Abstract No. : **OP-0247**

Topic : **Basic Research**

EX-VIVO GENE EDITING OF CHEMICALLY DERIVED HEPATIC PROGENITORS (CDHS) FOR TREATING MAPLE SYRUP URINE DISEASE (MSUD)

Haerin JEON [1], Soyoung JEON [1], Hyeon Woo IM [3], Soraya SALAS-SILVA [1], Ji Hyun SHIN [1], Sangsu BAE [3], Dongho CHOI [1]

[1] *Department of Surgery, , Republic of Korea,* [2] *Research Institute of Regenerative Medicine And Stem Cells, , Republic of Korea,* [3] *Department of Biomedical Sciences, , Republic of Korea,* [4] *Institute of Molecular Biology And Genetics, , Republic of Korea,* [5] *Cancer Research Institute, , Republic of Korea,* [6] *Medical Research Center of Genomic Medicine Institute, , Republic of Korea,* [7] *Hanyang University, Department of HY-KIST Bio-convergence, Republic of Korea*

Background : Maple syrup urine disease (MSUD) is a rare genetic metabolic disorder characterized by elevated levels of branched-chain amino acids (BCAAs) in the blood due to mutations in the BCKDHA, BCKDHB, or DBT genes. This metabolic dysfunction stems from a defective branched-chain keto acid dehydrogenase complex (BCKDC), often resulting in neonatal mortality if untreated. Current treatments involve strict dietary control and peritoneal dialysis, which do not fully prevent neurological damage. In this study, mouse chemically derived hepatic progenitors (mCdHs) were investigated as a potential therapeutic approach for MSUD.

Methods : BCKDHA+/- mice with a 266-bp deletion on Chromosome7 were bred to produce BCKDHA-/- mice. Phenotypic evaluation included plasma BCAA measurements and BCKDHA expression analysis. BCKDHA-/- mCdHs were then derived from neonatal mouse primary hepatocytes (mPHs) by magnetic associated cell separation using an E-cadherin antibody and subsequently reprogrammed by treating with HGF, A83-01, and CHIR99021. Afterward, human BCKDHA coding sequences were integrated into BCKDHA-/- mCdHs using twin prime editing system.

Results : Our research revealed that BCKDHA-/- mice exhibited decreased BCKDHA expression, resulting in elevated plasma BCAA levels and a survival limited to under 12 days. The gene-edited BCKDHA-/- mCdHs demonstrated stable expression of the human BCKDHA gene while maintaining the characteristic hepatic progenitor properties of mCdHs.

Conclusions : This study demonstrated the potential of ex vivo cell therapy for MSUD. The gene editing technology enables stable modifications with minimal off-target effects, without disrupting disease-related mutations, highlighting its promise for clinical application. Future transplantation of edited BCKDHA-/- mCdHs into MSUD mice is expected to provide therapeutic benefits.

Corresponding Author : **Dongho CHOI** (crane87@hanyang.ac.kr)