# Tandem mass spectrometry based analysis reveal relationship between active DNA demethylation and Krebs cycle in AML and MDS

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## Introduction

The most dynamic process which regulates DNA methylation is recently discovered active demethylation. It involves ten-eleven translocation (TET) enzymes to catalyze stepwise oxidation of 5-methylcytosine (5-mCyt) to 5-hydroxymethylcytosine (5-hmCyt) and further demethylation products 5-formylcytosine (5-fCyt) and 5-carboxylcytosine (5-caCyt). [Fig.1] [1] Mutations targeting TET genes are frequently observed in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). [2][3] Mutations in genes: succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH) are also found in acute leukemias. These mutations result in accumulation of the succinate (SA), fumarate (FA), 2-oxoglutarate (2-OG) and R-2-hydroxyglutarate (R-2HG). [Fig.2] It may deregulate the activity of TET enzymes and, in turn, DNA demethylation. Although the oncogenic mechanism of these mutations remains still under investigation, determine of these metabolites could be relevant for diagnosis, prognosis and treatment of a subset of patients with AML and MDS. [4]

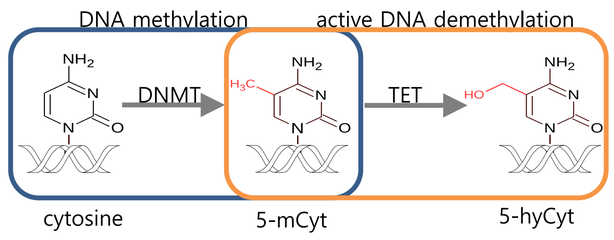


Fig. 1. Methylation of cytosine and active demethylation of 5-mCyt in DNA.

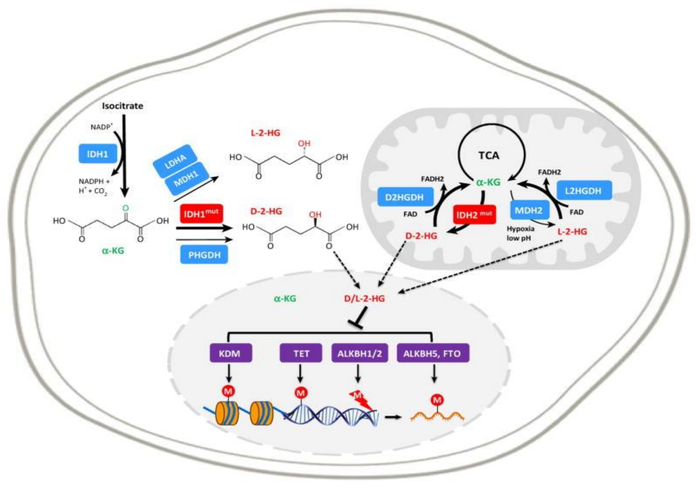


Fig. 2. Metabolism of 2-hydroksyglutarate. [4]

## Objectives

We would like to developed highly sensitive methods for separation and mass-spectrometric detection of sucinate, fumarate, 2-oxoglutate, L- and D-2-HG enantiomers. The main objective of this study is to find out the relationship between the level of 5-mCyt and the derivatives of active DNA demethylation process and the level of metabolites: SA, FA and 2-OG/2-HG in plasma/urine of patients developing AML and MDS.

## Material and methods

We have examined 3 groups: healthy controls, patients with AML and MDS at diagnosis de novo. In all groups we have measured the level of epigenetic DNA modifications in leukocytes using isotope-dilution automated online two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS) [Fig.3][Fig.5]. The level of R-2HG in urine and plasma has been measured using 2D-UPLC-MS/MS after derivatization with DATAN (Di-O-acetyl-L-tartaric anhydride). [Fig.6] Plasma concentrations of SA, FA and 2-OG have detected using UPLC- MS/MS method. [Fig.4]

Fig. 3. 2D-UPLC-MS/MS -XevoTQ-S /ACQUITY 2D-UPLC / APGC (Waters).

