UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
PhD SCHOOL

PhD THESIS

THE IMPORTANCE OF TUMOR ANGIOGENESIS IN CEREBRAL TUMOR DIAGNOSIS AND THERAPY

ABSTRACT

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Key words: brain tumors, glioblastoma, angiogenesis, VEGFR inhibitors, PDGFR inhibitors, ELTD1

The state of knowledge

Cerebral tumor is a mass of abnormal cells that develop in brain tissues or brain envelopes, called meninge. There are two main types of brain tumors: primary cerebral tumors that start in the brain and secondary tumors or brain metastases that develop in other areas of the body and spread to the brain, the latter being the most common, about half the metastasis from lung cancer.

Cerebral cancer is one of the top 10 causes of cancer deaths, and is considered a serious health threat.

There are a variety of therapies used in the treatment of brain tumors, namely surgery, radiotherapy and chemotherapy, targeted therapy, or a combination of these. The treatment differs from one patient to another and is chosen according to tumor location, volume and grade, growth rate, patient's health, and the wishes of the patient and his family. The purpose of the treatment is to remove the tumor, relieve symptoms, improve the function of the brain or comfort the patient.

Target therapy refers to the use of drugs or other substances to identify and attack cancer cells, leaving normal cells unaffected. One such therapy is the treatment with monoclonal antibodies administered by infusion using laboratory antibodies from a single cell type of the immune system.

Monoclonal antibodies attach to certain substances, then killing cancer cells by blocking their growth or preventing them from spreading. They can also be used for the transport of drugs, toxins or radioactive materials released directly into cancer cells. In the treatment of recurrent malignant gliomas, target agents, namely bevacizumab that bind to vascular endothelial growth factor (VEGF); as well as gefitinib, erlotinib and imatinib targeting epidermal growth factor receptors and platelet-derived growth factor (EGFR, PDGFR).

Angiogenesis is a complex, well-organized process that involves the formation of new blood vessels due to the migration, growth and differentiation of endothelial cells, thus forming the inner walls of the blood vessels. This process is controlled by different proteins called angiogenic activators and inhibitors. In the central nervous system, angiogenesis plays an important role in several conditions, such as hypoxia, infarctions, infections and cancers.

Studies have shown that tumors cause the spread of new blood vessels from surrounding vasculature, so suppression of the angiogenesis process could inhibit tumor growth. Glioblastomas represent the most malignant brain tumors characterized by extensive vascularization and high invasion.

In the angiogenesis of glioblastomas, angiogenic factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), angiopoietin-2, platelet derived growth factor (PDGF), growth factor basic fibroblast and hepatocyte growth factor (HGF). Along with the information on angiogenic mediators and signaling pathways, new targets have been identified that should be exploited therapeutically in brain tumors. Certain studies have shown that tumor cells promote angiogenesis because it secretes certain extracellular vesicles that carry long-coded "long-coding" RNAs and proteins, vesicles that are captured by endothelial cells inside which activate proangiogenic signaling.

II. Personal contributions

Purpose and objectives

O1. Evaluation of ELTD1 as a tumor marker and as a therapeutic target in brain tumors

O2. Evaluation of PDGFR as a tumor marker and as a therapeutic target in brain tumors

O3. Evaluation VEGFR as a tumor marker and as a therapeutic target in brain tumors
O4. Evaluation of concomitant inhibition of PDGFR and VEGFR on brain tumors
O5. Evaluation of EGFR as a tumor marker and as a therapeutic target in brain tumors
O6. Effect of intracellular signal inactivation on brain tumor viability

Glioblastoma, a highly malignant phenotype of the brain tumor, is generally incurable. ELTD1 is considered a new potential marker for glioblastoma, although functional data are not yet available to characterize this molecule. For example, it is not clear whether this receptor can be used as a target molecule in glioblastoma therapy.

In the current study, ELTD1, PDGFR, VEGFR and their P13K / mTOR common intracellular pathway were analyzed as potential targets in glioblastoma. Our results show that treatment with AG1433 and SU1498 have a moderate cytotoxic effect on glioblastoma cells. Also, in glioblastoma cells, a high percentage of BEZ235-induced cytotoxicity (a p13k / mTOR pathway inhibitor) at nanomolar concentrations compared to AG1433 (a PDGFR inhibitor) and SU1498 (a VEGFR inhibitor) were cytotoxic at micromolar concentrations. Our study also shows for the first time that inhibition of the siRNA receptor ELTD1 induces cell death in glioblastoma, suggesting that this receptor may be important in the treatment of glioblastoma.

Glioblastomas have generally undergone substantial changes in management approach to the development of new techniques of chemotherapy, neurosurgery and radiotherapy.

Despite these significant changes, GBM management and prognosis remain poor, with a survival rate of only 5% in five years.

