Abstract

One of the fundamental processes in the development and progression of colorectal cancer is the accumulation of a variety of genetic and epigenetic changes in colonic epithelial cells. Epigenetic processes, such as DNA methylation and demethylation, are natural and essential to many organism functions. Aberrant DNA methylation plays crucial role in cancer development. Another characteristic feature of cancer cells is significantly lower level of 5-hydroxymethylcytosine (5-hmCyt). This modification is a product of active DNA demethylation, involving enzymatic oxidation and deamination of 5-methylcytosine (5-mCyt) with subsequent formation of 5-hmCyt and its derivatives (5-formylcytosine (5-fCyt), 5-carboxycytosine (5-caCyt), 5-hydroxymethyluracil (5-hmUra)).

We applied isotope-dilution automated online two-dimensional ultraperformance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS) methodology in combination with isotopically labeled internal standards. This method allows us determination of several DNA modifications (5-mCyt, 5-hmCyt, 5-fCyt, 5-caCyt, 5-hmUra, uracil, 8-oxoguanine (8-oxoGua)), in the form of deoxynucleosides. This made it possible to compare the epigenetic profile between healthy individuals and patients with inflammatory bowel disease (IBD), colon polyps and colorectal cancer. Moreover, we analyzed plasma concentrations of antioxidant vitamins: ascorbate, retinol and α-tocopherol.

Patients from all groups presented with significantly lower levels of 5-mCyt and 5-hmCyt in DNA than the controls. Patients with IBD showed the highest levels 8-oxoGua, a marker of oxidative stress. Individuals with colorectal cancer presented with relatively high content of 5-caCyt and the lowest concentrations of ascorbate and retinol. A positive correlation was observed between plasma concentration of ascorbate and levels of two epigenetic modifications, 5-hmCyt and 5-hmUra in leukocyte DNA.

Each of the analyzed groups, healthy controls, individuals with IBD and adenomatous polyps and colorectal cancer patients, presented with a characteristic pattern of epigenetic DNA modifications. This study provided the first *in vivo* evidence for the relation between ascorbate concentration and levels of epigenetic DNA modifications. These findings suggest that deficiency of ascorbate in the blood may be a marker of its shortage in other tissues, which in turn may correspond to deterioration of DNA methylation-demethylation.

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