Optimizing Small Molecule-driven Hepatic Differentiation of Human iPSCs

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Abstract

The hiPSCs are ethically non-controversial pluripotent cells reprogrammed from somatic cells, enabling scalable in vitro modelling of diseases and study of different human systems. However, the current hepatic differentiation protocols often exhibit limited efficiency and incomplete cellular functionality. In this study, we aimed to enhance the hepatic differentiation of hiPSCs through small molecule induction by modifying established protocols. The concentration of small molecules and culture conditions will be adjusted to evaluate their effects on differentiation outcomes. The resulting cell morphology and hepatic marker expression will be analyzed. Our findings indicate that specific condition modifications significantly enhanced the differentiation efficiency, yielding more consistent hepatic cells. This study underscores the critical role of small molecules in protocol optimization and provides a foundation for doing differentiation and research on the hiPSC-derived liver organoid.

Materials and Methods

(1) Established Protocol

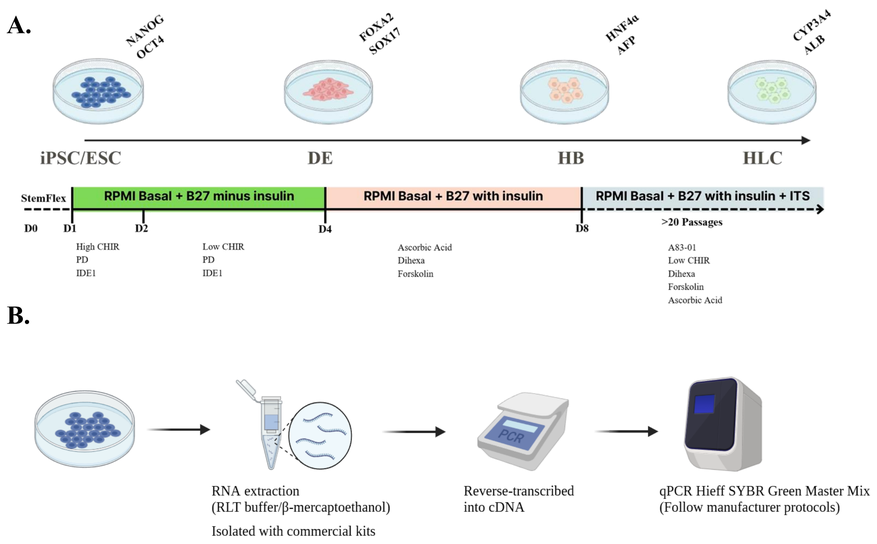

Figure 1: (A) Flow diagram and materials for differentiating HB by using the RUES2 cell line hiPSCs (B) Flow diagram of RNA extraction, purification, and RT-qPCR.

(2) Changing cell seeding density

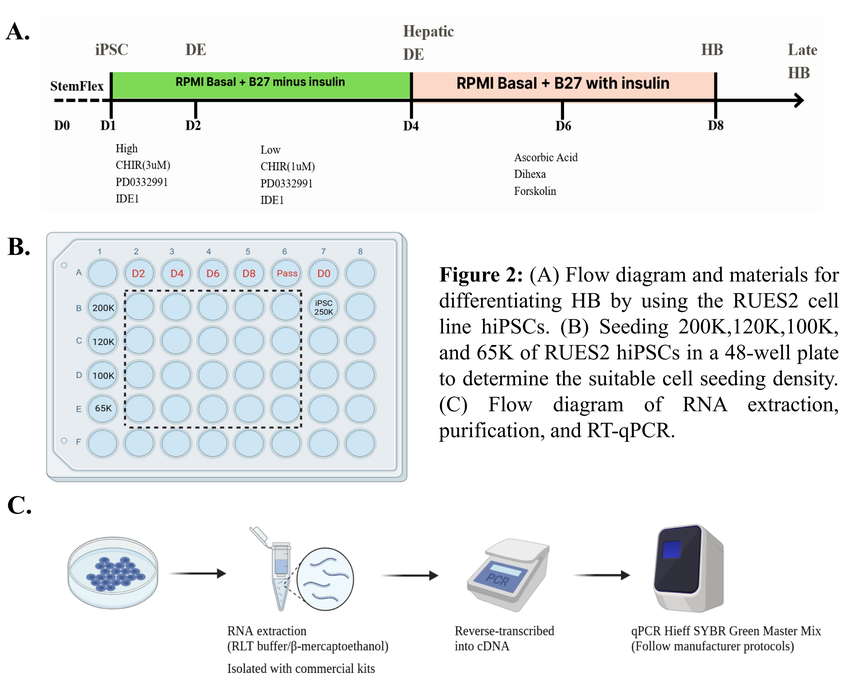

Figure 2: (A) Flow diagram and materials for differentiating HB by using the RUES2 cell line hiPSCs. (B) Seeding 200K,120K,100K, and 65K of RUES2 hiPSCs in a 48-well plate to determine the suitable cell seeding density. (C) Flow diagram of RNA extraction, purification, and RT-qPCR.

