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POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Polycom Group Protein Mel18 Inhibits Hematopoietic Stem Cell Self-Renewal through Repressing the Expression of Genes Important for Cellular Senescence and Proliferation

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Polycomb group (PcG) proteins are epigenetic gene silencers that have been implicated in stem cell maintenance and cancer development. Genetic and biochemical studies indicate that Polycomb group proteins exist in at least two protein complexes, Polycomb repressive complex 2 (PRC2) and Polycomb repressive complex 1 (PRC1), that act in concert to initiate and maintain stable gene repression. Bmi1 and Mel18 are two major homologs of the PCGF subunit within the canonical PRC1 complex. While Bmi1 is a positive regulator of hematopoietic stem cell (HSC) and leukemia stem cell (LSC) self-renewal, the role of Mel18 in normal and malignant hematopoiesis is not fully understood.

To further determine the role of Mel18 in hematopoiesis, we have generated *Mel18* conditional knockout mice (*Mel18* $^{f/f}$-*Mx1Cre* $^{+}$). Acute deletion of *Mel18* in the hematopoietic compartment did not affect the frequency and survival of hematopoietic stem and progenitor cells (HSPCs). To determine the impact of Mel18 deficiency on HSPC proliferation, we performed serial replating assays and found that loss of Mel18 increases the replating potential of HSPCs *in vitro*. To determine the role of Mel18 in HSC self-renewal, we first performed serial bone marrow (BM) transplantation assays and found that Mel18 null BM cells show increased engraftment in both primary and secondary transplantation assays. Limiting dilution transplantation showed that there were more functional HSCs in the BM of Mel18 knockout mice. We then performed HSC transplantation assays and found that loss Mel18 enhances HSC self-renewal. Thus, we demonstrated that Mel18 inhibits HSC self-renewal and decreases the number of functional HSCs *in vivo*.

To understand the mechanism by which Mel18 inhibits HSC self-renewal and proliferation, we performed RNA-seq using *Mel18* $^{+/+}$ and *Mel18* $^{-/-}$ HSCs. We then performed gene set enrichment analysis (GSEA) to identify potential Mel18 target genes important for HSC behavior. We found that HSC gene signatures are enriched in *Mel18* $^{-/-}$ HSCs. We also found that leukemia stem cell gene signatures and CBF-mutant-AML genes are significantly enriched in *Mel18* $^{-/-}$ HSCs, suggesting that loss of Mel18 may prime normal HSCs for leukemic transformation. Notably, our RNA-seq assay showed that senescence-related genes, including *S100a9* and *S100a8*, were down-regulated in *Mel18* $^{-/-}$ HSPCs. Importantly, loss of Mel18 significantly decreased the number of senescent HSPCs. To understand how Mel18 regulates gene transcription, we performed ATAC-seq assays in *Mel18* $^{+/+}$ and *Mel18* $^{-/-}$ HSPCs. Mel18 null HSPCs showed decreased chromatin accessibility near both *S100a8* and *S100a9* genes. We confirmed that both *S100a8* and *S100a9* are downregulated in *Mel18* $^{-/-}$ HSPCs compared to *Mel18* $^{+/+}$ HSPCs. Given that cell cycle arrest is a common feature of cellular senescence, we also examined the expression of cell cycle related genes in *Mel18* $^{-/-}$ HSPCs. The expression of cell cycle regulators, including *CDK4* and *CCND2*, is increased in *Mel18* $^{-/-}$ HSPCs. Mel18 can bind to a specific DNA sequence, 5'-GACTNGACT-3', thereby repressing the expression of target genes. Mel18 directly binds to the *CCND2* gene locus as revealed by ChIP-seq assays in embryonic stem cells, suggesting that Mel18 may directly repress *CCND2* expression in HSPCs.

In summary, we demonstrate that Mel18 inhibits HSC self-renewal via repressing the expression of genes that are important for cellular senescence and proliferation.

Disclosures No relevant conflicts of interest to declare.

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