Reg. No: 2015/18/E/NZ1/00737; Principal Investigator: dr Bogusz Stanisław Kulawiak

Ischemia of brain and heart tissue is the one of the most common causes of death worldwide. Recent studies have revealed that mitochondria play crucial role in cytoprotection against ischemia induced cell death. These organelles are surrounded by two membranes containing various proteins and protein complexes. Basic function of mitochondria, called cellular powerplants, is energy production used in many processes in the cell. These organelles take also part in other metabolic events such as the Krebs cycle, lipid beta-oxidation and apoptosis. In the inner mitochondrial membrane several potassium channels have been identified whose activation lead to cytoprotection during ischemic event. These proteins regulate potassium fluxes across the mitochondrial inner membrane. The basic properties have been found to be similar to the potassium channels from the plasma membrane. It was found that activation of mitochondrial large conductance calcium activated potassium channel (mitoBK$_{Ca}$) preserves brain and heart muscle cells. The pivotal role of mitoBK$_{Ca}$ channel in cytoprotection pathways is quite well described but detailed mechanisms of this phenomenon remains unclear. The channel forming subunit (called a subunit) is encoded by KCNMA1 gene. Many different splice variants of gene product were identified till today. Recently, the molecular identity of the mitoBK $_{Ca}$ channel was described raising fundamental questions about interactions of the channel subunits and influence of these interactions on channel regulation. It has been shown that a BK-DEC splice variant of BK$_{Ca}$-type channels alfa subunit localizes to mitochondria. Additionally, data obtained in the Laboratory of Intracellular Ion Channels showed that in brain tumor cells, the respiratory chain modulates activity of the channel and might interact with the regulatory subunit of the mitoBK$_{Ca}$. These findings open a new stage in the field of mitochondrial cytoprotection. Therefore the aim of the presented project is to characterize the regulation and interactions of mitoBK$_{Ca}$ channel subunits with other mitochondrial proteins.

We expect that the BK-DEC isoform of BK$_{Ca}$-type channel alfa subunit assemble in mitochondria to form a functional channel. We expect that assembled mitoBK$_{Ca}$ channel directly interacts with the respiratory chain and this interaction is important for channel formation and activity. We also expect to identify new partner proteins interacting with mitoBK$_{Ca}$ channel complex.

We plan to use two cell lines for our study HEK293 cells commonly used as a model for expression of functional BK$_{Ca}$ channels localized to the plasma membrane because they do not express functional BK channel proteins. Second cell line will be the human astrocytoma U-87 MG cells, since high activity of the mitoBK$_{Ca}$ channel was observed in these cells using mitoplast patch-clamping.

Workplan of this project will include three major steps. First, it is planned to generate cell lines expressing tagged mitoBK $_{Ca}$ channel subunits and cell knockouts of genes encoding subunits of the channel and respiratory chain. Here, we plan to use the newest techniques of mammalian genom editing (like CRISPR/Cas9 system). Then we plan to characterize mitochondria isolated from generated cell lines using electrophysiological (patch clamp of isolated mitoplasts) and biochemical methods. Finally, using tagged proteins we will purify and characterize protein complexes formed by the mitoBK$_{Ca}$ channel subunits. To achieve the goal of the project we will combine variety of techniques including electrophysiology, biochemistry and molecular biology.

Results of the planned research will help to understand natural cellular mechanisms of cytoprotection. It is possible, that obtained data will provide hints for development of new therapeutic strategies against consequences of damage induced by ischemia of heart and brain tissue.