Streszczenie w języku angielskim

The analysis of expression of genes encoding proteins involved in active DNA demethylation processes in breast cancer

5-methylcytosine (5-mC) is a central epigenetic mark, which is involved in regulation of crucial genes in cell. Breast cancer, similarly to other malignancies, displays global DNA methylation. Level of 5-mC in cell is dependent on active DNA demethylation, which involves TET family proteins, and deamination, which is driven by AID family proteins. The cancer cell displays altered energetic metabolism, which is reflected i.e. by changes of Krebs cycle enzymes' activity: isocitrate dehydrogenases (IDH1, IDH2) or alterations in oxygen status followed by activation of hypoxia inducible factors (HIF1-α, HIF2-α). The abovementioned processes may be involved in impaired balance between DNA methylation and demethylation. The main objective of the study was to determine whether breast cancer patients exert changes in expression of genes involved in active DNA demethylation as well as key metabolites arranged in this process, in leukocytes. The treatment process of breast cancer patients is conditioned by specific biological subtypes of this malignancy. It was determined that biological subtypes display distinct, specific methylation profiles. The epigenetic processes may be potentially involved in regulation of therapy outcome, therefore, the next objective of the study was evaluation if neoadjuvant treatment of breast cancer patients may evoke changes in genes and metabolites of DNA demethylation. The aberrant DNA methylation of crucial genes implicated in breast carcinogenesis may be involved in tumor progression. The final objective of the study was to assess whether the expression level of genes involved in active DNA demethylation and products of this process may have the prediction power of breast cancer therapy. The study involved 60 breast cancer patients and 33 healthy controls. The blood samples of breast cancer patients were collected before the treatment (sample A), after doxorubicin/cyclophosphamide treatment (sample B), after completion of taxanes treatment (sample C) and one month after surgery (sample D). Additionally, cancer and marginal tissue were obtained from 22 patients. Expression of *TET1, TET2, TET3, TDG, AID, IDH1, IDH1, HIF1-α, HIF2-α* was evaluated using qRT-PCR with Universal Probe Library probes. The quantitative level of 5–mC and its derivatives was measured by ultra-performance liquid chromatography system with tandem mass spectrometry detection (2D-UPLC-MS/MS). The decreased level of *TET1* expression and low 5-hydroxymethylcytosine (5-hmC) level in leukocytes and tissues of breast cancer patients and their correlation with Ki-67 proliferation index may indicate their potential role as predictive markers. The low level of *AID* expression with concurrent 5-hydroxymethyluracil (5-hmU) increase may suggest the minimal role of AID in 5-mC and its derivatives conversion in breast cancer patients. The high level of TET3 expression and increased levels of 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) may indicate the preferential further hydroxylation of 5-hmC driven by this gene. High expression of *IDH1* in leukocytes of breast cancer and its lower level in those with complete tumor remission may indicate its possible role in therapy outcome. The alterations in active DNA demethylation after doxorubicin treatment, which were evinced by lower expression of *TET1* and *TDG* and decreased level of 5-mC derivatives, may suggest alternative, possible mechanism of doxorubicin interaction on target cells. The slight tendency of genes' expression levels and intermediates of active DNA demethylation, which were observed amongst breast cancer patients with different biological subtypes, may indicate their potential to become standard diagnostic tool as well as innovation in personalized medicine approaches.

Key words: breast cancer, epigenetics, active DNA demethylation, neoadjuvant chemotherapy, RT-qPCR.

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