Estrogens mainly estradiol are important regulators of cell proliferation, survival and differentiation in various tissues. One of the metabolites of estradiol is 2-methoxyestradiol (MOE), which inhibits cell proliferation in various types of cancer. An important function of estrogens is the regulation of connective tissue metabolism. Estradiol stimulates collagen turnover by enhancing the biosynthesis and degradation of this protein. An indicator of collagen turnover is increase of prolidase activity (the enzyme releasing proline from imidodipeptides for collagen resynthesis).

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). In the first case, activation of PRODH / POX leads to the production of ATP for survival, in the second one, ROS induces apoptosis. The mechanism of switching the PRODH / POX function from inhibitory to stimulatory for tumor cell growth is not known. Probably the availability of proline in this process may play an important role in the mechanism of regulation of apoptosis/autophagy.

The link between estradiol and its metabolite (MOE), PRODH/POX and proline with apoptosis/autophagy in tumor cells allows to present a hypothesis on the role of estradiol and its metabolite (MOE) in regulation of apoptosis/autophagy. The intracellular concentration of proline and its conversion to P5C may play a key role in this process. It contributes to induction apoptosis in cancer cells by activation of signaling pathways including PRODH/POX activity and some transcriptional factors.

The purpose of the research project is to verify the hypothesis that the activation of estrogen receptor stimulates proline metabolism (increase in collagen biosynthesis and proline utilization in this process) leading to a reduction in the amount of free proline (as a substrate for PRODH/POX-dependent apoptosis) and creating a pro-survival phenotype of breast cancer cells (MCF-7). In contrast, the estradiol metabolite, MOE, by impaired collagen biosynthesis leads to increase in intracellular proline concentration and inducing PRODH/POX-dependent apoptosis. The impairment of estrogen receptor function through MOE should therefore contribute to the induction of the pro-apoptotic phenotype of the cell.

In order to carry out the project, clones of MCF-7 breast cancer cells with modified PRODH / POX expressions will be prepared. The effect of estradiol and its metabolite (MOE) on some metabolic processes in these cells will be studied. It is planned to evaluate the effect of estradiol and MOE on cell proliferation, collagen biosynthesis, prolidase activity, expression of some apoptosis and autophagy markers, PRODH/POX, some growth factor receptors, transcription factors, as well as proteins of signaling pathways by RT-qPCR technique, and Western immunoblot. Protein expression will be also assessed by immunocytochemistry using confocal microscopy and flow cytometry. The impact of estradiol and MOE 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 estradiol and MOE on the amino acid profile MCF-7 cells with modified PRODH/POX expression.

This project aims to clarify the molecular impact of estradiol and its metabolite (MOE) on PRODH/POX-dependent apoptosis/autophagy in the experimental model of breast cancer cells. The biological effects of estrogen and MOE on breast cancer cells will be assessed with respect to the potential use in development of a new targeted cancer pharmacotherapy. Understanding the role of estradiol and MOE on PRODH/POX-dependent apoptosis/autophagy control help 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.