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POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Ncrna-a3 Epigenetically Regulates *TAL1* and Its Target Genes to Drive Erythroid Differentiation

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Erythropoiesis is the development of enucleated red blood cells from multipotent hematopoietic stem cells. This complex process is tightly regulated by the dynamic changes in occupancy of tissue specific transcription factors (TFs) that activate and repress genes appropriately. TAL1, a critical regulator is expressed throughout the development of mature erythroblasts from multi-progenitors. Loss of TAL1 leads to defective erythropoiesis. As opposed to TAL1's role in erythropoiesis, itis an extraneous factor in developing lymphoid cells and aberrant expression of *TAL1* in T-cells is associated with T-ALL. The differential regulation during normal and malignant hematopoiesis remains largely unclear. We found that a distil RNA - ncRNA-a3, is transcribed from erythroid specific enhancer, 50kbp downstream of the *TAL1* TSS. We hypothesized that ncRNA-a3 plays an important role in regulating *TAL1* and its target gene transcription in an erythroid specific manner. To test this hypothesis, we examine the regulatory potential of ncRNA-a3 towards the activation of *TAL1* expression in the context of hematopoiesis and leukemogenesis.

We used RNA interference to deplete ncRNA-a3 expression across several hematopoietic and leukemic cells. The decrease in ncRNA-a3 resulted in significant reduction of *TAL1* transcript and protein levels in hematopoietic cells while *TAL1* transcripts showed little or no reduction in leukemic cells. Subsequently, transcriptomic profile changes in erythroleukemic K562 cells showed downregulation of erythroid specific targets of TAL1. To ascertain the role of ncRNA-a3 in erythropoiesis, we used siRNA against ncRNA-a3 in primary CD34+ cells and subjected cells to differentiation. Quantification of early and late erythroblasts by colony forming assay and flow cytometry indicated impaired erythroid differentiation and maturation in orthochromatic and polychromatic stages. Further, RNA-seq analysis of ncRNA-a3 knockdown cells revealed defects in globin gene function, heme biosynthesis and nucleosome condensation compared to control CD34+ cells during time course EPO induced erythroid differentiation.

Mechanistically, we observed ncRNA-a3s occupancy at *TAL1* regulatory regions from ChIRP-qPCR across *TAL1* locus. NG Capture-C data depicting changes in interaction frequencies indicated that ncRNA-a3 maintains canonical interactions at the *TAL1* locus. Knockdown of ncRNA-a3 negatively impacted the chromatin accessibility in *TAL1* and TAL1 target loci. Perturbation of chromatin landscape affected TAL1 and TAL1 binding partner - P300 and BRG1 occupancy at TAL1 target sites. To distinguish reduced TAL1 binding as a consequence of lower levels of TAL1, from RNA dependent binding of TAL1, we examined TAL1, P300 and BRG1 chromatin occupancy through Cut&Run in conjunction with RNase treatment. Effective depletion of global RNAs resulted in reduced enrichment of TAL1, P300 and BRG1 bound fragments indicating possible RNA dependent binding of TAL1 and its cofactors. TAL1 coimmunoprecipitation using RNase treatment (1) and ncRNA-a3 KD (2), demonstrated that TAL1s interaction with P300 and BRG1 is RNA dependent, but ncRNA-a3 independent. RNA-IP results further confirmed that ncRNA-a3 is associated with TAL1 through its interacting epigenetic cofactors P300 and BRG1. Together, our data indicate that although RNA dependent binding of TAL1 and its epigenetic cofactors is cell type or target locus specific, ncRNA-a3 modulates the occupancy of TAL1, P300 and BRG1 at the *TAL1* locus during erythroid specific *TAL1* transcription.

Disclosures No relevant conflicts of interest to declare.

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