Abstract of doctoral dissertation entitled "Regulation of glioblastoma multiforme cells invasiveness, apoptosis and metabolism by Regnase-2"

Regnase-2 (Reg-2), also known as MCPIP2 and ZC3H12B, is an RNA-binding protein that is expressed primarily in the brain. Its structure has a CCCH zinc finger domain and a highly conserved PIN domain with RNAse activity. Previously published papers indicate the involvement of Reg-2 in the negative regulation of inflammation through the degradation of transcripts of pro-inflammatory cytokines, such as *IL-6* and *IL-1β*. The aim of this study was to investigate the role of Reg-2 in the regulation of processes related to the progression of the most common brain tumors - gliomas. The study focuses on the analysis of the influence of Reg-2 on invasiveness, stress response, apoptosis, proliferation and metabolism of glioblastoma multiforme cells.

The results obtained in this study indicate that Reg-2 reduces the ability of glioma cells to migrate. At the molecular level, this effect may be due to the negative regulation of metalloprotease 2 (*MMP2*) transcript level by Reg-2. In cells overexpressing Reg-2, the level of protein secreted into the medium is also reduced, which translates into lower MMP2 activity examined by gelatin zymography. Reg-2 is also involved in regulating the amount and/or composition of chemoattractants secreted by glioma cells, which is reflected in the number of endothelial cells migrating into the post-culture medium collected from above cells with reduced Reg-2 expression. This leads to the conclusion that this protein may be involved in the regulation of factors important for the induction of angiogenesis.

Cancer cells are exposed to many factors that induce a stress response. Due to local hypoxia and intense cellular metabolism, it is often oxidative stress associated with the generation of reactive oxygen species. One way to protect cells from the effects of such stress is the formation of stress granules (SGs). Among cells overexpressing Reg-2, the percentage of cells lacking SGs increases compared to control cells. Additionally, the number of granules in these cells is also reduced. Western blot analysis showed that the level of a key SGs component, the G3BP1 protein, is reduced in cells overexpressing Reg-2. RNA immunoprecipitation (RIP) confirmed that Reg-2 directly interacts with *G3BP1* mRNA. The formation of SGs is one way to avoid apoptosis. This study shows that signals indicating the induction of apoptosis can be detected in cells with Reg-2. It has also been shown that one of the mechanisms responsible for this may be direct binding of the transcript of the apoptosis inhibitor *BIRC5* by Reg-2. In glioma cell lines overexpressing Reg-2, *BIRC5* mRNA level is significantly decreased, whereas in high-grade tumor samples that have decreased *Reg-2* mRNA level, *BIRC5* mRNA level is

increased. The results obtained in this study also showed that Reg-2 is an important factor regulating cell proliferation, as both overexpression and knock-down of affected this process.

Metabolic changes occurring in cancer cells are a way to adapt to the constantly changing conditions of the tumor microenvironment. Their aim is to provide ingredients for the construction of macromolecules and to meet the energy needs of intensively proliferating cells. The studies carried out in this study showed that inhibition of glycolysis leads to phosphorylation and degradation of Reg-2. At the same time, an increase in the mRNA level of *IL-6*, which is a well-described substrate for Reg-2, was observed. Moreover, Reg-2 affects the functionality of mitochondria, causing cells to obtain less energy through oxidative phosphorylation.

The research obtained in this study indicates that Reg-2 may acts as a glioblastoma multiforme suppressor because it induces apoptosis, limits migration, and negatively affects the proliferation, stress response and cell metabolism.