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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Mechanisms of PML::Rara and GATA2 Protein Interactions in Hematopoietic Cells

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Despite excellent outcomes for Acute Promyelocytic Leukemia (APL) patients with combined ATRA and Arsenic Trioxide therapy, the underlying molecular mechanisms by which the PML::RARA fusion oncoprotein initiates APL are not yet clear. We recently found that the ability of PML::RARA to initiate aberrant serial replating (a surrogate for self-renewal) is dependent upon its ability to bind to DNA, since PML::RARA with a missense mutation (C88A) that prevents the binding of RARA to DNA eliminates aberrant replating. Currently, however, there is no consensus regarding the genomic binding sites of PML::RARA in myeloid progenitor cells and APL cells. Also, the transcriptional and epigenetic consequences of PML::RARA expression in primary hematopoietic cells remain unclear.

To define the genomic binding sites of PML::RARA, we generated V5 epitope tagged PML::RARA constructs in MSCV-based retroviral vectors, and transduced mouse hematopoietic stem progenitor cells (HSPCs) with retroviruses containing V5-PML::RARA^{WT}, V5-PML::RARA^{C88A}, or an empty vector. Anti-V5 ChIP-seq revealed that PML::RARA binds to 2,677 distinct loci in primary mouse hematopoietic cells, and 2,064 sites in primary human hematopoietic cells. We previously demonstrated that Gata2 expression is induced with PML::RARA, and indeed, we identified strong PML::RARA binding to the distal Gata2 enhancer in both human and mouse HSPCs. Surprisingly, ATAC-seq revealed that the vast majority of PML::RARA binding sites are found in regions that are chromatin-accessible even in WT promyelocytes, suggesting that these regions are "open" prior to PML::RARA expression, perhaps because of the normal binding of "pioneer" transcription factors to these regions in WT cells. Indeed, evaluation of transcription factor binding motifs in the regions occupied by PML::RARA revealed significant enrichment for RARA binding motifs, and also GATA motifs. We therefore performed ChIP-seq on GATA2 in HSPCs, which confirmed enrichment in binding at the same sites. These data suggested that PML::RARA and GATA2 may bind in close proximity at hundreds of loci in the genome. To test this, we performed proximity labeling studies followed by mass spectrometry using PML::RARA^{WT} vs. PML::RARA^{C88A} fused to the biotin ligase TurboID in primary HSPCs. This approach revealed that the interactions of several proteins with PML::RARA (including GATA2) are dependent on the binding of PML::RARA to DNA; this suggests that DNA may in fact act as a scaffold to stabilize these protein:protein interactions (**Figure 1**).

To determine whether Gata2 may induce increased chromatin accessibility following PML::RARA expression, we performed ATAC-seq following Gata2 or PML::RARA overexpression in WT hematopoietic progenitor cells. 3,157 regions shared increased accessibility with either PML::RARA or Gata2 overexpression. Moreover, at a transcriptional level, we found that 535 (27.4%) of PML::RARA differentially expressed genes were coordinately regulated by Gata2 in WT cells. This suggests that PML::RARA indirectly regulates many of its gene targets via the upregulation of Gata2, and that Gata2 may be required for PML::RARA to initiate its transcriptional program. To test this, we performed serial replating assays using cells where Gata2 was knocked out either before or after PML::RARA expression. Consistent with our previous study, knock out of Gata2 following PML::RARA expression led to increased self-renewal capacity. However, knock out of Gata2 before PML::RARA expression prevented self-renewal. These data suggest a model in which GATA2 "opens" specific chromatin regions in early myeloid cells, which are then accessible to RARA or PML::RARA. The two proteins interact at these sites, tethered by their mutual interactions to their respective DNA binding motifs. PML::RARA binding promotes a pre-leukemic transcriptional program mediated in part by positively regulating Gata2. Gata2 is therefore initially required for PML::RARA to establish its transcrip-

tional program. However, once that program is established, *Gata2* paradoxically acts as a tumor suppressor, performing a normal role to limit proliferative excess in hematopoietic stem and progenitor cells.

