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POSTER ABSTRACTS

112.THALASSEMIA AND GLOBIN GENE REGULATION

Identifying Novel Regulators of γ-Globin Expression Using a Genome-Scale CRISPR Activation Screen

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β-hemoglobinopathies are the most common monogenic disorders worldwide, and are defined based on whether patients have quantitative (β-thalassemia) or qualitative (Sickle Cell Disease (SCD)) defects in β-globin synthesis. Unfortunately, there are limited treatment options for β-hemoglobinopathies, and the majority of patients continue to develop life-threatening complications from their diseases. An alternative β-like subunit, γ-globin, has been shown to inhibit pathogenic hemoglobin polymerization in SCD and to functionally replace β-globin in β-thalassemia. The discovery of novel strategies to upregulate γ-globin expression may lead to effective therapies for β-hemoglobinopathies.

To identify novel regulators of γ-globin expression, we performed a genome-wide pooled CRISPR activation (CRISPRa) screen, using the Synergistic Activation Mediator (SAM) system. The screen was performed in the HUDEP-2 cell line, which expresses adult β-globin.

We generated a clonal HUDEP-2-MPH cell line, which stably expresses the transcriptional activator complex MPH (MS2-P65-HSF1). This cell line was transduced with a viral library that delivers a VP64 activation domain fused to a catalytically dead Cas9 (dCas9-VP64), a blasticidin resistance cassette, and one of 3 unique sgRNAs targeting virtually every coding gene in the human genome (the library consists of 70,290 sgRNAs). At day 8 of erythroid differentiation, the top and bottom 10% γ-expressing cells were sorted, and integrated sgRNAs were sequenced using NextGen sequencing.

As expected, sgRNAs targeting *BCL11A* and *ZBTB7A* (known negative regulators of γ-globin) were enriched in the bottom 10% γ-expressing cells, while sgRNAs targeting *HIF1A* (known positive regulator of γ-globin) were enriched in the top 10% γ-expressing cells. Notably, this screen identified several novel candidate positive regulators of γ-globin expression, including the transcriptional repressor Hypermethylated in Cancer 1 (HIC1).

In preliminary validation studies, we used 2 independent sgRNAs to activate *HIC1* in HUDEP-2-MPH cells and measured the percentage of γ-globin expressing cells (F-cells) by flow cytometry. Compared to cells transduced with control sgRNAs, increased *HIC1* transcription (>200-fold) resulted in a profound increase in the proportion of F-cells, from a baseline of ~6% up to ~76%. Similarly, *HIC1* overexpression in HUDEP-2 cells resulted in a ~13-fold increase in γ-globin mRNA levels and reduction in *BCL11A* mRNA level to ~30% of normal. These results suggest that the increased γ-globin expression resulting from *HIC1* overexpression may result, at least in part, from reduced *BCL11A* levels (which we are currently validating). Recently, increased expression of HIC2, a paralogous protein for HIC1, was reported to result in increased γ-globin expression. To exclude the possibility that *HIC1* targeting sgRNAs may also target *HIC2*, we measured the *HIC2* mRNA level in HUDEP-2 cells targeted with sgRNAs aimed at increasing *HIC1* expression. *HIC2* mRNA was not increased in the latter cells.

We next overexpressed *HIC1* cDNA in erythroid cells differentiated from primary human CD34+ hematopoietic stem and progenitor cells (HSPCs). In early preliminary results, we found that *HIC1* overexpression resulted in increased γ-globin expression, validating the HUDEP-2 data. Additional studies are ongoing to define the role of *HIC1* in the regulation of γ-globin expression, and to define the impact of *HIC1* overexpression on erythroid differentiation.
In summary, our screen uncovered HIC1, and other potential novel regulators of γ-globin expression, which we are currently validating. These findings may result in future therapeutic approaches for β-hemoglobinopathies.

Disclosures No relevant conflicts of interest to declare.

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