(3) IWR-1 affects induction of HB

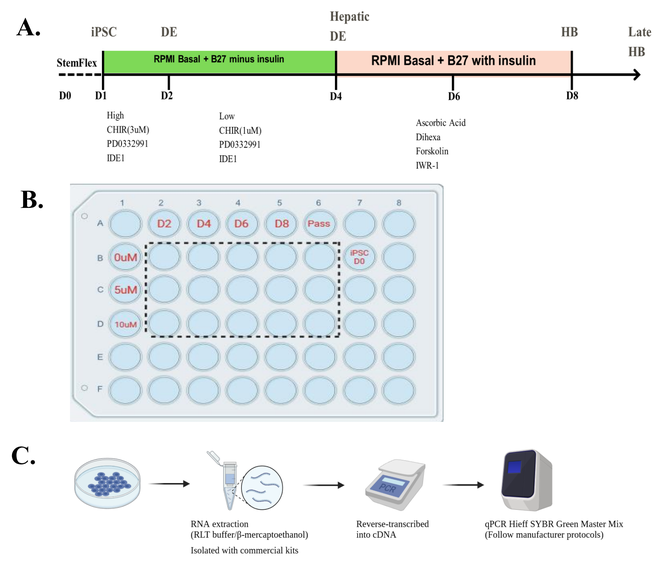

Figure 3: (A) Flow diagram and materials for differentiating HB by using the RUES2 cell line hiPSCs (B) Seeding 75K of RUES2 hiPSCs in a 48-well plate and IDE-1 was added individually at concentrations of 0 μM, 5 μM, and 10 μM. (C) Flow diagram of RNA extraction, purification, and RT-qPCR.

Results

(1) Established Protocol

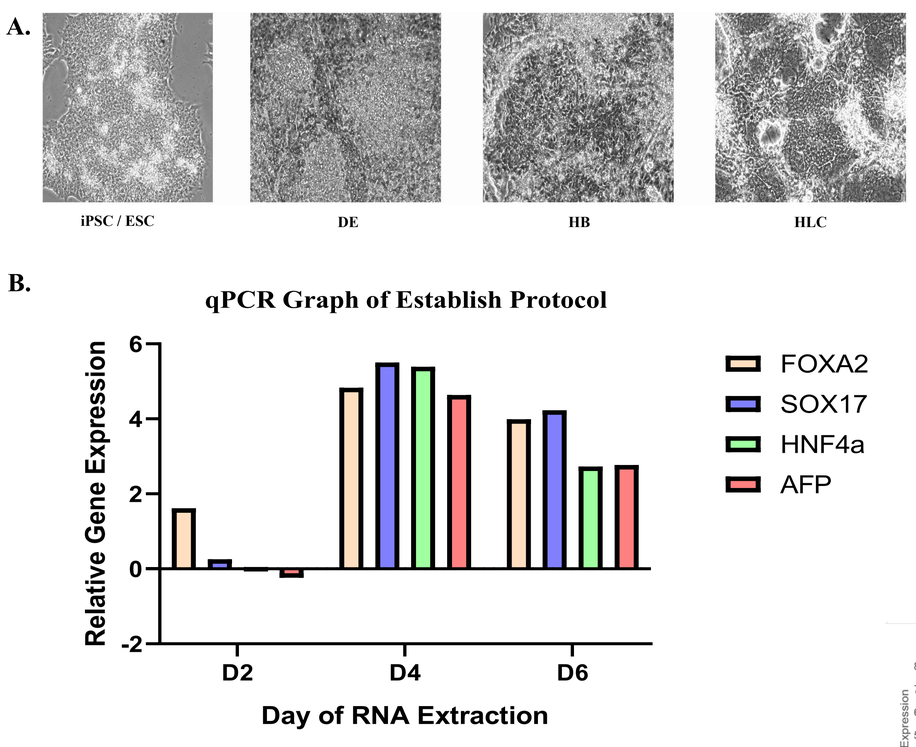

Figure 1: (A) Morphology of differentiating DE, HB, and HLC using the RUES2 cell line hiPSCs (B) qPCR relative gene expression graph.