Disclosures No relevant conflicts of interest to declare.

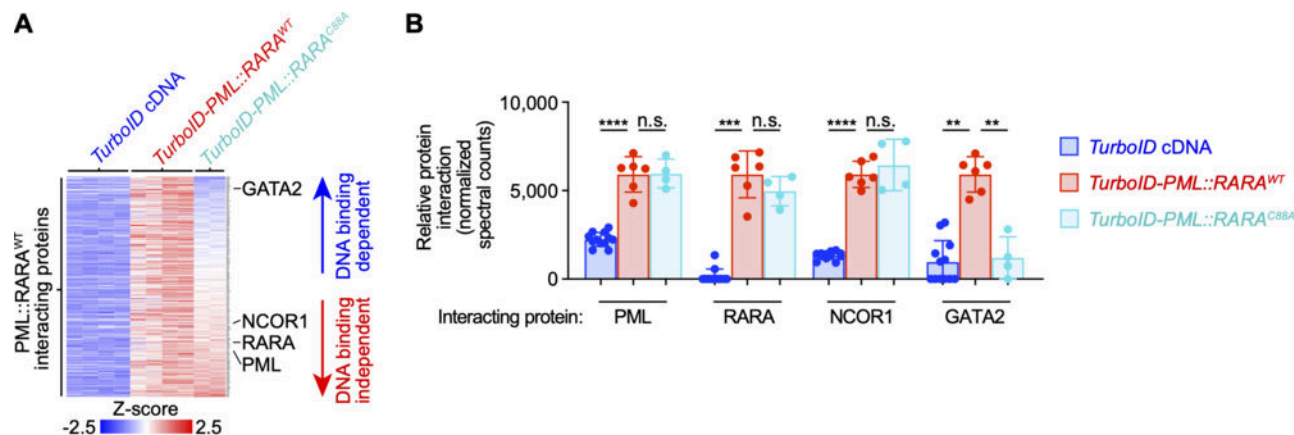


Figure 1. PML::RARA interacts with GATA2 protein in a DNA binding dependent mechanism, and PML::RARA requires *Gata2* to induce aberrant self-renewal. Proximity labeling assays in which lineage-depleted mouse bone marrow cells were transduced with MSCV-IRES-GFP based retroviruses containing a cDNA for a highly active biotin ligase called 'TurboID' (TurboID cDNA, which serves as a background control), wild-type PML::RARA fused to TurboID (TurboID-PML::RARA^{WT}), or a DNA binding-deficient mutant PML::RARA (C88A in the RARA DNA binding domain) fused to TurboID (TurboID-PML::RARA^{C88A}). GFP+ cells were flow purified and labeled with biotin for four hours. Biotin labeled proteins were enriched with streptavidin bead pulldowns and identified using mass spectrometry. **Panel A:** Heatmap of the proteins that were identified as interacting with TurboID-PML::RARA^{WT} (relative to TurboID cDNA alone; FDR ≤ 0.05 and fold-change ≥ 2 using edgeR package). Proteins are plotted in order of relative interaction with TurboID-PML::RARA^{C88A}, and the TurboID cDNA alone and TurboID-PML::RARA^{WT} samples are passively plotted. Each column is an independent biological replicate, and data are representative of 12, 12, and 4 independent biological replicates of TurboID cDNA alone, TurboID-PML::RARA^{WT}, and TurboID-PML::RARA^{C88A} respectively. **Panel B:** Relative protein interactions of each TurboID construct with PML, RARA, NCOR1, or GATA2 are shown in normalized spectral counts (Y-axis). Note that the interaction of GATA2 with PML::RARA requires the DNA binding activity of PML::RARA, suggesting that this interaction requires binding to a DNA scaffold. Each data point is an independent biological replicate. ****FDR<0.0001, ***FDR<0.001, **FDR<0.01 using edgeR package. Error bars represent standard deviation.

Figure 1

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