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POSTER ABSTRACTS

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

MED16 Negatively Regulates Erythropoiesis and Myelopoiesis through Modulation of Chromatin Accessibility

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Tissue-specific transcription factors work in conjunction with numerous coregulators to modulate transcription networks during hematopoiesis; disruptions in this process may lead to hematopoietic disorders, including myelodysplastic syndromes (MDS). While the Mediator complex is known to regulate various aspects of hematopoiesis, the mechanisms underlying subunit-specific functions have not been fully understood. Here, we uncovered that MED16, a core component of Mediator tail module, serves as a crucial negative regulator of erythropoiesis and myelopoiesis by controlling chromatin accessibility at specific target genes.

To investigate the function of MED16 *in vivo*, we transplanted control (shSCR) or MED16-deficient (shMED16) human cord blood CD34+ hematopoietic stem and progenitor cells (HSPCs) into immunodeficient mice. Mice receiving shMED16 HSPCs showed a higher proportion of human erythroid and myeloid cells in the bone marrow (BM), while B cell and T cell reconstitution remained unaffected. MED16 deficiency led to an increased number of myeloid colonies and a more pronounced increase in erythroid colonies in the colony-forming assay. During erythroid differentiation, MED16 deficiency resulted in an elevated ratio of CD235a+ cells and increased enucleation. Furthermore, hematopoietic-specific Med16 knockout (*Mx1-Cre* × *Med16* $^{-/-}$ *Med16 KO)*mice showed normal BM and spleen cellularity, with an increased frequency of common myeloid progenitors (CMPs). Med16 KO mice exhibited a higher frequency of CD11b+ myeloid cells and Ter119+ erythroid cells in the BM and spleen. A higher percentage of enucleated erythrocytes were observed in the BM of Med16 KO mice. Additionally, in the model of phenylhydrazine-induced hemolytic anemia, Med16 KO mice showed accelerated red blood cell recovery and pronounced splenomegaly, highlighting the crucial role of Med16 in stress erythropoiesis. Our findings demonstrate that MED16 plays a repressive role in hematopoietic development.

To elucidate how MED16 regulates gene expression, we conducted single-cell RNA-seq and ATAC-seq of cultured CD34+ cells after MED16 knockdown in primary human CD34+ HSPCs. MED16 deficiency resulted in upregulation of erythroid and innate immunity genes in erythroid and myeloid cells, respectively. Interestingly, MED16 deficiency led to significantly increased chromatin accessibility around the transcription start sites of upregulated genes. Considering the Mediator complex often interacts with transcription factors to convey information, we conducted MED16 chromatin IP sequencing (ChIP-seq). The majority of MED16 occupancy peaks were located at promoters (59.68%). Colocalization analysis of MED16 with chromatin occupancy of hematopoietic-related transcription factors revealed significant overlap with RUNX1. To unveil the mechanism of MED16-mediated transcriptional repression, we conducted immunoprecipitation coupled with mass spectrometry on cultured CD34+ cells to identify MED16-interacting nuclear proteins. We identified the FAcilitates Chromatin Transcription (FACT) complex as a potential candidate. ChIP-seq of the FACT complex subunit SPT16 showed weakened chromatin occupancy signals upon MED16 deficiency. Similar results were observed in mouse BM Ter119+ erythroblasts. These findings indicate that MED16 plays a crucial role in modulating chromatin accessibility and transcriptional repression, potentially through cooperation with RUNX1 and the FACT complex.

Furthermore, we found that MED16 overexpression impeded erythroid cell enucleation, similar to MDS erythroid dysplasia. We observed higher MED16 expression in BM-derived CD34+ cells from 183 MDS patients across different subtypes compared to healthy individuals. Interestingly, knocking down MED16 in bone marrow mononuclear cells from MDS patients restored normal erythropoiesis. Transcriptome analysis revealed that MED16 deficiency upregulated innate immunity and erythroid genes. Together, these findings shed light on the previously unknown role of MED16 in hematopoiesis and its asso-

ciation with MDS. Our study provides mechanistic insights into the lineage commitment and developmental progression of hematopoiesis governed by Mediator and may have implications for the treatment of hematological disorders.

Disclosures No relevant conflicts of interest to declare.

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