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POSTER ABSTRACTS

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Prime Editing of the α-Thalassemia Hb Constant Spring Mutation

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Hemoglobin Constant Spring (HbCS) is the most common non-deletional α-thalassemic mutation and is an important cause of HbH-like disease in Southeast Asia. Although the α-globin is expressed from two copies of homologous genes (*HBA2* and *HBA1*), the constant spring (CS) mutation CD142 (UAA > CAA) occurs only in *HBA2* gene that leads to the instability of both mRNA and protein in erythroid cells. The newly developed gene editing technology prime editors (PEs) can generate all types of substitutions, providing therapeutic potential in different types of diseases. However, the efficiency of primer editor in primary hematopoietic stem cells is still a big challenge and lack of study in the HbCS mutation.

We first created the endogenous HbCS mutation in human umbilical cord blood-derived erythroid progenitor-2 (HUDEP-2) cells, providing as a model for prime editing optimization. The protein of α^{cs} chain (a larger protein with extra 31 amino acids) was detected in the model cells with *HBA2* bi-allelic CS mutation and normal *HBA1*, which exhibited the delay of expansion, higher apoptosis rate and altered erythroid maturation markers after induced differentiation. To correct the CS mutation for converting HbCS to HbA, we performed ex vivo delivery of mRNA encoding the prime editor together with a pegRNA and a nick guide RNA into the Hb CS HUDEP2 model cells and achieved the successful in-situ correction with an efficiency of 39.883±9.720% of CS alleles without enrichment, while no measurable editing in the same loci of *HBA1* as evaluated by both Sanger sequencing and NGS. More importantly, the correction resulted in a significant decrease of α^{cs} chain, thereby alleviated erythroid phenotype after induced maturation with reducing the apoptosis rate. After that, we collected the primary Hb H-CS patients derived CD34$^+$ hematopoietic stem/progenitor cells (HSPCs) and confirmed that the defect on erythroid expansion, differentiation in Hb H-CS patients derived CD34$^+$ HSPCs compared to the normal donor, especially demonstrated by lower enucleation rate and higher apoptosis. Moreover, the protein of α^{cs} chain was detected in the erythroblast from Hb H-CS CD34+ HSPCs. We further successfully corrected the CS mutation in Hb H-CS patient derived CD34$^+$ HSPCs with an efficiency of 23.68% by mRNA electroporation. At the same time, the editing efficiency was achieved with minimal generation of insertions and deletions and a low level of Cas9-dependent DNA off-target editing. Importantly, we observed an ~23% decrease of α^{cs} chain protein and significant restoration of the enucleation defect from the differentiated erythroblast after PE correction in CD34$^+$ HSPCs.

In summary, these results suggests that the delivery of PE mRNA together with synthesized pegRNA and nick sgRNA can successfully achieve the in-situ correction in Hb HbCS mutation site in the model cells and the primary Hb H-CS patient derived CD34$^+$ HSPCs. Our results provide proof of principle for prime editing for its therapeutic potential in directly and precisely correction of the Hb-CS mutation.

Disclosures No relevant conflicts of interest to declare.

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