Cancer cells energy metabolism have been reported to be strongly dependent on glucose in comparison to normal cells. This characteristic feature is associated with mitochondrial dysfunction. Mitochondria are described as "the powerhouse of the cell" because they supply most cells with most of their ATP. The machinery that the mitochondria use to make ATP is called the electron transport chain. This chain is made up of 5 complexes which are groups of proteins that work together to generate ATP. ATP production rate in mitochondria is regulated by activity of mitochondrial mega-channels that are localized to contact sites between the inner and outer mitochondrial membranes. Due to mitochondrial dysfunction cancer cells shift the burden of energy metabolism to cytoplasmic fermentation for ATP synthesis. To compensate the inefficient way of producing energy from glucose, malignant cells have increased rate of glucose uptake than normal cells. Within a cell, small fraction of glucose is converted to uridine disphospate N-acetylglucosamine (UDP-GlcNAc) which is substrate for O-GlcNAc transferase (OGT). This enzyme is responsible for the attachment of O-GlcNAc moieties to cellular polypeptides. The reports related to role of O-GlcNAcylation in mitochondria are very limited. However, recent data indicates that respiratory chain complexes and core mega-channels proteins are O-GlcNAc modified. At the same time, growing body of evidence shows that mitochondrial dysfunction may be caused by hyperglycemia, although mechanism of this regulation is still not fully understood. Many studies have shown strong relationship between glucose availability and intracellular level of O-GlcNAcylation. Therefore, this post-translational modification may be considered as a nutrient sensor that participates in adaptation of cells to changes of energy metabolism. Thus, in this project we will focus on the role of poorly investigated mitochondrial isoform of O-GlcNAc transferase.

Our preliminary study have shown glucose dependent OGT expression in mitochondria of breast cancer cells. Based on available data and already conducted own research, we hypothesize that mitochondrial O-GlcNAc transferase participates in adaptation of breast cells to changes of glucose availability and regulates glucose consumption. Thus in this project we would like to verify the impact of mOGT-dependent O-GlcNAcylation on activity of respiratory chain complexes and mitochondrial mega-channels. The experiments will be performed using various breast cancer cell lines as well as non-tumor mammary gland cell line. In order to determinate the role of mOGT dependent of glucose availability cells will be growing in hypo-, normo- and hyperglycemia. Altered expression of mOGT allow to determine whether this enzyme affect respiratory chain complexes activity or mitochondrial mega-channels phosphorylation. Increased expression of mOGT gene will be conducted by an appropriate method, using special expression vectors (plasmids). In cells with altered mOGT expression will be determined e.g.: cellular mOGT localization, mitochondrial metabolic state, O-GlcNAcylation of mitochondrial electron transport chain proteins and activity of respiratory chain complexes, mitochondrial mega-channels O-GlcNAcylation and glucose consumption. The results of our work may shed light on the molecular links between hyperglycemia and cancer.