2-ketoglutarate and 2-hydroxyglutarate as a potential metabolic modulators of an active DNA demethylation process among colorectal cancer patients.

The central epigenetic mark is 5-methylcytosine (5-mCyt), the fifth DNA base, and disturbance of DNA methylation is known as a main reason of tumor progression. It has been known that hypermethylation, as well as global hypomethylation of DNA, may contribute to genetic instability and malignant transformation. However, the mechanisms of hypo- and hyper-methylation are unclear. It has been demonstrated that Ten-Eleven-Translocation (TET) enzymes oxidized the DNA 5-mCyt residue to 5-hydroxymethylcytosine (5-hmCyt) which can be further oxidized to the 5-formylcytosine and 5-carboxycytosine. The TET enzymes depend on 2-ketoglutarate (2-KG) and oxygen and are involved in an active DNA demethylation process, responsible for DNA hypomethylation. It is possible that their activity is inhibited by 2-hydroxyglutarate (2-HG).

The main objective of this project is to find out whether 2-KG and 2-HG, which are potential TETs modulators, may serve as cancer markers? Specifically we would like to:

- know whether there are differences in the level of 2-KG and 2-HG in plasma and/or urine among colorectal cancer patients (CRC) and healthy individuals?

- determine concentrations of 2-KG/2-HG in plasma and urine among healthy individuals useful for comparative purposes in future studies.

- know whether the plasma/urinary level of 2-KG/2-HG reflects activity of TET proteins expressed by the level of 5-methylcytosine derivatives?

- know whether 2-KG plasma level in CRC patients/control correlates with its level in tumor/peripheral tissue?

In order to answer the aforementioned questions we are going to measure and compare the concentrations of 2-KG/2-HG in biological material from patients included in this project. Our proposal provides the use of the most reliable and sensitive methodology: UPLC-MS/MS with stable isotope-labeled internal standards. The answer to the last question will provide us an attempt to correlate the results of this study with results from another project in which we have analyzed the level of epigenetic modifications in DNA isolated from leukocytes and urine of the same groups of patients.

The aspects included in this project should allow a better understanding recently discovered pathways of active DNA demethylation, their relationship with the pathogenesis of CRC. Colorectal cancer is the second most common cancer in women and the third in men worldwide. Early–stage cancer detection is critical for reducing incidence and mortality. In practice, the most commonly used CRC screening tools have insufficient predictive power or are invasive for patient. Lack of sensitivity and specificity precludes the use of carcinoembryonic antigen (CEA), as well as, all other serum markers for early detection of CRC. The combination of quantitative measurements of 2-KG/2-HG in plasma and/or urine may become minimally- or even completely non-invasive biomarker of development and/or relapse of cancer.