ABSTRACT

DNA methylation is a well-established epigenetic mechanism regulating gene expression. It has essential functions in cells under physiological as well as pathological conditions. In acute myeloid leukemia (AML), aberrant DNA methylation has been confirmed in the pathogenesis and progression of the disease. Changes in DNA methylation of promoters, or other regions, are studied primarily with respect to pathways that are involved in tumor transformation and DNA methylation impact on prognosis. Clinical importance of DNA methylation has been confirmed by a number of recent publications. According to the Cancer Genome Atlas (TCGA) Research Network, mutations of genes involved in DNA methylation are found in 44% of AML patients at diagnosis. However, the impact of these mutations on specific DNA methylation and gene expression remains controversial.

We examined 79 AML patients at diagnosis for DNA methylation of 12 selected genes (*CDKN2B*, *CALCA*, *CDH1*, *ESR1*, *SOCS1*, *MYOD1*, *DAPK1*, *TIMP3*, *ICAM1*, *TERT*, *CTNNA1*, *EGR1*) – some of them proved as tumor suppressor genes and 24 *HOX* genes, and in parallel for mutations in *DNMT3A*. We observed lower levels of DNA methylation ($P<0.0001$) as well as lower numbers of concurrently hypermethylated genes ($P<0.0001$) in patients with *DNMT3A* mutations. Our study of the impact of DNA methylation on prognosis revealed a relation between higher DNA methylation and better patients' outcome. Lower levels of methylation were connected with higher relapse rates and inferior overall survival.

By the use of targeted bisulfite sequencing and microarray expression profiling, we analyzed 14 AML patients at diagnosis and CD34+ pool of healthy donors. Hierarchical clustering analysis of DNA methylation and expression data revealed a novel cluster specific to *CBFB-MYH11* fusion gene resulting from inversion of chromosome 16 - inv(16) or translocation of chromosome 16 - t(16;16). Regions unique for this cluster were preferentially hypomethylated and enriched for genes previously described as overexpressed in *CBFB-MYH11* AML. Further, by comparing all targeted methylation and microarray expression data, *PBX3* differential methylation was found to correlate with its gene expression. PBX3 has recently been shown to be a key interaction partner of HOXA9 during leukemogenesis and we revealed higher incidence of relapses in *PBX3*-overexpressing patients.

Altogether, we showed a clear connection between hypomethylation of selected genes and *DNMT3A* mutations. We also discovered new genomic regions with aberrant DNA methylation associated with expression of genes involved in leukemogenesis.