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The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

203.LYMPHOCYTES AND ACQUIRED OR CONGENITAL IMMUNODEFICIENCY DISORDERS

PRDM1 Promotes Primary Human Circulating CD56 [dim] NK-Cell Differentiation

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Background: Natural killer (NK) cells are innate lymphocytes that mount immune responses against viral infection and malignant cells. Circulating NK-cells can be generally divided into two groups, CD56 [bright] and CD56 [dim], with the latter constituting ~90% of NK-cells in peripheral blood. While CD56 [bright] NK-cells are considered less mature and capable of producing more cytokines, CD56 [dim] population represents more differentiated cells with greater cytotoxicity. Transcription factor (TF) regulatory circuits were proposed for CD56 [bright] and CD56 [dim] NK-cells, indicating that TCF1/LEF1 and BACH2 are important in the former population and that Blimp1 and MAF are master TFs in the latter group. Blimp1 (encoded by *PRDM1*) has been previously found to regulate terminal differentiation of B-cells and CD8 [+] T-cells. In NK-cells, deletion of *PRDM1* has been shown to promote cell growth and survival in vitro, and its loss of function was commonly detected in NK-cell lymphomas. Here, we employed a multi-omics approach to investigate the role of *PRDM1* in the differentiation of human primary NK-cells.

Methods: We used CRISPR-Cas9 system to knock out *PRDM1* in primary human NK-cells isolated from PBMCs of healthy donors and performed whole transcriptome sequencing. ChIP-seq was employed to investigate the global binding spectrum of PRDM1 in primary human NK-cells cultured with K562-mbIL21-Cl9 feeder cells or with IL-2 alone. We also performed integrative genomics analyses to examine the transcriptomic regulation of NK-cell differentiation by PRDM1.

Results: Gene set enrichment analyses (GSEA) demonstrated that *PRDM1* KO resulted in enrichment of genes highly expressed in CD56 [bright] cells and depletion of genes upregulated in CD56 [dim] NK-cells. Specifically, we found that *PRDM1* deficient NK-cells expressed CD56 [bright] NK-cell master TFs at higher levels, such as *TCF7*, *RUNX2*, and *MYC*. In contrast, TFs that play an important role in more differentiated CD56 [dim] NK cells were downregulated in PRDM1-KO cells, including *IKZF3*, *MAF*, and *TBX21*. Notably, *BCL11B*, a TF that has been reported to promote canonical and adaptive NK-cell differentiation, was significantly downregulated along with its target gene *ZBTB16* when *PRDM1* was deleted. Moreover, genes encoding cytotoxic molecules such as *PRF1* and *GZMB* were also repressed in *PRDM1*-KO NK-cells. Together, these results indicate that *PRDM1*-KO NK-cells closely resembled the less mature CD56 [bright] NK-cells than the CD56 [dim] terminally differentiated counterpart.

ChIP-seq analysis of PRDM1 revealed that many of the differentially expressed genes (DEGs) between *PRDM1* KO and WT cells were directly bound by PRDM1. When integrated with publicly available ATAC-seq datasets, we found that some of these PRDM1 bound sites showed differential chromatin accessibility between CD56 [bright] and CD56 [dim] NK-cells. To explore the transcriptional program regulated by PRDM1, we utilized a regulated system to re-express *PRDM1* in KHYG1, an NK-cell lymphoma cell line that is sensitive to *PRDM1* overexpression. RNA-seq analysis showed that the DEGs between KHYG1 with short-term re-expression of PRDM1 and control cells overlapped with DEGs between *PRDM1* KO vs WT NK-cells. These genes included *BCL11B*, *MAF*, *PRF1*, and *GZMB*. Therefore, PRDM1 directly regulates genes that are crucial in NK-cell differentiation. Furthermore, we detected upregulation of memory T-cell or progenitor exhausted T-cell signature genes in *PRDM1* KO NK-cells compared with WT, including *TCF7*, *SELL*, *CCR7*, and *IL7R*. Consistent with this, GSEA analysis demonstrated enrichment of TCF1 [+] progenitor exhausted T cell or memory T cell upregulated genes in *PRDM1* deleted NK-cells, suggesting that *PRDM1* KO NK-cells may share some features with memory T-cells or progenitor exhausted T cells. Importantly, *MYB*, which was found to be essential in progenitor exhausted T cells, was also a direct target of PRDM1 and was upregulated in *PRDM1*

KO NK-cells. GSEA also showed that *PRDM1* depletion in NK-cells led to upregulation of genes highly expressed in mast cell, Th1 cell, B cell, or CD8 [+] T cell compared to NK cells, which may indicate a role of *PRDM1* in maintaining lineage commitment. **Conclusions**: Our findings collectively show that PRDM1 may be a master TF that regulates human NK-cell differentiation and functions through direct transcriptional regulation of key target genes.

