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POSTER ABSTRACTS

112.THALASSEMIA AND GLOBIN GENE REGULATION

Genome-Scale CRISPR-Cas12a Screen Identifies Novel Fetal Hemoglobin Regulators

Elizabeth A. Traxler, MD PhD[1,2], Chad Komar[3], Megan Saari[2], A Josephine Thrasher[4], Kunhua Qin, PhD[2], Eugene Khandros, MD PhD[2], Quynn Hotan[4], Osheiza Abdulmalik, DVM[2], Claire Shao[5], Qingzhou Chen, PhD[6], Andy J. Minn, MD PhD[7], Cheryl A. Keller, PhD[8], Junwei Shi, PhD[9], Gerd A. Blobel, MD PhD[2]

[1] Division of Hematology/Oncology, Department of Medicine, Hospital of University of Pennsylvania, Philadelphia, PA
[2] Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, PA
[3] Perelman School of Medicine at University of Pennsylvania, Philadelphia
[4] Division of Hematology, Children's Hospital of Philadelphia, Philadelphia
[5] Department of Radiation Oncology, University of Pennsylvania, Philadelphia
[6] Department of Cancer Biology, Perelman School of Medicine at University of Pennsylvania, Philadelphia
[7] Department of Radiation Oncology, Perelman School of Medicine at University of Pennsylvania, Philadelphia, PA
[8] Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA
[9] Department of Cancer Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Reversal of the postnatal hemoglobin switch in red blood cells is a promising approach to therapies for sickle cell disease and β-thalassemia, as reactivating fetal hemoglobin (HbF, $\alpha 2\gamma 2$) in adult red blood cells ameliorates disease complications. Studies over several decades have illuminated the paradigm of this fetal-to-adult hemoglobin transition, including central roles of transcriptional repressors BCL11A, LRF, and NFIA/X, which recruit repressive chromatin modifiers to the γ-globin locus. To elucidate undescribed regulatory mechanisms of these molecules and reveal new targets for therapies, we performed the first genome-wide genetic screen to pinpoint specific pathways or protein complexes that regulate HbF expression in adult erythroid cells.

Because Cas9 technology is limiting at the genome-wide scale, we conducted a CRISPR-Cas12a genetic screen targeting all known coding genes in the human genome using a novel custom array directed towards 18,292 genes. The single CRISPR RNA (crRNA) library was cloned into a lentivirus scaffold and transduced into the adult-type erythroid cell line, HUDEP2, engineered to stably express Cas12a. HUDEP2 cells express little HbF, allowing for positive phenotypic selection (HbF+) by fluorescence-activated cell-sorting. By isolating the top 10% and bottom 10% of HbF-expressing cells and determining the representation of crRNAs via deep sequencing, we identified a crRNA subset enriched in the high-HbF population encompassing 211 candidate genes, representing potential regulators of HbF. To prioritize these genes for follow up studies, we conducted a domain-focused, CRISPR-Cas9 screen of all 211 candidate genes. Using 2,184 sgRNAs, this validation Cas9 screen in HUDEP2 cells nominated 18 high-confidence targets.

Two of the top candidate genes were identified as modulators of the phosphatase PP2A, *PPP2R4* and *TIPRL*. PP2A is a multi-subunit complex with diverse cellular functions as a serine/threonine phosphatase. To our knowledge, it has no previously known role on globin gene production. In validation experiments, disruption of either *PPP2R4* or *TIPRL* in HUDEP2 cells significantly increased γ-globin mRNA and protein expression. Using sgRNA-Cas9 ribonucleoprotein complex nucleofection, *PPP2R4* gene editing in primary human CD34+ cell derived erythroblasts significantly increased γ-globin mRNA and protein expression without overtly affecting erythroid maturation. Notably, the degree of effect is similar to that of BCL11A +58 enhancer disruption. Depletion of either PP2A regulator in HUDEP2 cells resulted in markedly reduced BCL11A protein levels. Furthermore, BCL11A overexpression in *PPP2R4* or *TIPRL*-targeted cells reduced γ-globin mRNA commensurate to parental control cells, thereby demonstrating both TIPRL and PPP2R4 mediate γ-globin silencing predominantly via BCL11A. RNA-sequencing of *TIPRL* and *PPP2R4*-edited HUDEP2 cells revealed strong overlap of differentially expressed genes, consistent with convergent function. Altogether, our studies utilizing a novel Cas12a-based, genome-wide screen nominate a new pathway mediating γ-globin silencing via regulation of BCL11A.

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