

New insights into multifaceted targeted therapy for treatment of glioblastoma multiforme.

Cancer is a major health problem worldwide. According to the World Health Organization reports, cancer accounts for over 13% of all deaths, which puts them in second place, immediately after cardiovascular diseases. Despite the knowledge, experience and a considerable amount of produced pharmaceuticals, as well as the huge investment in research, some types of cancer are still considered incurable. One of them is glioblastoma multiforme (GBM) - the primary malignant tumors of the central nervous system, which is characterized by poor prognosis and extreme malignancy. The median survival of patients with GBM is 12–15 months with a 5-year survival rate that remains at less than 5%, despite the use of intensive treatment modalities. Current therapy consists of surgical resection, radiotherapy together with concomitant chemotherapy. Innovative treatment strategies that have been implemented have contributed to extending the survival time, which is still far from satisfactory. Paradoxically, the increase in knowledge in the field of cancer biology and mutational landscapes in GBM is not accompanied by the progress associated with the therapeutical progress.

The most frequently alterations in GBM includes amplification or aberration within the epidermal growth factor receptor (EGFR) gene, which results in overexpression of the EGFR tyrosine kinase. Due to the presence of this kinase in the cell membrane, it is the first and the main element of the signal pathway. Dysregulation of EGFR is associated with increase tumor growth, adhesion, migration, and angiogenesis. Unfortunately, attempts to implement drugs with high specificity - selective EGFR inhibitors did not bring the expected effect. The main reasons limiting the efficacy of this therapy include GBM molecular heterogeneity, a frequent genomic alteration in the EGFR/PI3K/AKT pathway, which cause generation of alternative kinase signaling pathways and the acquisition of cellular resistance to currently available EGFR inhibitors. Moreover, one of the major challenge for modern science is development of innovative therapeutic strategies using *in vitro* GBM models, especially those that would reflect GBM molecular heterogeneity.

Our idea to overcome these problems is to use multitargeted kinase inhibitors that can interact with the EGFR signaling pathway and its downstream targets. Our assumptions are linked to the polypharmacological approach to the design of new drugs. Drugs that interact with multiple targets may possess better selectivity profile and effectiveness than agents aimed at single target only. The benefit of multidirectional targeting approach is to overcome problem of drug resistance. Moreover, in the case of many molecules that overexpressed in GBM guarantee a high selectivity, thus minimizing side effects of the therapy.

The main aim of the current project focused is design and synthesize inhibitors that will interact with the EGFR signaling pathway, and specifically at its first (EGFR) and last element (mTOR). We plan to conduct a comprehensive biological tests that will include antiproliferative activity on the panel of glioblastoma cells with different expression of EGFR, PTEN, IDH1, mTOR, as well as *in vitro* inhibition tests against tyrosine, serine-threonine and lipid kinases, which are closely associated with EGFR/mTOR signaling pathway. In addition, we plan to evaluate the ability to across the blood-brain-barrier for the most promising inhibitors, which is important element from the point of view of achieving the therapeutic concentration of the drug in GBM cells. Moreover, for the selected most active compounds, we plan to determine the exact mechanism of action, including the impact on interaction in many signaling pathways and the regulation of molecules involved in cell cycle progression and proteins involved in cell death by apoptosis or autophagy. Due to the heterogeneity of the glioblastomas, we perform studies on different models, such as 3D spheroids, patient-derived primary cell lines, that may better reflect the microenvironment of the brain tumor. In the final stage of project, we will verification of the efficiency of the therapy with most promising kinase inhibitor on *in vivo* model.