Generation of Induced Pluripotent Stem Cells from Mouse Hepatocytic Lineage Cells

Department of Surgery, Soonchunhyang University College of Medicine, Seoul, Korea[1], Stem Cell Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA[2], Department of Molecular Biology, College of Medicine, Kosin University, Pusan, Korea[3], Department of Biomedical Engineering, New Jersey Institute of Technology, NJ[4], Laboratory of Experimental Carcinogenesis, CCR, National Cancer Institute , National Institutes of Health, Bethesda, MD[5], Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI, USA[6]

Dongho Choi[1], Kye-Yoon Park[2], Jeonghoon Heo[3], Cheul Hyung Cho[4], Marian Durkin[5], Agnes Holczbauer[5], Jens U. Marquardt[5], Valentina M. Factor[5], Karim Si-Tayeb[6], Stephen A. Duncan[6], Snorri S. Thorgeirsson[5]

Background: Induced pluripotent stem cells (iPS) are recently identified pluripotent stem cells by functional hepatocytes for drug discovery, developmental biology, and therapeutic use. In this study, we aimed to verify the possibility of hepatocytic lineage cells as a cell source for iPS generation.

Method: 16.5 days old hepatoblasts and neonatal hepatocytes were harvested with dispase treatment. E-cadherin MACS (magnetic activated cell sorting) were used for purification of pure hepatocyte population. 3 month old hepatocytes were collected by two step collagenage methods. Using KOSM lentiviral vectors, we transduced mouse hepatocytic lineage cells from C57/B6 mice. Mouse embryonic fibroblasts (MEF) from syngeneic mouse were used as a control.

Results: After 2～3 weeks of cultivation period, iPS cell lines were established from mouse hepatocytic lineage cells and MEF. They showed typical morphological characteristics of pluripotent cells and were stained with alkaline phosphatase (AP). Pluripotent stem cell markers (Oct4, Nanog, Klf4, Sox2, SSEA-1) were expressed and identified with immnunohistochemical staining. They formed embryoid bodies (EB) in the ultra-low attatchment dishes. iPS cell lines developed teratoma in the nude mice buttock 4～5 weeks after transplantation. In addition, hepatocyte like cells were differentiated from iPS cell lines by adding specified media for 3 weeks.

Conclusion: iPS cell lines were established effectively from hepatocytic lineage cells by KOSM lentiviral systems. They were pluripotent and showed hepatocyte differentiation potential *in vitro*. Comparison of each cell lines from different cell sources are underway to formally test their iPS generation efficiency and ability to give rise to differentiated hepatocytes *in vitro*.