Abstract

The cytosine methylation process, catalyzed by DNA methyltransferases, is a key epigenetic process whose marker is 5-methylcytosine (5-mCyt). The process of an active DNA demethylation, in part controlled by Ten-eleven-translocation (Tet) proteins, is responsible for hypomethylation of DNA. In 2009, it has been demonstrated that Tet1 enzyme oxidized the DNA 5-mCyt residue to 5-hydroxymethylcytosine (5-hmCyt) which was conclusively detected in human DNA. It is believed that, global hypomethylation, as well as hypermethylation of some areas of DNA, may contribute to an activation and silencing of respectively proto-oncogenes and suppressor genes in cancer cells.

This doctoral dissertation presents the results of 2-ketoglutarate (2-KG), L-, D-2-hydroxyglutarate (L-, D-2-HG) and vitamin C levels, potential modulators of Tet proteins activity, using the model of large intestine diseases. This model was chosen because of the possibility to analyze the progression of clinical changes from chronic inflammation, through benign proliferative lesions (polyps) to cancer (colorectal cancer, CRC). There are no experimental results in the literature testing the possibility of using quantitative analyses of 2-KG/2-HG in plasma/urine as a marker of intestinal diseases.

To accomplish the research aims, quantitative methods for metabolites level analysis in plasma, urine and tissue homogenates (tumor fragment and surgical margin) using ultraperformance liquid chromatography with UV, MS/MS detection were developed, validated and optimized.

This doctoral thesis in quantitative manner characterizes adults in terms of the "physiological" level of 2-ketoglutarate and vitamin C in plasma and L-, D-2-hydroxyglutarate in plasma and urine. There is a weak correlation at the level $r=0.3992$, $p=0.012$, $n=39$, between the content of 2-KG in the tumor and corresponding healthy tissue, however, there is no relationship between the plasma and intracellular level of 2-KG, which can be an individual property. Significantly decreased level of 2-KG in plasma of patients with inflammatory bowel disease in relation to other studied groups may be related to its excessive consumption by changes that occurring in the Krebs cycle under conditions of hypoxia and chronic inflammation.

The plasma/urinary of D-2HG level is not significantly different between the studied groups, it can be considered that its "physiological" concentration was measured, which is in accordance with the literature data (except for two patients with polyp and CRC who exhibit its spectacularly high level and for which mutations within the *IDH* or *2-*

HGDH genes can be suspected). Moreover, significantly higher concentration of L-2HG in urine and plasma of patients with CRC with respect to the control group was found. There is a strong positive correlation between L-2HG level in plasma and urine $r=0.6084$, $p<0.001$, $N=82$ and moderate positive correlation between L-2HG and D-2HG level, both in plasma ($r=0.4516$, $p<0.001$, $N=82$) and urine ($r=0.5746$, $p<0.001$, $N=82$). The highest plasma level of vitamin C was found in the control group and the lowest in CRC group.

Based on the literature data it can be assumed that the role of D-2HG is limited only to the poor competitive inhibition of 2-ketoglutarate-dependent dioxygenases (2-KGDO), such as Tets or PHDs enzymes. L-2HG, in addition to acting as a weak inhibitor of 2-KGDO, can be attributed an additional function in the adaptation of cells to hypoxia conditions. It may contributes to stabilization of HIF1 protein by inhibiting PHDs. It is quite probable that it is easier to "capture" the inhibitory effect of D-2HG on Tet's enzymes than L-2HG, which is multidirectional.

The modified bases/nucleosides are released into the bloodstream and eventually appear in the urine after theirs excision from DNA. With reference to the 2-HG oncometabolite, there is a strong negative correlation between the concentration of D-2HG in the plasma and 5-hmCyt urinary level for both control and CRC (respectively: $r=-0.6357$, $p=0.001$, $N=23$ and $r=-0.6064$, $p=0.037$, $N=12$).

The higher plasma concentration of D-2-HG, which is likely to be "released" from cells into the bloodstream, less 5-hmCyt level appears in the urine. This would indicate the inhibition of Tets by D-2-HG, then less 5-hmCyt is released to the circulation, which is reflected in the urinary level.

Key words: large intestine diseases, epigenetics, an active DNA demethylation, cellular metabolites, UPLC-MS/MS/UV.

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