P1436 DEVELOPMENT OF A BETA-GLOBIN GENE REPLACEMENT STRATEGY AS A THERAPEUTIC APPROACH FOR BETA-TALASSEMA

Topic: 24. Gene therapy, cellular immunotherapy and vaccination - Biology & Translational Research

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Background:

β-thalassemia is a genetic disorder characterized by reduced production of β-globin, a protein that forms functional, oxygen-carrying hemoglobin with α-globin (HbA, α2β2). In its most severe form, β-thalassemia is caused by mutations in both alleles of the β-globin gene. These patients fail to produce functional β-globin, resulting in severe anemia. More than 300 β-thalassemia mutations are known; most are small nucleotide insertions, substitutions, or deletions within or directly adjacent to the β-globin (HBB) gene. A gene editing platform using homology directed repair (HDR) that replaces the HBB gene to achieve a normal or β-thalassemia trait phenotype with HbA expression similar to healthy individuals is an ideal strategy. The challenge is achieving high levels of gene replacement that result in physiologic HbA expression.

Aims:

We designed a homologous DNA donor that can replace a mutated HBB gene with a functional HBB gene and overcomes the two main challenges with gene replacement at this locus. First, to avoid sequence homology of the insert with the endogenous locus, we used a diverged HBB coding sequence. Second, because introns are required for high HBB expression, we hypothesized that incorporation of non-homologous introns would result in physiologic HbA production.

Methods:

We developed an HBB gene replacement strategy using a high-efficiency gene editing platform that combines: 1) a high fidelity Cas9 precomplexed with chemically modified guide RNA, 2) a DNA template delivered by AAV6, and 3) CD34+ hematopoietic stem and progenitor cell culture optimization. To identify DNA templates resulting in high HBB expression, we screened 39 versions of T2A-EGFP-tagged HBB coding sequences containing various heterologous introns and polyadenylation signals. Primary CD34+ hematopoietic stem and progenitor cells (HSPCs, n = 3 healthy donors) were edited with different β-globin homologous DNA donors, differentiated into red blood cells (RBCs) in vitro, and then evaluated for EGFP mean fluorescence intensity (MFI) by flow cytometry as a surrogate for beta-globin expression. To evaluate if the MFI positively correlated with HbA expression, the T2A-EGFP sequence was removed from the DNA donors and HbA expression was measured by HPLC in HSPC-derived RBCs. To increase gene replacement frequencies, the top DNA donors were further optimized by truncating the introns to create a smaller insertion cassette.

Results:

Adding heterologous introns to the HBB coding sequence significantly increased EGFP MFI by three-fold (p=0.0008). MFI and HbA output were positively correlated, and the top DNA donors resulted in HbA expression on par with physiologic levels. Using this gene replacement strategy, we achieved HDR frequencies of up to 40% in CD34+ HSPCs. Optimized smaller DNA templates resulted in higher HDR and maintained high levels of HbA expression. Using HSPCs from patients with sickle cell disease as a therapeutically relevant model, we investigated if this DNA
donor can replace the non-functional $HBB$ gene that produces HbS. Gene replacement of the sickle allele using the optimized DNA donor restored physiologic HbA expression (by HPLC) comparable to an HDR gene correction strategy that corrects the HbS point mutation. Future experiments are investigating $in~vitro$ and $in~vivo$ stem cell repopulation capacity of HSPCs targeted for $HBB$ gene replacement.

**Summary/Conclusion:**

In summary, we developed a precise $HBB$ gene replacement strategy that is highly efficient and results in high HbA production, offering a potential differentiated approach for treating β-thalassemia.