ELSEVIER

Blood 142 (2023) 5690

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

602.MYELOID ONCOGENESIS: BASIC

Myeloid Master Regulators PU.1 and CEBPA Suppress Leukemogenesis through Downregulating Cell Cycle Related Genes in NPM1 Mutated Leukemia

Karina Hamamoto, PhD[1], *Julia Lesperance*[1], *Huacheng Luo, PhD*[1], *Zachary Zaroogian*[2], *Olga A. Guryanova, MDPhD*[2], *Yi Qiu, PhD*[3], *Suming Huang, PhD*[1]

[1] Division of Pediatric Hematology/Oncology, Pennsylvania State University College of Medicine, Hershey, PA
[2] Department of Pharmacology and therapeutics, University of Florida College of Medicine, Gainesville, FL
[3] Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, PA

Nucleophosmin1 mutation (NPM1c) is the most common mutation in adult acute myeloid leukemia (AML) and known as a driver mutation causing leukemogenesis through upregulating homeotic gene transcription. Mutated NPM1 is reported to mis-localize in the cytoplasm. However, how cytoplasmic NPM1c regulates leukemic transcription remains poorly understood. We previously reported that NPM1c reprograms homeotic and leukemogenic transcription through reshaping CTCF-defined three-dimensional topology associated domain (TAD). Also, re-localization of cytoplasmic NPM1c to the nucleus switches MIZ1/MYC repressive transcription regulatory axis to MIZ1/NPM1 active transcription regulatory axis at CDKN locus and *CEBPA* locus leading to cell cycle arrest and myeloid differentiation in NPM1 mutated AML. In this study, we are focused on regulation of myeloid differentiation and hypothesized upregulation of myeloid master regulators, PU.1 and CEBPA may suppress the leukemogenic transcription and induce myeloid differentiation in NPM1 mutated AML cells.
To confirm NPM1c regulates TAD formation at *CEBPA* locus, we performed HiC-seq in OCI-AML3 with nuclear re-localized NPM1c using XPO1 inhibitor treatment and NPM1-wildtype overexpression. Nuclear re-localized NPM1 upregulates CTCF-defined TAD interaction at *CEBPA* locus and upregulates *CEBPA* and *CEBPD* transcription which indicates NPM1c regulates *CEBPA* transcription via altering CEBPA TAD formation. We then test whether activation of *CEBPA* or *SPI1* in NPM1 $^{C+}$ AML cells induced myeloid differentiation and blocked NPM1 $^{C+}$ driven leukemogenesis. We transcriptionally activated *SPI1* or *CEBPA* gene in OCI-AML3 using CRISPR-dCas9-VP160 system to determine how these myeloid regulators changed the leukemic transcription and leukemic cell phenotype. PU.1- or CEBPA- activated OCI-AML3 reduces cell proliferation, especially affecting the G2/M phase in cell cycle. They have monocyte-like nuclei with large cytoplasm and induce myeloid differentiation with upregulating both of CD11b and CD14 myeloid cell surface markers. Moreover, compared to parental OCI-AML3 cells, Pu.1- or CEBPA-activated cells showed lower colony formation ability and in xenograft mouse models produced diminished leukemic symptoms and prolonged survival. We further conducted RNA-seq to explore how PU.1 or CEBPA changed the gene transcription leading to the blockage of leukemogenesis. Between PU.1- and CEBPA-activated cells, 58% of differentially expressed genes overlap and GO or GSEA analysis revealed that both of them downregulates G2M checkpoint/mitotic-spindle cell cycle related gene sets, indicating that PU.1 or CEBPA activation controls cell cycle control rather than myeloid cell differentiation in NPM1 $^{C+}$ AML. In summary, our data revealed that re-localization of NPM1 $^{C+}$ into nucleus activates master myeloid transcription factors, PU.1 and CEBPA, which, in turn, suppress leukemogenesis via inducing cell cycle arrest and myeloid cell differentiation in NPM1 $^{C+}$ AML.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-189988