Abstract: PB1914

Title: UNPRECEDENTED ACCUMULATION OF 5-HYDROXYMETHYLURACIL IN CHRONIC LYMPHOCYTIC LEUKEMIA –POTENTIAL SOURCES AND CLINICAL IMPLICATIONS

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Background:

Methylation and demethylation of cytosine, usually within CpG dinucleotides, is one of the most important epigenetic processes leading to gene repression or activation. It was suggested that chronic lymphocytic leukemia (CLL) lymphocytes DNA methylation is relatively stable over time what indicated aberrant methylation as an early leukemogenic event. One of the mechanisms responsible for the active demethylation of 5-methylcytosine involves its oxidation by ten-eleven translocation proteins (TET) to 5-hydroxymethylcytosine and later on to 5-formylcytosine and 5-carboxylcytosine. TET proteins are also able to oxidize thymine to 5-hydroxymethyluracil (5hmU), which may be also produced alternatively in the process of deamination of 5-hydroxymethylocytosine. Thymidine DNA glycosylase and SMUG1 glycosylases are involved in the base excision repair process leading to replace modified bases with cytosine.

Aims:

We previously discovered an unprecedented accumulation of 5hmU in the DNA of CLL cells, correlating with Rai's stage and being a predictor of shortened time-to-treatment. To elucidate the potential sources of 5hmU accumulation we investigated a broad spectrum of the factors which may influence its content in the DNA: expression of the enzymes responsible for its generation and repair, as well as intracellular content of vitamin C, the most potent activator of TET enzymes.

Methods:

The blood samples were collected from 122 patients of the Department of Hematology University Hospital No2 Jan Biziel Memorial Hospital in Bydgoszcz between 2018 and 2021 year. The median age of CLL patients was 63 (range 36 – 84), and gender: female/male: 63/59. Each of the participants voluntarily signed a consent form before any procedures started. The control group consisted of 74 healthy persons (53 female, 21 male), a median age of 54 (a range 33-71). The lymphocytes were isolated using the standard density gradient method. Global content of DNA modifications in DNA and urinary excretion of 5hmU repair products were analyzed using an isotope-dilution two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry or gas chromatography with mass spectrometry after pre-purification by liquid chromatography. The expression of proteins potentially involved in 5hmU generation and repair was estimated using multicolor flow cytometry. The intracellular content of vitamin C was analyzed by ultra-performance liquid chromatography with tandem mass spectrometry.

Results:

At the time of assessment, 46 (43%) were stage 0, 22 (21%) stage 1, 16 (15%) stage 2, 12 stage 3 (11%) and 11 stage 4 (10%) according to the Rai's classification. Comparison of the CLL group with the control confirmed accumulation of the 5hmU in the DNA of CLL patients. Moreover, the content of this molecule DNA was the best biomarker of disease when assessed by ROC analysis (AUC=0.962, $p<0.0001$, sensitivity 90%, specificity 93% for cut-off value 0.871 determined using Youden's index) and correlated with Rai stage. It was found that TET2 and TDG protein expression was higher in the lymphocytes of CLL patients compared to the control group, while AID was lower. The patients' group presented also with higher urinary excretion of 5-hydroxymethyluracil, the main product of its excision from DNA via base excision repair. Leukocytes of CLL patients had also significantly higher content of vitamin C than healthy persons.

Summary/Conclusion:

Our results suggest that the accumulation of 5-hydroxymethyluracil in DNA of CLL cells is not caused by defective DNA repair or aberrant deamination. The main contributor may be an aberrant expression of TET2, additionally stimulated by an exceptionally high concentration of intracellular vitamin C.

Keywords: DNA methylation, Hydroxymethylation, Demethylation, Chronic lymphocytic leukemia