Metformin is currently the first-line drug in the treatment of type II diabetes. Antineoplastic activity of metformin has been documented in pharmaco-epidemiological study in 2005 showing a reduced risk of cancer in patients with type II diabetes who received metformin. Numerous experimental and clinical studies are currently underway. However, the molecular mechanism of anti-cancer activity of metformin is not yet known.

One of the effects of metformin is activation of AMP kinase (AMPK). When the AMP / ATP ratio increases, AMPK is activated to restore normal ATP levels and inhibit cell energy expenditure. AMPK is therefore regulated especially in conditions of energy shortage (eg during starvation) and hypoxia (low oxygen level). On the one hand, AMPK inhibits anabolic processes and stimulates catabolism on the other. One of the energy substrates under glucose deficiency is proline, derived from protein degradation products, mainly collagen. Proline is degraded in mitochondria only by proline dehydrogenase / proline oxidase (PRODH / POX). Particularly interesting in this context is observation of the induction of PRODH / POX by AMPK. The main process of proline utilization is the collagen biosynthesis, which may limit the availability of proline to its degradation in mitochondria. On the other hand, prolidase activity (proline releasing enzyme from imidodipeptides) is an important regulator of free proline concentration in the cytoplasm.

PRODH / POX is a mitochondrial enzyme catalyzing the conversion of proline to pyrrolidine-5-carboxylic acid (P5C). During the conversion of proline to P5C, electrons are transported to the respiratory chain producing ATP or reactive oxygen species (ROS) are generated. In the first case, activation of PRODH / POX leads to the production of ATP for survival, and in the second case, ROS induces apoptosis. Although the mechanism of switching PRODH / POX from inhibitory to stimulatory for growth of tumor cells is not known, it seems that proline availability plays important role in this process. The above data suggest potentially important role of proline availability in the regulation of apoptosis / autophagy.

The link between AMPK, PRODH / POX and proline with apoptosis / autophagy in tumor cells and stimulatory effect of metformin on AMPK allows to present a hypothesis on the mechanism of metformin-induced apoptosis / autophagy. The intracellular concentration of proline and its conversion to P5C may play a key role in this process. For this reason, proline metabolism may play an important role in the regulation of apoptosis / autophagy. It can be regulated by some growth factor receptors and transcription factors such as HIF-1α, NF-κB.

The purpose of the research project is to identify the mechanism of anticancer activity of metformin as an apoptosis inducer in breast cancer cells by analyzing the expression of some receptors, transcription factors, signaling proteins, AMPK, PRODH / POX, and apoptosis and autophagy markers, under controlled conditions of cytoplasmic proline level.

In order to carry out the project, clones of MCF-7 breast cancer cells with modified PRODH / POX and prolidase expressions will be prepared. The effect of metformin on some metabolic processes in these cells will be studied. It is planned to evaluate the effect of metformin on cell proliferation, collagen biosynthesis, prolidase activity, expression of AMPK, PRODH/POX, some growth factor receptors, transcription factors, as well as proteins of signaling pathways by RT-qPCR technique, and Western immunoblot. Protein expression levels will be assessed by immunocytochemistry using confocal microscopy and flow cytometry. The impact of metformin on the cell cycle will also be assessed. Analysis of amino acids by high performance liquid chromatography or gas chromatography coupled with mass spectrometry (LC- MS / GC-MS) will be performed at the final stage of the study, evaluating the effect of metformin on the amino acid profile of PRODH / POX-modified MCF-7 cells.

This project aims to clarify the molecular mechanism of anti-cancer action of metformin in the experimental model of breast cancer cells. The biological effects of metformin on breast cancer cells will be assessed with respect to the potential use in development of a new targeted cancer pharmacotherapy. Knowing the target of antineoplastic action of metformin allows to improve the pharmacotherapy of cancer. The scientific results of the research project will be documented in journals with a high impact factor and posters presented at national and international scientific conferences.