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Blood 142 (2023) 553

The 65th ASH Annual Meeting Abstracts

# ORAL ABSTRACTS

## 112.THALASSEMIA AND GLOBIN GENE REGULATION

### Selective Globin Gene Regulation By the Non-Canonical Baf Chromatin Remodeling Complex

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The regulation of the beta-type globin genes has served as a powerful model for developmental transcriptional control, with humans expressing embryonic (HBE), two fetal (HBG1/2) and two adult (HBB and HBD) versions at the corresponding developmental stages. Reactivation of HBG in adult erythroid cells is of benefit to patients with sickle cell disease and $\beta$-thalassemia. While past efforts focused on the identification of repressors of the HBG genes, including BCL11A, ZBTB7A, and NFI-A/X, to date no transcriptional activators have been identified with selectivity for any of the globin genes. Using a CRISPR-Cas9 screen in the HUDEP2 erythroid cell line, we recently identified BRG1/SMARCA4 as a potential activator of HBG production. BRG1 is a core ATPase component of the multi-subunit BAF (BRG1/BRM-associated factor) complex; different subtypes of this large chromatin remodeler family are specific to particular cell types or developmental stages. BRG1 was previously implicated in globin gene regulation but its molecular composition and whether it has selectivity for any of the genes in the cluster is unknown. Notably, we found that loss of BRG1 in both HUDEP2 and primary erythroid cells impairs transcription of HBG to a much greater extent than that of HBB. Here we functionally dissected BAF subunits to decipher composition of the relevant BAF complex and the mechanism for this unexpected HBG gene selectivity.

We used CRISPR-Cas9 in HUDEP2 cells and human primary erythroid cultures to test the requirement for 14 different BAF subunits for selective HBG transcription. Among these, the BAF60A component emerged as critically required for the normal production of HBG but not HBB in HUDEP2 cells. Depletion of BAF60A in human primary erythroid cultures reduced HBG by up to 80% with no reduction in HBB as assessed by western blot, flow cytometry, HPLC, RT-PCR, and droplet digital RT-PCR. We also observed a reduction in HBD expression, suggesting that BAF60A functions in a gene selective manner but not necessarily favoring the fetal over the adult-type globin genes. $\alpha$-type globin genes were unaffected by BAF60A loss, further demonstrating the gene selectivity of BAF60A action. RNA-seq of BAF60A-targeted cells showed that the most downregulated transcripts were from the two HBG genes and the BGLT3 long non-coding RNA, which typically parallels HBG in expression. These results revealed an unexpected gene selectivity of the BAF complex, conveyed at least in part by BAF60A.

To assess global chromatin accessibility upon BAF60A loss in primary erythroid cells we utilized ATAC-seq. Among ~132,000 accessibility peaks, ~10,000 were reduced in BAF60A targeted cells. Interestingly, we saw a strong decrease in chromatin accessibility at the HBG and HBD genes with minimal to no effect on the HBB or $\alpha$-globin genes, in line with gene expression data, again reflecting the gene specificity of BAF60A.

BAF60A can be found in different classes of BAF complexes, but is absolutely required for the non-canonical BAF (ncBAF) subtype. ncBAF is a relatively recently described BAF complex that is distinguished from other classes of BAF complexes by the presence of the bromodomain protein BRD9. Therefore, we hypothesized that ncBAF is the relevant BAF complex required for selective HBG transcription. Genetic or pharmacologic disruption of BRD9 in HUDEP2 cells recapitulated the BAF60A phenotype, with decreased HBG and HBD expression but sparing of the HBB and $\alpha$-globin genes.

This is the first study of the role of the non-canonical BAF complex in hemoglobin gene regulation. Our results demonstrate remarkable selectivity of this subtype of a broadly active chromatin remodeling complex for specific hemoglobin genes in adult erythroid cells. Further work on the ncBAF complex will focus on rerouting the BAF complex to promote the transcription of HBG and HBD in hemoglobinopathies.

**Disclosures Blobel:** *Fulcrum Therapeutics:* Research Funding; *Blueprint Medicine:* Research Funding.

https://doi.org/10.1182/blood-2023-179001