Analysis of active DNA demethylation products in urine of patients with colorectal cancer and acute myeloid leukemia

In addition to genome-wide hypomethylation, cancer cells are also characterised by site-specific hypermethylation of promoter regions of CpG island. The pattern of DNA methylation is not only a consequence of the attachment of methyl groups to cytosines but also of demethylation. Active DNA demethylation is a multistep process in which occurs the enzymatic removal of the methyl group from 5-methylcytosine (5-mCyt) with the involvement of TET proteins, excision and replacement of 5-mCyt by normal cytosine via base excision repair, deamination of 5-mCyt to thymine or excision of an oligonucleotide fragment containing 5-mCyt. Active demethylation products of 5-mCyt and oxidatively damaged nitrogenous bases of DNA or nucleosides, after removal from DNA by repair systems, can be released into the bloodstream and ultimately excreted in the urine.

This study aimed to develop a method to analyze quantitatively the level of epigenetic DNA modifications in urine and assess their usefulness as potential non-invasive biomarkers for the development of colorectal cancer (CRC) and acute myeloid leukaemia (AML).

Using the technique of two-dimensional ultraperformance liquid chromatography-tandem mass spectrometry (2D-UPLC-MS/MS), we optimized the separation and detection conditions, which allowed us to identify and quantify modified nitrogenous bases in urine such as 5-hydroxymethylcytosine (5-hmCyt), 5-formylcytosine (5-fCyt), 5-carboxylcytosine (5-caCyt) and the nucleosides: 5-methyl-2'-deoxycytidine (5-mdC), 5-hydroxymethyl-2'-deoxycytidine (5-hmdC), 5-hydroxymethyl-2'-deoxyuridine (5-hmdU) and 8-oxo-2'-deoxyguanosine (8-oxodG). Since commercially available standards are lacking for some of the analysed compounds, we decided to synthesise them in our laboratory.

The first study group included patients with acute myeloid leukemia (AML) and patients with myelodysplastic syndromes (MDS). Analysis of epigenetic modifications in the urine of patients with AML showed higher concentrations of all modified nitrogenous bases and deoxynucleotides compared to healthy subjects. Patients with MDS had also higher levels of active DNA demethylation products in urine compared to the control group. Urinary levels of 5-fCyt, 5-hmdC and 5-hmUra reached similar values in AML and MDS patients. Urinary excretion for 5-hmCyt, 5-caCyt and 5-hmdU was characterised by a gradual increase in concentration from the control to the MDS group, with the highest concentrations observed

in patients with AML. Statistical analysis with the usage of the ROC test allowed us to assess the diagnostic power and identify the most promising biomarkers for the development of AML and MDS and the transformation of MDS to AML. Urinary levels of 5-hmCyt and 5-hmdU were found to be the most diagnostically useful to distinguish AML patients from controls, while urinary 5-hmdC in DNA and urinary 5-hmCyt were found to be the most useful for MDS patients. Multivariate classification tree models allowed correct classification of AML and MDS patients in 95,7% and 94,7% of cases, respectively. The highest predictive value among the analysed parameters in predicting MDS transformation into AML was observed for 5-cadC and 5-hmdU in DNA.

The second study group included patients with colorectal cancer (CRC), inflammatory bowel disease (IBD) and adenoma (AD). Analysis of active demethylation products in the urine of patients with CRC and AD showed significantly lower levels of 5-fCyt than in the control group. Urinary excretion of 8-oxodG was significantly higher in CRC and IBD patients. The level of 5-hmCyt was significantly higher in the group of CRC patients than in the control group.

Key words: active DNA demethylation, biomarkers, urine, acute myeloid leukemia, colorectal cancer

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