Fig. 4. UPLC-MS/MS -AcquityUPLC with Quattro Premier TM XE (Waters/Micromass).

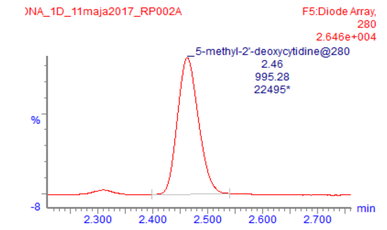


Fig 5. Representative chromatographic separation of 5-hmdC in leukocyteDNA from paient with AML by 2D-UPLC-MS/MS.

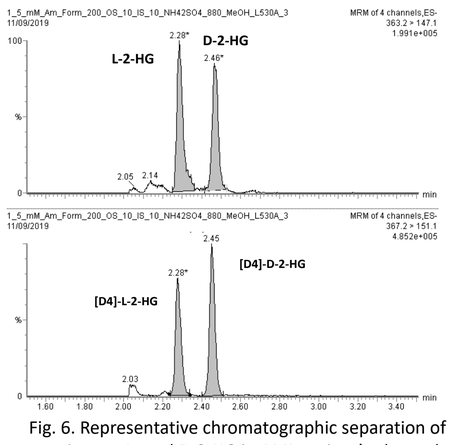


Fig. 6. Representative chromatographic separation of enantiomers L- and D-2-HG in AML patient's plasma by 2D-UPLC-MS/MS.

## Results

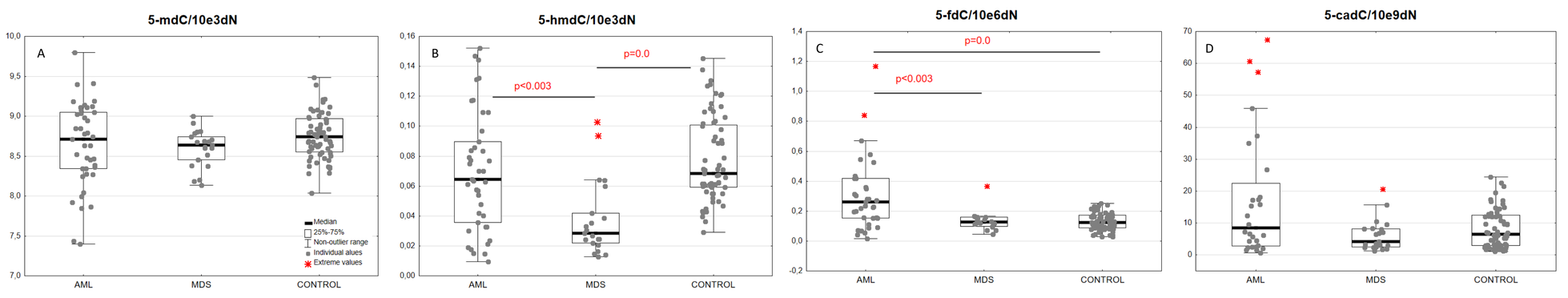


Fig. 7.The level of 5-mdC (A) and its oxidation products 5-hmdC (B), 5-fdC(C) and 5-cadC(D); patients with AML, MDS and healthy controls. Statistically significant differences: Mann-Whitney U Test p<0.05.

