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Optimization Of Differentiation Methods From Various Humanderived Pluripotent Stem Cell Lines To Hepatocyte-like Cells (HLCs)

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Background : For decades, the study of human liver physiology and development, as well as drug discovery, have been limited by the lack of representative models. Pluripotent stem cells (PSCs), such as embryonic stem cells (ESCs) and induced PSCs (iPSCs), represent an unlimited source from which hepatocyte-like cells (HLCs) can be produced. IPSC-derived HLCs have shown great potential as an alternative to primary hepatocytes (PHHs). Various studies have been conducted to obtain hepatocytes, but there is no definitive method yet. Therefore, we have been trying to find a better protocol.

Methods : Our hiPSC line-1 was reprogrammed from a human fibroblast cell line and hiPSC line-2 provided by other companies was maintained. Both iPSCs were differentiated with ActA, BMP2, FGF4, KGF, HGF, and OSM for about 28 days to HLCs. The differentiation protocol for the induction of definitive endoderm cells, hepatoblasts, and HLCs was based on our previous reports with some modifications. Cells were characterized by real-time qPCR, flow cytometry, immuno-fluorescence, CYP activity assay, Albumin production assay, etc.

Results : First, we describe a protocol that allowed us to generate high-quality HLCs from both different induced pluripotent stem cell lines. We also found that there were differences in differentiation potential among cell lines, and hiPSC line-1 tended to have higher differentiation potential than hiPSC line-2, according to real-time qPCR and flow cytometry results.

Conclusions : Although HLCs differentiated from induced pluripotent stem cells are still less mature than primary hepatocytes in terms of differentiation efficiency, we further upgraded the method to increase efficiency with hepatic properties through the optimization of various protocols. Supported by: This research is funded by grant from National Research Foundation of Korea (2022R1A2C2004593) and Korea Evaluation Institute of Industrial Technology (20012378).

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