ABSTRACT

Acute myeloid leukemia (AML) is a hematopoietic malignancy characterized by great heterogeneity and clonal nature. In recent years, rapidly evolving next-generation sequencing methods provided a deep insight into the mutational background of AML. It was shown that ~ 44 % of AML patients harbor mutations in genes that regulate DNA methylation. So far, many researchers have tried to evaluate the prognostic significance of DNA methylation changes in AML, however, due to a great inconsistency in these studies, none of the reported markers were implemented into clinical practice.

The aim of this work was to further investigate the DNA methylation changes in AML patients with specific mutations and their prognostic effect. Next, we wanted to develop a new approach for a complex evaluation of prognostically significant DNA methylation aberrations.

In our first project, we assessed the overall DNA methylation, hydroxymethylation, and gene expression in AML patients with mutations in either *DNMT3A* or *IDH1/2* or their combinations. We discovered that each genetic aberration is connected with a distinct pattern of DNA hydroxy-/methylation changes that are not entirely reflected in altered gene expression. Patients with mutations in both genes exhibited a mixed DNA methylation profile most similar to healthy controls. Furthermore, we found a prognostically significant hypermethylation in an upstream enhancer of *GZMB* gene ($p = 0.035$). Prior to validation of the DNA hydroxy-/methylation levels measured with arrays in the first project, we compared four most common methods for DNA methylation validation: analysis with methylation specific restriction enzymes, pyrosequencing, methylation-specific high-resolution melting, and methylation-specific PCR. Pyrosequencing proved to be the most convenient method due to its single base resolution and easy implementation. Next, we focused on a comprehensive evaluation of prognostically significant DNA methylation changes using a custom sequencing panel. To assess a summarizing influence of various aberrations in DNA methylation on patients' prognosis we developed MethScore, a simply computed value that reliably stratified the patients with better and worse survival ($p < 0.001$). MethScore significance was verified in multivariate analyses and validated on an independent cohort of AML patients. We further showed that MethScore may be primarily helpful for stratifying the patients with intermediate risk.

Our research contributed to the knowledge of AML epigenetic background and the prognostic significance of DNA methylation. MethScore may serve as a new surrogate marker that can specify the prognosis of AML patients within the intermediate risk group.