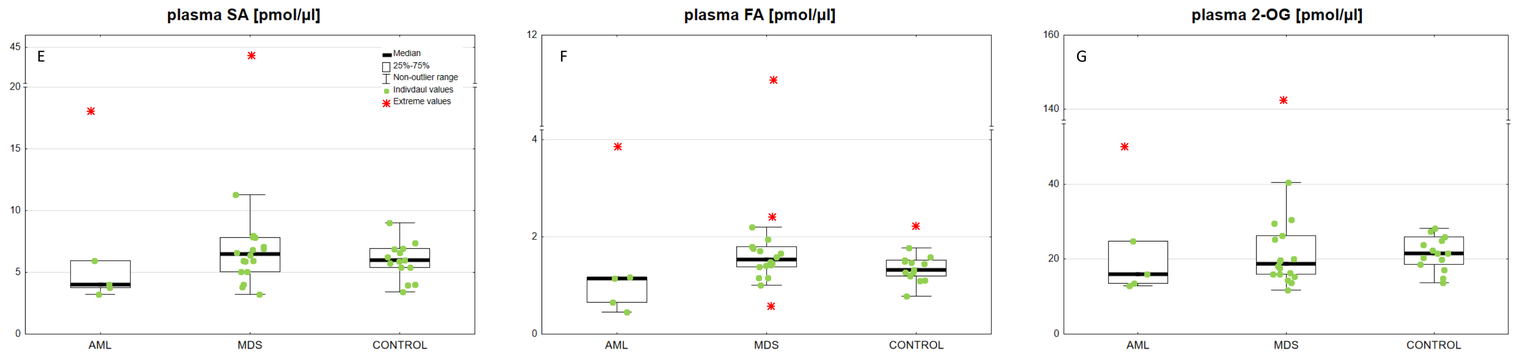


Fig. 8.The level of succinate (E), fumarate (F) and 2-oxoglutarate (G); patients with AML, MDS and healthy controls.

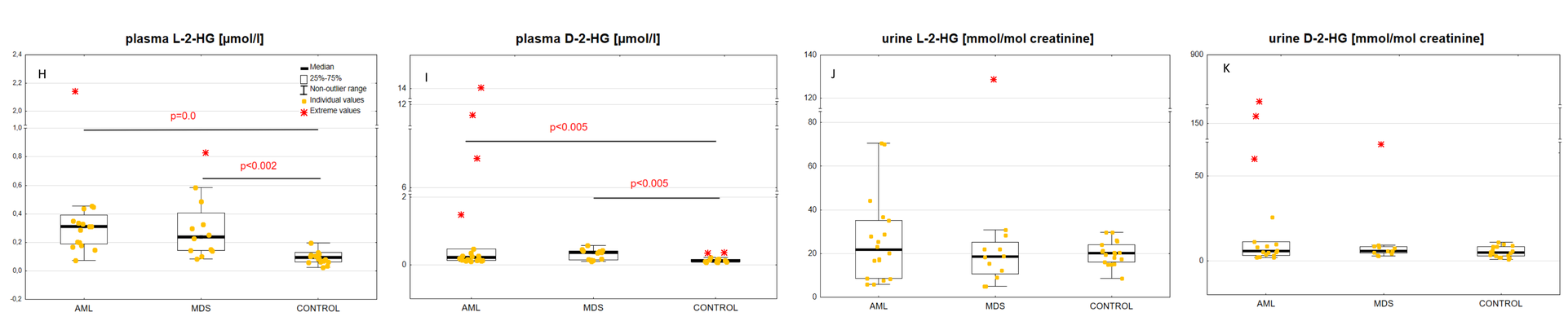


Fig. 9. The level of L- and D-HG in plasma (H)(I) and urine (J)(K); patients with AML, MDS and healthy controls. Statistically significant differences: Mann-Whitney U Test p<0.05.

