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RESEARCH ARTICLE

Gene editing of *PKLR* gene in human hematopoietic progenitors through 5' and 3' UTR modified TALEN mRNA

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Abstract

Pyruvate Kinase Deficiency (PKD) is a rare erythroid metabolic disease caused by mutations in the *PKLR* gene, which encodes the erythroid specific Pyruvate Kinase enzyme. Erythrocytes from PKD patients show an energetic imbalance and are susceptible to hemolysis. Gene editing of hematopoietic stem cells (HSCs) would provide a therapeutic benefit and improve safety of gene therapy approaches to treat PKD patients. In previous studies, we established a gene editing protocol that corrected the PKD phenotype of PKD-iPSC lines through a TALEN mediated homologous recombination strategy. With the goal of moving toward more clinically relevant stem cells, we aim at editing the *PKLR* gene in primary human hematopoietic progenitors and hematopoietic stem cells (HPSCs). After nucleofection of the gene editing tools and selection with puromycin, up to 96% colony forming units showed precise integration. However, a low yield of gene edited HPSCs was associated to the procedure. To reduce toxicity while increasing efficacy, we worked on i) optimizing gene editing tools and ii) defining optimal expansion and selection times. Different versions of specific nucleases (TALEN and CRISPR-Cas9) were compared. TALEN mRNAs with 5' and 3' added motifs to increase RNA stability were the most efficient nucleases to obtain high gene editing frequency and low toxicity. Shortening ex vivo manipulation did not reduce the efficiency of homologous recombination and preserved the hematopoietic progenitor potential of the nucleofected HPSCs. Lastly, a very low level of gene edited HPSCs were detected after engraftment in immunodeficient (NSG) mice. Overall, we showed that gene editing of the *PKLR* gene in HPSCs is feasible, although further improvements must to be done before the clinical use of the gene editing to correct PKD.