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POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

The Condensin II Complex Subunit NCAPH2 Regulates Patterns of H4K20me1 Occupancy and Erythroid Gene Expression

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During terminal maturation, erythroid precursors upregulate erythroid-specific genes while silencing non-erythroid genes in the setting of a rapid cell division and a nucleus that is dramatically condensing in preparation for enucleation. Setd8, the sole histone methyltransferase that can generate H4K20me1, plays an essential role in both chromatin condensation and the regulation of gene expression during erythropoeisis (Malik Cell Reports 2019, Myers Epigenetics and Chromatin, 2020). H4K20me1 regulates chromatin structure and gene expression through interaction with multiple partners, including the Condensin II Complex. The Condensin II complex is a ring-like structure composed of two conserved SMC components (SMC2 and SMC4), two HEAT subunits (NCAPG2 and NCAPD3), and a kleisin subunit NCAPH2. The Condensin II complex plays an important role in chromatin condensation during mitosis. It also regulates higher-order chromatin interactions and gene expression in interphase cells (Yuen Science Adv 2017; Iwasaki Nature Comm 2019). Similar to Setd8, many subunits of the Condensin II complex are highly expressed in erythroid cells (biogps.org; bloodspot.eu). We hypothesized that the Condensin II complex, Setd8, and H4K20me1 work in tandem to establish appropriate patterns of chromatin architecture and gene expression in maturing erythroblasts. To address this hypothesis, we first depleted NCAPH2 in erythroid cells by crossing mice with floxed alleles of NCAPH2 with mice expressing cre-recombinase under the direction of the Erythropoietin receptor promotor (EpoRCre). Homozygous disruption of NCAPH2 (NCAPH2 Δ/Δ) was embryonic lethal by E13.5. NCAPH2 Δ/Δ embryos were similar in appearance to littermate controls until E12.5 when they developed notable pallor and a dramatic decline in the number of benzidine positive cells. In contrast to cells from littermate controls, primitive erythroid NCAPH2 Δ/Δ cells at E11.5 were heterogenous in cell and nuclear size. NCAPH2 Δ/Δ embryos also had a dramatic failure of definitive erythropoiesis, as evidenced by significant pallor of the fetal liver at E13.5.

To gain insights into the mechanisms underlying these findings, we used CUT&RUN to identify the genomic targets of NCAPH2 in E11.5 erythroblasts and to identify changes in H4K20me1 occuapncy in E11.5 NCAPH2 Δ/Δ erythroblasts compared to littermate control. In addition, the NCAPH2 and H4K20me1 CUT&RUN studies were compared to transcriptomic analyses of NCAPH2 Δ/Δ, NCAPH2 Δ/+, and NCAPH2 +/+ erythroblasts from E11.5 embryos. The majority of NCAPH2 peaks were located at transcripton start sites (TSS). Genes that were enriched for NCAPH2 at the TSS were also enriched for H4K20me1 across the gene body. At NCAPH2 target genes, enrichment for H420me1 occupancy increased significantly in NCAPH2 D/Derythroblasts compared to control. NCAPH2 was present at the TSS of differentially expressed genes that control cell cycle progression and erythroid maturation, and there were dynamic changes in H4K20me1 over the body of these genes following NCAPH2 deletion. Together, these results demonstrate that NCAPH2 is essential for erythropoiesis, and works in conjunction with Setd8 and H4K20me1 to establish appropriate patterns of gene expression in maturing erythroblasts.

Disclosures No relevant conflicts of interest to declare.

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