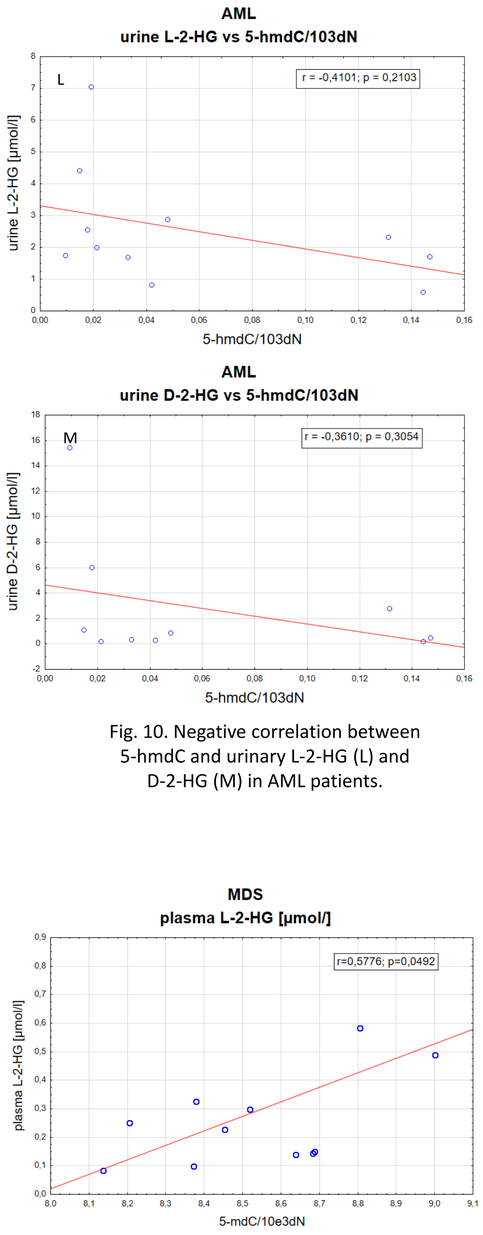


Fig. 10. Negative correlation between 5-hmdC and urinary L-2-HG (L) and D-2-HG (M) in AML patients.

Fig. 11. Positive correlation between 5-mdC and plasma L-2-HG in MS patients.

## Conclusions

• Our study has show statistically significant diferences between the level of derivatives of active DNA demethylation process in AML and MDS patients compare to healthy controls. [Fig.7] We have noticed some extreme values of the level of 5-hmdC [Fig.7.B], 5-fdC [Fig.7.C] and 5-caC [Fig.7.D]. These differences could be related to mutations of genes involved in DNA methylation/demethylation process like: DNA methylotransferases, TETs, IDH1/IDH2. It is still unclear wheather hyper-and hypomethylation of DNA is cause or effect of malignant transformations.

• The extreme values of succinate [Fig.8.E], fumarate [Fig.8.F], 2-oxoglutarate [Fig.8.G] in plasm, 2-HGs in plasma [Fig.9.H][Fig.9.I] and urine [Fig.9.J][Fig.9.K] of patients with AML and MDS could be related to mutations in succinate dehydrogenase , fumarate hydratase and isocitrate dehydrogenase genes what can affect TET proteins and may lead to malignancy.

• We have observed negative correlation between global level of 5-hmdC and 2-HGs in AML [Fig.10.L][Fig.10.M]. It can suggest that L-2-HG as well as D-2-HG are potent inhibitors of TETs activity in vivo and may play significant role in development of AML and MDS. We suggest that non-invasive quantification of L-and/or D-2-HG in plasma and/or urine may serve as a screening indicator of people with altered IDH activity.

• Our initial research have shown weak correlation between the level of the derivates of active DNA demethylation process and metabolites. Larger studies need to be performed to revealed how the concentrations of SA, FA, 2-OG and R-2HG influence key enzymes of active DNA demethylation pathway.

[1] Tahiliani M., et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009:324,930–935. [2] Harinder G., et al. Molecular and Cellular Mechanisms of Myelodysplastic Syndrome: Implications on Targeted Therapy. Int J Mol Sci. 2016 Apr; 17(4): 440. [3] Younguk S. Epigenetic Regulators in the Development, Maintenance, and Therapeutic Targeting of Acute Myeloid Leukemia. Front Oncol. 2018; 8: 41. [4] Dan Y, et al. Metabolism, Activity, and Targeting of D-and L-2-Hydroxyglutarates.Trends Cancer. 2018 Feb;4(2):151-165.