical steps of malignant tumorigenesis, such

as cell proliferation, stimulation of angiogenesis, enhancement of cell motility, cellular invasion and the production of key proteins involved in metastasis development.

Taken together, the aims of my study were to determine the following:

1. Is Hsp 16.2 present in different types of brain tumors?
2. Can a correlation be found between the expression of Hsp 16.2 and the grades of different brain tumors?
3. Are the pGHRH receptor and its main splice variant, SV1, expressed in glioblastoma cell lines?
4. Do GHRH antagonists have an effect on the cell survival of glioblastoma cell lines?
5. Do GHRH antagonists have an effect on the cell signalling pathways of glioblastoma cell lines?
6. How do GHRH antagonists influence the mitochondrial membrane potential of glioblastoma cells?
7. Do GHRH antagonists decrease the rate of glioblastoma tumor growth in a nude mouse animal model?
8. Do GHRH antagonists have an effect on invasion and metastasis development *in vitro*?
9. Could GHRH antagonists be a possible therapeutic tool for the treatment of malignant gliomas?

General conclusions

1. Hsp 16.2 was detected in different types and grades of brain tumors, however, the level of expression varied according to the type and grade of the tumor. All ninety-one tumor samples were labeled equally intra-nuclearly; they varied in their cytoplasmic labeling of Hsp 16.2.

2. Hsp 16.2 could not be detected in a significant quantity in normal brain tissue, it was only present in tumor cells in significant quantity and its level increased with the increase of cell anaplasia. The cytoplasmic expression of Hsp 16.2 correlates directly with the grade of the different types of brain tumors. Based on our findings, Hsp16.2 could become a valuable marker for primary brain tumor diagnosis and the anti-apoptotic activity of sHsp16.2 could become the target of drug therapy.

3. Both the pGHRH receptor and its main splice variant, SV1, were detected on the two glioblastoma cell lines,DBTRG-05 and U-87MG, pGHRH-R at 60 kDa and SV1 at 39.5 kDa.

4. GHRH antagonists, JMR-132 and MIA-602, decreased the cell viability of both DBTRG-05 and U-87MG glioblastoma cancer cell lines.

5. GHRH antagonists affect cell death through the following key pro-apoptotic pathways: the reduction of phosphorylated Akt, GSK3β and ERK 1/2, the cleavage of PARP and caspase-3 and through the intracellular translocation of proteins AIF, EndoG and cyt c.

6. GHRH antagonists abolish mitochondrial membrane integrity, through the depolarization of the mitochondrial membrane potential, thus leading to the release of pro-apoptotic signals from the mitochondrial inter-membrane space.

7. The treatment of experimental glioblastoma *in vivo* with the GHRH antagonist, MIA-602, resulted in the considerable (69%) reduction of tumor growth, demonstrating a significant inhibitory effect of this GHRH antagonist.

8. The new GHRH antagonist, MIA-602 decreased the proliferation, migration, invasion and MMP production in three cancer cell lines representing three different cancers (brain, ovarian, breast cancers) *in vitro*. Exposure to MIA-602 upregulated the expression of caveolin-1 and E-cadherin, and led to the powerful downregulation of β-catenin and NF-κB.

9. The current investigation indicates that GHRH antagonists, such as MIA-602, might be useful for the treatment of patients suffering from malignant brain cancer by the reduction of tumor growth and through the inhibition of cancer cell metastasis.