In recent years there has been an increasing interest in epigenetic processes. Epigenetics is defined as heritable changes in gene expression that are not encoded in the genomic DNA. One of the best characterised epigenetic mechanism is cytosine methylation in DNA, which is considered as a stable epigenetic mark involved in regulation of gene expression. Recent studies have revealed active DNA demethylation – the most dynamic process which regulates methylation pattern through hydroxylation/deamination of 5-methylcytosine (5-mC) and subsequent excision of generated derivatives in DNA repair process. Pathways of DNA demethylation are mediated by ten-eleven translocation (TET) enzymes and thymine DNA glycosylase (TDG). The alterations in DNA methylation pattern are considered as a hallmark of malignant transformation, including breast cancer.

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer death amongst women worldwide. According to the American Cancer Society, nearly 1.7 million new breast cancer cases are diagnosed each year globally. It is highly heterogeneous disease characterized by many subtypes that have different treatment responses and clinical outcomes. Treatment of breast cancer is mainly determined by expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2) in cancer cells. Selective estrogen receptor modulators (SERMs) are group of drugs used in hormone-dependent breast cancer treatment in the course of hormone therapy. The most used representative of SERMs is tamoxifen. Since its discovery, the use of the tamoxifen was evolved by embracing its ability to act like estrogen or antiestrogen depending on receptor type and its localization around the body.

The main objective of the project is to find out whether selective estrogen receptor modulators (SERMs) or estrogens exposure may evoke changes in DNA methylation pattern in breast cancer cell lines. There have been no studies concerning this subject. Our research may contribute to explain mechanisms of selective estrogen receptor modulators and their potential impact on epigenetic processes in DNA, including demethylation/deamianation within breast cancer cells.

Our research will include breast cancer cell lines which express different types of receptors: estrogen (ER), progesterone (PR), and G protein–coupled estrogen receptors (GPER). Our project comprises four different experiments using cell culture: without supplementation (A), with β-estradiol exposure (B), with SERMs exposure (C), with β-estradiol and SERMs exposure (D). We are going to quantify 5-methylcytosine (5-mC), and products of DNA demethylation/deamination: 5-hmC (5–hydroxymethylcytosine) and 5-hmU (5–hydroxymethyluracil) in cells isolated from abovementioned experiments. Furthermore, we would like to evaluate the expression of TET1, TET2, TET3 and TDG genes and of corresponding proteins, which play a crucial role in demethylation pathways. In order to determine DNA modification we will use a highly advanced technique– automatic online two-dimensional ultra-high-performance liquid chromatography with tandem mass spectrometry with isotopically labelled internal standards (2D-UPLC/MS/MS). For the analysis of expression of genes involved in epigenetic process expression, we intend to use the qRT-PCR technique, and proteins levels will be determined by Western Blot analysis.

Hence, findings from our project may shed a new light on underlying mechanism of breast cancer and the approach to prevention and treatment of this malignancy. If successful, findings from the project may provide new information, which may pave the way for new diagnostic and therapeutic methods as well as innovations in personalized medicine approaches.