SUMMARY

INTRODUCTION

Development of acute myeloid leukemia and myelodysplastic syndromes is mediated by the somatic mutations that disrupt the processes of maturation and apoptosis of hematopoietic stem cells. Epigenetic factors are also crucial in leukemogenesis, and disruption of CpG island methylation is one of the most important ones. The repair mechanism for the impaired methylation process is TET-TDG-BER-dependent DNA demethylation, the activity of which leads to the removal of demethylated 5-methylcytosine derivatives from DNA and their elimination with urine, in which TET2 dioxygenase and TDG glycosylase play a key role.

OBJECTIVES OF THE STUDY

The aim of the study is to evaluate epigenetic processes in adult patients with AML and MDS, including analysis of levels of active demethylation products in leukocyte DNA and concentrations of these products in urine compared to the control group, analysis of mRNA expression of genes involved in epigenetic processes and their impact on the TET-TDG-BER-active DNA demethylation pathway, and assessment of the prevalence of epigenetic gene mutations. Assessment of the impact of baseline levels of demethylation products in leukocyte DNA and concentrations of these products in urine on clinical data: the risk of transformation of MDS to AML, response to induction chemotherapy and overall survival of AML patients, were also planned.

MATERIALS AND METHODS

62 patients with untreated AML and 42 patients with untreated MDS were included in the study. Values of active demethylation products in leukocyte DNA and urine were determined in all patients, and dedicated methodology based on two-dimensional ultraperformance liquid chromatography-tandem mass spectrometry (2D UPLC-MS/MS) was used for analysis. The mRNA expression of *TET1, TET2, TET3, TDG, IDH1* and *IDH2* genes was also measured. Results were compared to the control group (n=52). The mutation status of the *TET2*, *IDH1* and *IDH2* genes was also determined in all patients.

RESULTS

There was a reduction in the level of 5-hmdC in the leukocyte DNA of AML patients relative to

the control group, and of MDS patients relative to the control group. The highest levels of active demethylation products in urine were observed in AML patients, intermediate levels in MDS patients, and the lowest levels in the control group. AML patients showed lower expression of *TET2* mRNA than in the control group, and at the same time a higher expression of *TET1*, *TET3*, *TDG* and *IDH1/2* mRNA. Genetic mutations were found in AML and MDS patients: *IDH1 in* 3.23% and 2.4% of patients, respectively, *TET2 in* 6.5% and 11.9% of patients, respectively, and *IDH2* in 4.8% of AML patients. There was no correlation between the values of demethylation products and the response rate to intensive chemotherapy in patients with AML. Patients with MDS who transformed to AML had lower baseline 5-mdC levels in leukocyte DNA than patients who did not transform. AML patients with low baseline urinary 5-mdC levels had longer overall survival than patients with high 5-mdC levels.

CONCLUSIONS

Hypermethylation expressed by 5-hmdC depletion has been shown to be a hallmark of AML and MDS. Evidence was provided that one of its causes may be a decrease in mRNA expression of the *TET2* gene. By analyzing concentrations of demethylation products entering urine, differences in the severity of epigenome disruption in the study groups were demonstrated: the highest among AML patients, intermediate among MDS patients, and the lowest among healthy subjects. By demonstrating increased *TDG* mRNA expression in the AML patient group, a significant enhancement of methylation disorder repair processes by TET-TDG-BER-dependent active DNA demethylation was confirmed. In addition, evidence was provided to support the hypothesis assuming a compensatory function of *TET1* and *TET3* genes in response to *TET2* depletion, and the frequency of epigenetic mutations in the AML and MDS patient population was measured. The baseline value of active demethylation products in DNA and urine had no effect on the success of induction chemotherapy in AML patients. The baseline level of 5-mdC in leukocyte DNA in MDS patients may have prognostic value in assessing the risk of transformation to AML, and urinary levels of 5-mdC may affect the length of overall survival of AML patients, but these conclusions should be confirmed on a larger group of patients in a multivariate analysis.