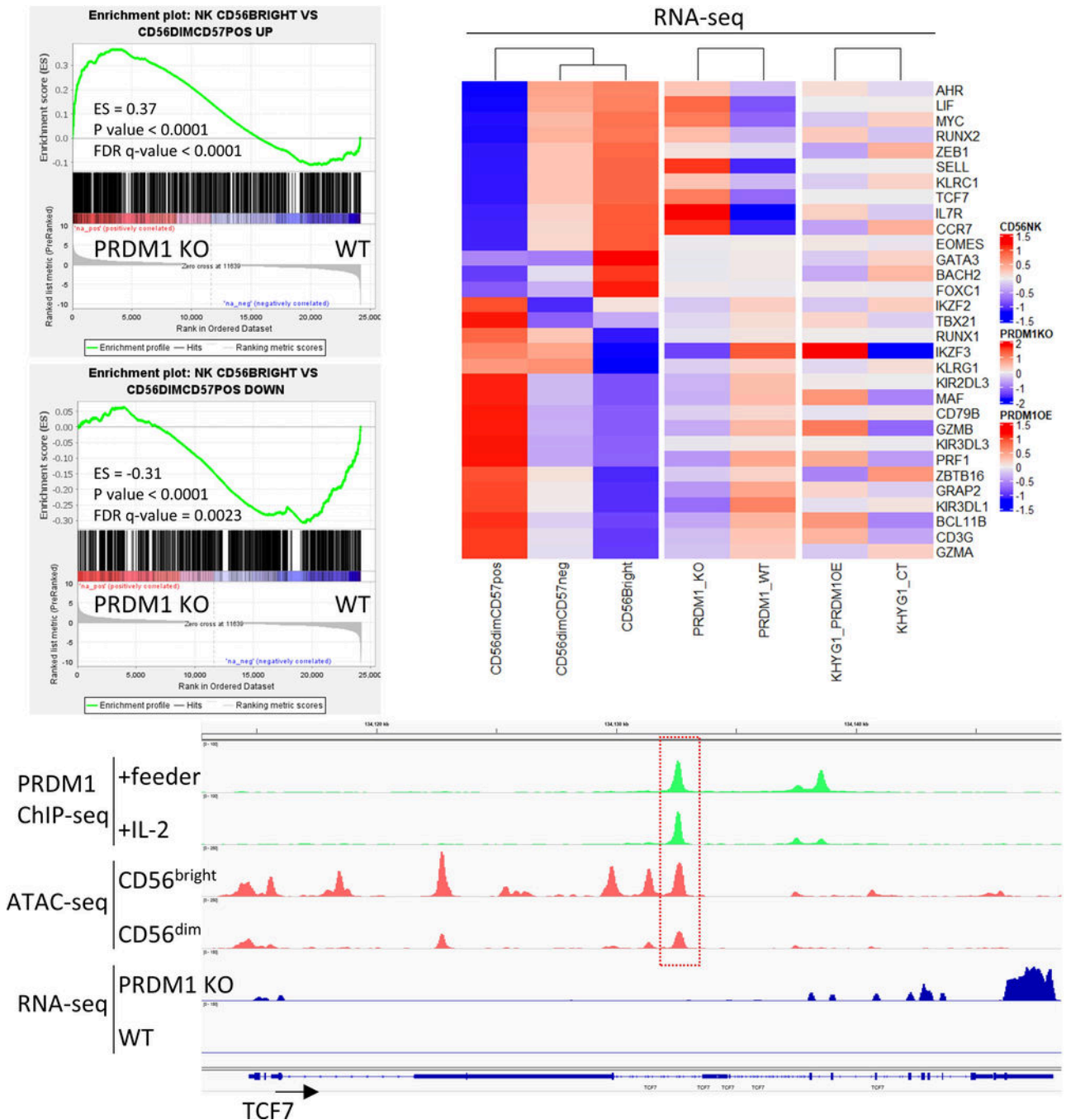**Disclosures** No relevant conflicts of interest to declare.

Figure 1