ASSESSMENT OF THE MECHANISM OF R132H MUTATION OF ISOCITRATE DEHYDROGENASE 1 GENE ($IDH1^{R132H}$) ON DIFFERENTIATION AND SURVIVABILITY OF HUMAN INDUCED NEURAL STEM CELLS

There are three human isoenzymes of isocitrate dehydrogenase, differing in their localization within the cell. In cancers, the genes encoding isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) are most frequently mutated. These enzymes catalyze the process of isocitrate conversion to α-ketoglutarate (αKG). The vast majority of mutations in *IDH1* gene are mutations of amino acids located in the active site of the enzyme. In more than 90% of cases it is a substitution of arginine to histidine at codon 132 (R132H). This mutation results in the loss of enzyme's proper function and reduction of αKG cellular level as well as in the gain of a new function, leading to production of 2-hydroxyglutarate (D2HG), which is thought to be an oncometabolite.

$IDH1^{R132H}$ mutation seems to be characteristic for gliomas, which are one of the most common primary brain tumors. Occasionally, it is also observed in other tumors, including acute myeloid leukemia, chondrosarcoma, thyroid cancer, prostate cancer and cholangiocarcinoma. Over the last three decades, a two fold increase in the number of brain cancer incidents has been observed in Poland (over 2700 cases in 2010 [*KRN*]). Over 15% of new cases is diagnosed in children; it is the second most common type of cancer in this group, constituting the cause of 1/3 cancer-related deaths. The mechanisms of glioma tumorigenesis and development still remain unknown. However, high incidence of $IDH1^{R132H}$ (observed in 70-80% of II/III gliomas and secondary glioblastoma (IV)), suggests that this mutation plays an essential role in this process. In addition, recent data also indicate the role of neural stem cells and progenitor cells populations in glioma genesis.

Both effects of the mutation seem to be involved in IDH1-mediated regulation of differentiation, growth, migration, proliferation and survivability of cancer cells. Nonetheless, defining their exact role in the process is exceptionally difficult since the problem itself is multithreaded and there is no appropriate experimental model. Vast majority of studies on the $IDH1^{R132H}$ mutation is focused on the inhibition of numerous αKG-dependent dioxygenases, via both reduction in their co-substrate level (αKG) and production of D2HG, which is similar in structure and, thus, inhibits the enzymes. Altered activity of dioxygenases due to $IDH1^{R132H}$ mutation predisposes the cell to malignant transformation via abnormal collagen maturation, histone and DNA methylation, which may lead to cell differentiation inhibition and stabilization of hypoxia inducing agent (HIF1α), which regulates expression of genes related to metabolism, angiogenesis, apoptosis and cell growth. Negative influence of $IDH1^{R132H}$ on differentiation and survivability of neural stem cells was demonstrated in preliminary studies (results published by the grant application author in PLOS One, 2016).

The innovatory aim of the study is to determine the relation between the effects of $IDH1^{R132H}$ mutation (loss or gain of function), cellular level of metabolites (αKG and D2HG), activity of αKG-dependent dioxygenases and inhibition of induced neural stem cells differentiation or increased apoptosis susceptibility. Identification of these relations will form a basis for the identification of the potential mechanism responsible for these processes impairment as well as will further the understanding of causes of gliomagenesis and will aid in the selection of new therapeutic targets. Moreover, the improved experimental model will constitute a valuable platform for studies on molecular basis of glioma, and, thus, will form a basis for broadening the knowledge of the genesis and biology of these tumors.