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DEVELOPMENT OF A HYBRID PKU:FRG MOUSE MODEL FOR ENHANCED EX VIVO CELLULAR THERAPY IN PHENYLKETONURIA

Dayoung KIM [1], Myounghoi KIM [1], Soyoung JEON [1], Sung-Ah HONG [2], Tae Hun KIM [1], Michael ADISASMITA [1], Elsy Soraya Salas SILVA [1], Ji Hyun SHIN [1], Sangsu BAE [2], Dongho CHOI [1]

[1] *Department of Surgery, Hanyang University Medical Center, Republic of Korea,* [2] *Department of Biomedical Sciences, Seoul National University College of Medicine, Republic of Korea*

Background : Phenylketonuria (PKU) is a metabolic disorder caused by Pah gene mutations, leading to PAH deficiency and L-phenylalanine (L-Phe) accumulation. Gene editing technologies enable in situ correction of disease-causing mutations, but translating these strategies into effective ex vivo therapies requires an appropriate in vivo platform for cell transplantation in PKU.

Methods : Primary hepatocytes from PKU mice were reprogrammed into chemically derived hepatic progenitors (PKU-mCdHs). Pah gene was corrected using base editing and prime editing. We developed a hybrid PKU:FRG mouse model (Pah-/-, Fah-/-, Rag2-/-, IL2ɣg-/-) by crossing PKU (Pah-/-) and FRG (Fah-/-, Rag2-/-, IL2ɣg-/-) mice. Genotyping and sequencing were performed to identify PKU:FRG mouse.

Results : Genetically edited PKU-mCdHs (Pah-/-) exhibited increased PAH activity and reduced L-Phe levels, effectively restoring PKU phenotypes in vitro. F1 (first filial generation) hybrids were generated by crossing PKU male with FRG female, leading to heterozygous individuals for the four critical genes: Pah, Fah, Rag2, and IL2ɣg. After breeding, in F7 (seventh filial generation) hybrids, we identified the PKU:FRG mouse in which all four genes were knocked out.

Conclusions : The PKU:FRG model effectively combines metabolic PKU defects with FRG-mediated cell engraftment, providing a promising platform for ex vivo cellular therapy studies.

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