Check for updates

ELSEVIER

Blood 142 (2023) 1378–1379

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Co-Analysis of Genome-Wide DNA Methylation and Gene Expression Suggests Novel Regulatory Mechanism for RUNX1 in Inv(16) Acute Myeloid Leukemia

Ana Catarina Menezes, PhD[1], *Tao Zhen, PhD*[1], *Derek E. Gildea, PhD*[2], *Tyra G. Wolfsberg, PhD*[2], *Nisc Comparative Sequencing Program*[3], *Paul Pu Liu, MD PhD*[1]

[1] Oncogenesis and Development Section, National Human Genome Research Institute, NIH, Bethesda, MD
[2] Bioinformatics and Scientific Programming Core, National Human Genome Research Institute, NIH, Bethesda, MD
[3] NIH Intramural Sequencing Center (NISC), National Human Genome Research Institute, NIH, Bethesda, MD

Acute myeloid leukemia (AML) is characterized by recurring chromosomal abnormalities that encode oncogenic fusion proteins. Approximately 10% of AML cases are associated with a chromosome 16 inversion [inv(16)(p13q22)] or translocation t(16;16)(p13q22). These chromosomal rearrangements results in the formation of the fusion oncogene *CBFB-MYH11* and ultimately the fusion protein CBFβ-SMMHC. Although CBFβ-SMMHC was initially considered a dominant negative repressor of RUNX1, we have recently shown that it works together with RUNX1 to activate gene expression through direct target gene binding. RUNX1 is known to regulate gene expression at both transcriptional and epigenetic levels. Aberrant DNA methylation patterns have been reported across different cancer types including AML. Recent studies have shown that DNA methylation negatively influences RUNX1 DNA binding. We hypothesize that changes to DNA methylation and consequently dysregulated RUNX1 DNA binding contribute to the pathogenesis of inv(16) AML. In this study, we examined the epigenetic and transcriptomic landscapes of *CBFB-MYH11* knockin mice and assessed the contribution of the resulting fusion protein CBFβ-SMMHC to RUNX1 DNA target binding.

To assess the effects of DNA methylation on RUNX1 DNA binding *in vitro*, we initially performed fluorescence polarization and electrophoretic mobility shift assays using unmethylated and methylated RUNX1 DNA target sequences. RUNX1, RUNX1/CBFβ and RUNX1/CBFβ-SMMHC showed significantly lower binding affinity for methylated DNA compared to unmethylated DNA probes. To determine genome-wide DNA methylation changes, we performed enzymatic methyl sequencing on sorted Lin $^{-}$Sca1 $^{-}$c-Kit $^{+}$ bone marrow cells from control and pre-leukemic inv(16) mice. Inv(16) mice showed a significant genome-wide DNA hypermethylation compared to controls (13,478 hyper- vs 705 hypomethylated regions; $q\text{-value} < 0.05$), with 14% of the differentially methylated regions (DMRs) lying in promoters. Genes associated with these DMRs were enriched for gene ontology (GO) processes related to hematopoiesis, such as myeloid cell homeostasis and erythrocyte differentiation, and DNA replication, such as mitotic DNA replication initiation, among others. In addition, several RUNX1 target genes showed promoter hypermethylation, such as *Klf1* or the thrombopoietin receptor *Mpl*. To understand the impact of aberrant DNA methylation on gene expression, we performed RNA-seq using the same cell population. Gene expression changes were observed in inv(16) mice compared to controls, with 741 genes being upregulated and 278 downregulated from a total of 1019 differentially expressed genes (DEG; absolute fold-change ≥ 2, $q\text{-value} < 0.05$). Enriched GO processes identified from the DEGs were associated with immune responses, such as response to interferon-alpha (and beta) and leukocyte activation involved in inflammatory response. We then intersected the DMR (focusing on promoters) and DEG lists to identify genes whose expression might be directly influenced by DNA methylation changes in inv(16) leukemia. Hypermethylation of promoters was associated with 102 genes, with 67% of them showing downregulation of gene expression in inv(16) mice, whereas hypomethylation was associated with 18 genes with 94% showing upregulation of gene expression. These results show that in inv(16) mice, promoter hypermethylation is associated with downregulation of gene expression, and hypomethylation with increased expression. We also confirmed that several RUNX1 target genes showing promoter hypermethylation also had reduced gene expression in inv(16) mice.

The initial analysis suggests that CBFβ-SMMHC expression significantly affects the methylation landscape in mice, with important RUNX1 target genes exhibiting promoter hypermethylation and subsequent downregulation of gene expression. We are currently performing chromatin immunocleavage sequencing using the same cell population to determine RUNX1 binding *in vivo*. Overall, this study explores a novel regulatory mechanism of RUNX1 function through DNA methylation, and ultimately a new role for RUNX1 in the pathogenesis of inv(16) AML.

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/1378/2195536/blood-7981-main.pdf by guest on 08 June 2024

© 2023 by The American Society of Hematology

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-187840

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/1378/2195536/blood-7981-main.pdf by guest on 08 June 2024