Epigenetics refers to functionally relevant modifications of the genome which do not involve a change in the nucleotide sequence. One of the best characterized epigenetic modification is the methylation of DNA, which consists of methyl group binding to the carbon at the fifth position of the cytosine. 5-metCyt has a fundamental impact on cellular processes including genome stability, transcription/regulation of gene expression and development.

Process of the generation 5 methylcytosine (5-metCyt) is catalyzed by DNA methyltransferase(s) and it is well characterized while the pathways by which DNA is actively demethylated are not well understood. Recent discoveries demonstrated that TET (Ten-Eleven Translocation), enzymes are responsible for active demethylation process which involves conversion of 5-metCyt to 5-hydroxymethylcytosine (5-hmCyt) . These enzymes belong to 2-ketoglutarate, oxygen and iron-dependent dioxygenases. TET family proteins can further catalyze the oxidation of 5-hmCyt into 5-formylcytosine (5-fCyt) and 5-carboxycytosine (5-caCyt).

One of the general feature of many cancers, including breast cancer, is global hypomethylation which may be a result of aberrant activity of the demethylation process. Interestingly, 5-hmCyt level was shown to decrease in all studied cancers. In addition, drug induced changes in gene expression were linked with 5-metCyt decrease and simultaneous increase of 5-hmCyt, what suggest that active DNA demethylation may be directly involved in drug-induced transcription regulation

Recently it was demonstrated that the vitamin C induces TET-dependent DNA demethylation likely by regulating of the activity of TETs. The intermediate of the Krebs Cycle, 2-ketoglutarate may be converted to 2-hydroxyglutarate, which is considered as an “oncometabolite”. This metabolite is a competitive inhibitor of the dioxygenases, what in turn, impair the active DNA demethylation.

The aim of this study is to find out whether mentioned above the key intermediates of active DNA demethylation process may have prediction power of an outcome of breast cancer therapy. The study will comprise women diagnosed with breast cancer who are eligible for neoadjuvant (presurgery) chemotherapy in the AC-T regimen (doxorubicin, cyclophosphamide, taxanes - paclitaxel). Blood samples will be collected before the treatment – prior to neoadjuvant chemotherapy (NAC) (sample A), after completion of the first series of chemotherapy-doxorubicine, cyclophosphamide (sample B), after completion of paclitaxel treatment (sample C) and about one month after surgery (sample D). After surgery cancerous and marginal tissues will be collected and assessed. Patients will be also examined with mammography and USG in order to determine the stage of the tumour according to the Classification of Malignant Tumours (TNM). The study group will consist of 70-100 patients.

Determination of the derivatives of 5-hmetCyt will be performed in DNA isolated from leukocytes in all above mentioned time points and from tumour and surrounding normal tissues. Analyses will be carried out using two-dimensional liquid chromatography with mass detection (LC-2D/MS/MS). Proposed research may provide an answer to the question; whether there is a correlation between the level of the active demethylation DNA metabolites, and remission of the tumour and whether there are differences in the levels of derivatives of 5-metCyt linked with the response to chemotherapy. The prognostic evaluation is very valuable in making decisions concerning further treatment. If the role of 5-hmCyt (and/or its derivatives) as a predictive marker of the response to the systemic treatment for breast cancer, will be documented the level of this modification may be analyzed using commercially available assays (e.g. ELISA) to define a group of increased risk/ negative response to treatment. So far, no studies concerning the relationship between the level of vitamin C and 2-ketoglutarate/ 2-hydroksyglutarate in the blood and tissues and the level of the 5-hmCyt and its derivatives in the samples of patients before and after chemotherapy were performed. All these compounds will be analysed in our project. In order to clarify the participation of proteins, including AID, TET, TDG in the active demethylation process, in the context of cancer chemotherapy, we will also analyse the expression of these genes in the leukocytes, cancerous and normal cells using Real Time PCR technique. We would like to explain the relationship between the level of expression of these genes and the amount of active demethylation products in the investigated specimens before and after chemotherapy. The proposed analysis should provide new information about the role of abnormal metabolic pathways in the development of breast cancer and may allow to find out useful markers for early diagnosis.

It is commonly believed that 8-oxo-2'-deoxyguanosine (8-oksydG) is the best marker of oxidative stress at the level of the whole organism. Our previous studies shown a relationship between chemotherapy with an anthracycline and oxidative stress, which justifies investigation of the level of 8-oksydG excreted in the urine. We also want to know if there is a relationship between oxidative stress and the level of DNA repair products related to active demethylation (5-hmCyt and 5-hmUra) excreted into the urine. To answer the question, whether there is a relationship between oxidative stress induced by chemotherapy and an active demethylation process in patients with breast cancer, we are planning to perform the analysis of the aforementioned modifications in urine samples taken at different time points mentioned above.

Changes in the DNA methylation patterns are commonly observed in the time-course of cancer development, as well as a result of drug treatment, including chemotherapeutic agents. Therefore, given the plasticity of epigenetic marks, especially 5-metCyt derivatives, and their responsiveness to chemotherapy, proposed analyses should answer the important question whether the analysed compounds have prediction power of the therapy outcome. In addition the analyses may give some insight into mechanism of chemotherapy resistance.