Discussions

Despite the current therapeutic approaches, glioblastoma remains one of the most lethal forms of cancer. The two most important ideas in cancer treatment are targeted therapy and personalized treatment. While chemotherapy is generically designed and affects all cells in a similar way, specific therapies seek to target a specific alteration. They offer the chance to act on cancer cells only, leaving normal cells intact. Several signaling pathways have been identified in glioblastoma as playing a major role in

tumorigenesis, resistance to treatment and disease regression, such as growth factor receptors, intracellular signaling cascade molecules. PDGFR, VEGFR and other tyrosine kinase receptors have been reported to be overexpressed in glioma, while receptor inhibition has been found to induce cell death as a single therapy or in combinations. It has been previously shown that inactivation of PDGFR by AG1433 induces low cell death in high-grade glioma primary cell lines, whereas action on both PDGFR and VEGFR increases the risk of cell death compared to single receptor inhibition.

Using a low passage of glioblastoma cell line, in the current study it was also found that treatment with a PDGFR (AG1433) inhibitor alone used induces low cytotoxicity after 3 days.

In a study with stem-like cell glioma, treatment with SU1498 and bevacizumab showed significant effects in inhibiting tumor growth, especially in combination with radiotherapy.

In this study, it was found that treatment with SU1498 had a low cytotoxic effect on glioblastoma cell cultures.

Protein receptor tyrosine kinases (EGFR, PDGFR, VEGFR, etc.) overexpressed or gene mutations activate Ras-Raf-MEK-ERK and PI3K-Akt-mTOR, resulting in uncontrolled cell proliferation of tumor cells.

Inhibitors of a single molecule normally show modest or no anti-tumor activity when used as a single therapy, but when used in combination with other therapeutic methods such as radiotherapy or chemotherapy, the cytotoxic effect is improved.

Many studies have shown the effects of BEZ235 in inhibiting the PI3K / AKT / mTOR pathway in various cancers, such as cisplatin-resistant tumors where radiation and BEZ235 delayed tumor growth. In this study, the high rate of cytotoxicity in the GB8B cell lines after treatment with this inhibitor at nanomolar concentrations was observed compared to AG1433 and SU1498 which had cytotoxic effect only at micromolar concentrations.

In recent years, a number of other biomarkers with potential in the diagnosis and therapy of glioma have been studied. Recently, Rheal, A. et al. have described ELTD1 as an important biomarker in glioma, which differentiates a low grade glioma from a

high-grade glioma.

Since siRNAs have been better studied lately, their therapeutic potential has increased. However, there are still issues that need to be addressed in their target release to tumor cells.

Their mode of action has been identified as induced differentiation in tumor cells and inhibition of growth in normal glioblastoma cells. In our study, GB8B cells were transf ected with ELTD1SRNA to obtain the highest cytotoxicity of all treatments used to inhibit the action of cell membrane receptors or their common intracellular signaling pathways.

In conclusion, our results have shown that treatment with AG1433 and SU1498 induces moderate cytotoxicity in glioblastoma cells.

ELTD1-siRNA treatment is most effective in inducing cell death in glioblastoma cells.

Conclusions

Although actual data published in connection with the function and structure of ELTD1 are quite limited, the receptor appears to be very important, not only as a biomarker, but also as a molecular target in glioblastoma. Inhibition of ELTD1 protein expression resulted in a significant decrease in cell proliferation among the GB8B GBM line, the maximal ELTD1 siRNA dose causing more than half of the cells in the culture to die. Inhibition of VEGFR and PDGFR receptors using AG1433 (anti-PDGFR) and SU1498 (anti-VEGFR) tirofostens resulted in a modest cytotoxicity, both monotherapy and combined, on the GB8B and GB10B GBM cultures. This may be due to the redundancy of the signal produced by the blocked receptors and to the ability of the multiple-surface receptor recruitment malignant cell which in turn activates multiple signaling pathways.

EGFR is one of the most important receptors in the progression of GBM, both through the high percentage of mutations / overexpression found among tumors and the impressive number of recruited signaling pathways. Inhibition of EGFR in GBM 8, 18 and 38 cells using tyrosine AG556 produced a modest cytotoxic effect compared to that obtained with anti-VEGFR and anti-PDGFR tirofosin.

Inhibition of the PI3K / AKT / mTOR signaling pathway using the BEZ235 inhibitor in the GB8B cell lines produced a more pronounced cytotoxic effect than that obtained

with surface receptor tyrosine kinase inhibitors. For the maximum doses of 60nM and 100nM respectively, the BEZ235 inhibitor produced two-thirds of the GBM cells.

Producing a more intense cytotoxic effect by inhibiting downstream signaling pathways compared to inhibiting tyrosine kinase receptors demonstrates the ability of these receptors to simultaneously activate common signaling pathways. By looking at these receptors by an important signaling pathway leads to the death of cells whose proliferation is stimulated by the activity of one or more tyrosine kinase receptors.

Although the current treatment prospects in HGG are limited, one of the most researched approaches by the researchers is the combined treatment of both classical therapeutic agents such as chemotherapy and that using newer therapeutic agents such as tyrosine kinase inhibitors or immunotherapy. An approach with particular potential in GBM, explored in other cancers, is the simultaneous inhibition of both surface receptors and downstream signaling pathways, an approach that could improve the survival of patients suffering from this incurable neoplasia.

Selective Bibliography