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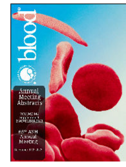

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

113.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: BASIC AND TRANSLATIONAL

Protein Phosphatase 6 Complex: Novel Regulator of Fetal Hemoglobin and Potential Therapeutic Target in Sickle Cell Disease

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Although increasing levels of fetal hemoglobin (HbF) in sickle cell disease (SCD) significantly reduces mortality, effective pharmacologic induction of HbF has remained elusive. To identify potentially druggable molecules involved in HbF control, we carried out a domain-focused CRISPR-Cas9-based genetic screen in HUDEP2 cells targeting all 218 serine/threonine protein phosphatases with 6 independent sgRNAs each. A single phosphatase, PPP6C, emerged as a potential HbF repressor. PPP6C is the catalytic subunit of protein phosphatase 6 (PP6), a serine/threonine phosphatase complex that has been implicated in cell cycle regulation, autophagy, and innate immunity mechanisms in other cell types, but its role in erythropoiesis and HbF regulation has not previously been described.

CRISPR-Cas9-based depletion of PPP6C in primary human erythroid cells elevated mRNA levels of HBG (the fetal form of β-globin) in a dose-dependent manner up to 3-4 fold, increased HbF levels 3-fold as measured by HPLC (up to 15-20% of total hemoglobin), and doubled the number of HbF-expressing cells. PPP6C depletion caused relatively few changes in the erythroid transcriptome by RNA-seq analysis, and did not measurably impair erythroid maturation. In addition, PPP6C loss in primary SCD patient-derived cells was well-tolerated, led to robust levels of HbF induction, and reduced cell sickling in vitro by up to 60%. Mechanistically, loss of PPP6C reduced the levels of the HbF repressor BCL11A by nearly 50% but left unchanged the levels of other HbF regulators, such as HRI, LRF, EKLF, NFIA/X, ZNF410, or HIC2, suggesting that PPP6C-mediated HbF regulation proceeds at least in part via loss of BCL11A. Importantly, xenotransplantation data (NBSGW) showed ˜4-fold induction of HBG at 16 weeks post-transplant, suggesting that PPP6C deficiency leads to effective, sustained HbF induction in vivo.

We next set out to determine whether any PP6 subunits have erythroid specificity that could be exploited therapeutically. In contrast to PPP6C, which is highly expressed in all hematopoietic lineages, the PP6 facultative subunit PPP6R1 appears to be enriched in erythroid precursors, suggesting that depletion of PPP6R1 may function as an erythroid-selective target to increase HbF levels. CRISPR-Cas9-based depletion of PPP6R1 in primary human erythroid cells showed a 2-3-fold increase in HBG and near-doubling of HbF-expressing cells, recapitulating the majority of PPP6C-mediated HbF induction. Functional studies are currently in progress to specifically determine whether additional PP6 subunits play a key role in HbF regulation. Furthermore, ongoing work utilizing dTAG-based acute depletion of PPP6C and PPP6R1 paired with phospho-proteomic studies will narrow down direct targets of the PP6 complex and provide insights into erythropoietic and HbF regulatory pathways impacted by PP6.

Taken together, our data indicate that PP6, including its catalytic subunit PPP6C and erythroid-enriched regulatory subunit PPP6R1, inhibit HbF production in part via modulating BCL11A levels, and may serve as a red-cell specific therapeutic target in the treatment of SCD.

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