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Gene Editing For The Rescue Of PKU Disease Phenotype Using Mouse Chemically Derived Hepatic Progenitors (mCdHs) In Vitro

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Background : Phenylketonuria (PKU) is an autosomal recessive liver disease caused by point mutation. The PKU patient is highly accumulated L-phenylalanine (L-Phe) in the blood by the dysfunction of phenylalanine hydroxylase (Pah). The base editor modifies single nucleotide using deaminase and prime editor promises precise editing using reverse transcriptase. For repairing genetic diseases, gene editors are the only curative treatments for PKU disease.

Methods : Mouse primary hepatocytes (mPHs) were isolated from the PKU disease mouse model liver. To generate mouse chemically derived hepatic progenitors (mCdHs), the mPHs were cultured with a reprogramming medium for 7 days. The mCdHs were transfected with CRISPR-Cas9 and gRNA plasmids by electroporation and performed sequencing of Pah locus to validate the gene correction.

Results : We generated disease mCdHs (Pah-/-, Homo-mCdHs) and showed similar properties compared with normal mCdHs (Pah+/+, Wild-mCdHs). After editing, the clonal expansion significantly enhanced the correction rate in the cytosine base editor (CBE) group (5.5% to 93%). Next, we used prime editor 5 (PE5) instead of PE3 to increase editing efficiency and showed a 5-fold increase (PE3,1.8% to PE5,10.7%) without clonal expansion. Finally, we analyzed the Tyr level of edited cells and indicated increased Tyr compared with Homo-mCdHs after hepatic differentiation indicating increased L-Phe metabolism. Therefore, we demonstrated that edited Homo-mCdHs using gene editors represented the restoration of PKU disease phenotypes.

Conclusions : These findings demonstrate an effective and safe gene editing system with high gene correction efficiency for the restoration of PKU disease phenotype in vitro. Supported by: This research is funded by grants from National Research Foundation of Korea (2021M3A9H3015390) and National Research Foundation of Korea (2022R1F1A1073058).

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