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MYELOID NEOPLASIA

Comment on Poplineau et al, page 2358

APL: Nemo finds its sea anemone

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In this issue of *Blood*, **Poplineau et al¹** identified a retinoic acid (RA) resistance network involving the E2F-EZH2 axis by utilizing a PLZF-RARA transgenic acute promyelocytic leukemia (APL) mouse model probed with multiomics and elegant functional assays. They also showed that nonenzymatic activity of EZH2 is critically involved in RA resistance. Targeting pan-EZH2 activity significantly improved survival in mice reconstituted by RA-resistant PLZF-RARA leukemia cells.

During the last 3 decades, the field witnessed miraculous improvements in the treatment of classical PML-RARA APL, through the usage of RA and arsenic trioxide (ATO), targeting the RARA and PML moiety of the PML-RARA fusion protein, respectively.² Mouse models of APL have provided unexpected insights into the mechanism of action for these 2 drugs. RA binding activates PML-RARA target gene transcription and initiates degradation of the fusion protein, and arsenic binding to PML-RARA triggers its conjugation by SUMO, ultimately resulting in full degradation of PML-RARA.³ Now, more than 90% of APL patients can be cured by the RA + ATO combination in a chemo-free treatment model.⁴

However, this miracle has not been replicated in the treatment of variant APL, which accounts for ~5% of all APL cases and is characterized by chromosomal translocations involving RARA and 18 different non-PML partner genes. As ATO

does not work on non-PML partner proteins,³ new treatment strategies are needed to overcome primary drug resistance in variant APL. PLZF-RARA, the most common APL variant, was first described in 1993 by Chen and colleagues.⁵ Although RA treatment in a PLZF-RARA mouse model triggers cellular differentiation, it does not clear leukemia-initiating cells or induce disease remission.⁶ Mechanistically, PLZF-RARA, but not PML-RARA, forms a stable complex with transcriptional corepressor SMRT. Transcriptional silencing plays a major role in RA insensitivity.⁷ However, it remains unknown whether cellular heterogeneity exists in PLZF-RARA leukemia cells and how to target RA resistance in a treatment-related setting. The current study by Poplineau et al provides novel insights into these important questions by pinpointing a unique subset of PLZF-RARA-driven leukemia cells. These cells are RA resistant and dependent on a finetuned E2F-EZH2 network. Targeting panEZH2 activity, in combination with RA administration, leads to PLZF-RARA degradation and prolonged survival of APL mice given RA-resistant PLZF-RARA leukemia cells.

Drug resistance is often mediated by a small subset of cells with potent tumor-initiating capacity within a mixture of functionally heterogeneous tumor cells. It is a challenge to pinpoint the critical molecular circuit(s) in the drugresistant cells that can drive disease progression. Using single-cell transcriptome sequencing, Poplineau et al first mapped out multiple leukemia cell subpopulations that were present before and after RA treatment in a PLZF-RARA APL mouse model. Development trajectory analysis and drug sensitivity assessment further identified an RA-resistant progenitor cell cluster (ReP), which maintained PLZF-RARA expression. To characterize molecular features associated with RA resistance, the authors combined singlecell transcriptome and chromatin accessibility profiling to dissect regulon activity. Activation of the E2F-EZH2 network was found to be most prominent in the ReP cells and was maintained after RA treatment

The functions of EZH2, as an enzymatic catalytic subunit of Polycomb repressive complex 2 (PRC2), include posttranslational methylation of histone and nonhistone protein substrates. Recent studies also reveal a noncatalytic function of EZH2 in solid tumors via transcriptional activation rather than H3K27me3-related repression.⁸ Interestingly, this current study discovered a functional and physical interdependency between EZH2 and PLZF-RARA proteins (see figure). The PLZF-RARA fusion stabilized the interaction of 2 PRC2 components, EZH2 and SUZ12, and depletion of EZH2 resulted in degradation of this fusion protein. Functionally, EZH2 was essential for initiation and maintenance of PLZF-RARA oncogenic activity ex vivo and in vivo. Targeting the pan activity of EZH2 with a commercial EZH2 degrader along with RA repressed the resistance-related biological processes such as cell proliferation and DNA replication and significantly prevented disease progression in mice transplanted with RA-treated PLZF-RARA leukemia cells. In contrast, inhibition of EZH2 methyltransferase activity did not reverse RA resistance or leukemia progression



Physical and functional interdependency between EZH2 and PLZF-RARA. (1) PLZF-RARA fusion stabilized the interaction of 2 PRC2 components, EZH2 and SUZ12, and the presence of EZH2 protected the fusion protein from degradation. (2) Targeting pan-EZH2 activity with its degrader, in combination with RA administration, induced restoration of RA sensitivity, cell differentiation and death, and transcriptional repression in cell cycle and DNA replication and prolonged survival of APL mice. (3) EZH2, as an enzymatic catalytic subunit of PRC2, posttranslationally trimethylates H3K27. However, inhibition of EZH2 enzymatic activity with small molecules did not reverse RA resistance. Replating capacity was sustained and cells were able to expand, which further resulted in leukemia progression. Professional illustration by Patrick Lane, ScEYEnce Studios.

in combination with RA. These data reveal an unexpected interdependent relationship between EZH2 and PLZF-RARA and highlight the multifaceted actions of EZH2 in leukemia development and drug resistance.

One unresolved issue in this study is how PLZF-RARA and EZH2 can protect the protein integrity of each other. The authors clearly showed that EZH2 does not directly bind to the fusion protein as it does for wild-type PLZF. Furthermore, genomic localization of PLZF-RARA and EZH2 was not shown in this study. It is not clear whether they co-occupy promoter and enhancer regions and impact on the observed histone modification changes. Intriguingly, recent studies demonstrated a critical requirement of EZH2 in stabilizing the MYC protein via direct protein-protein binding in cancer.⁹ Like the mutualistic relationship between clownfish (like Nemo) and sea anemone, there is a potential interdependency between EZH2 and key oncoproteins. Exploration of their "cohabitation" and interplay will likely open a branch of research for a new role of EZH2 in human cancer.

One important finding by the de Thé group demonstrated that PML-RARA could not be fully degraded in all cells unless arsenic was added.³ However, arsenic has little effect on PLZF-RARA.¹⁰ Therefore, depletion of EZH2 using proteolysis targeting chimeras (PROTACs) is a promising direction in achieving remission for this type of variant APL and warrants future investigation in the clinic. Finally, although this current study underscores the importance of epigenetic regulation of EZH2 in APL, it remains to be explored whether this model is unique to PLZF-RARA or shared by some or all of the remaining 17 APL variants and whether a similar mechanism involving EZH2 operates in relapsed classical APL patients.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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