(2) Changing cell seeding density

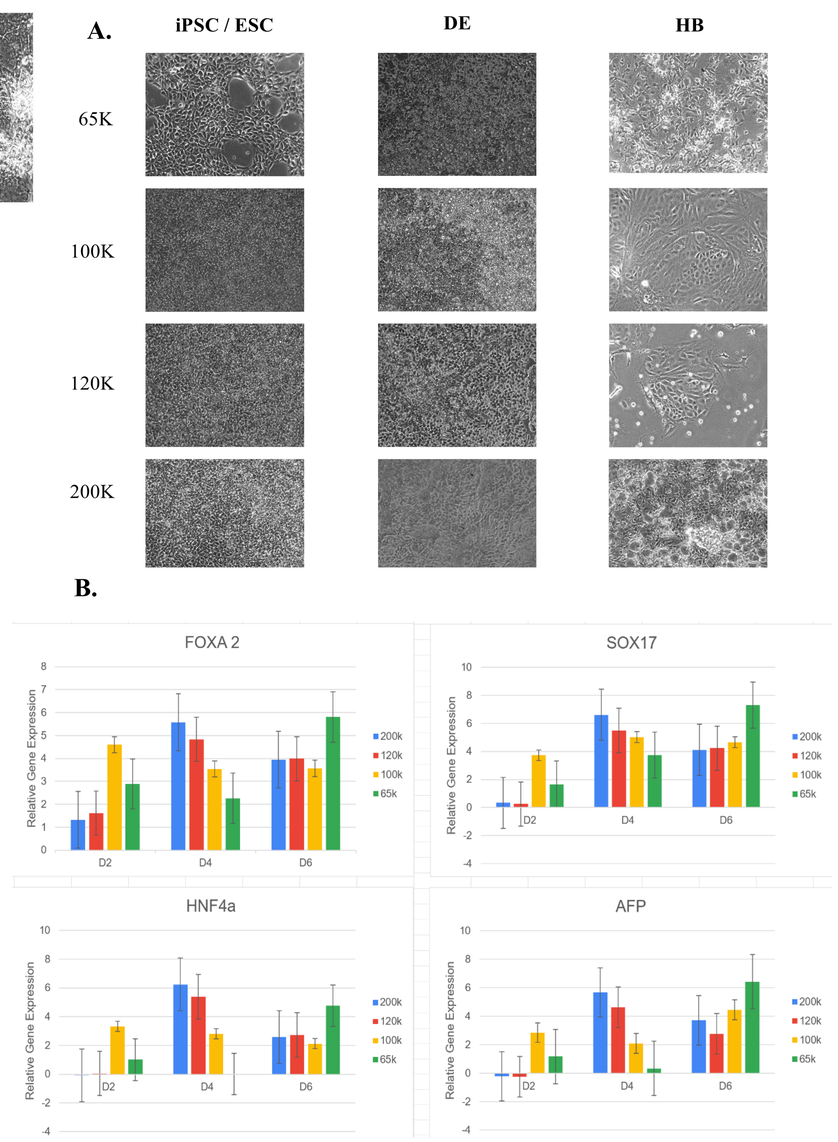

Figure 2: (A) Morphology of differentiating DE, HB, and HLC using the RUES2 cell line hiPSCs (B) qPCR relative gene expression graph.

(3) IWR-1 affects induction of HB

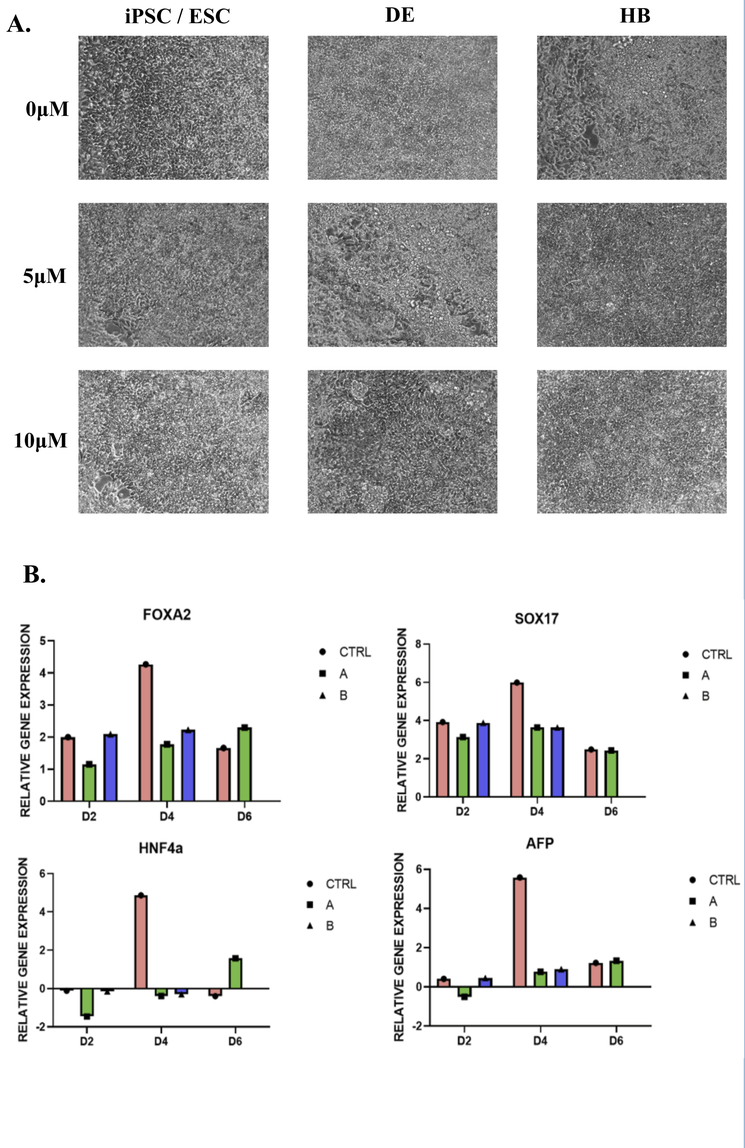

Figure 3: (A) Morphology of differentiating DE, HB, and HLC using the RUES2 cell line hiPSCs (B) qPCR relative gene expression graph.

Future Work

Future work will focus on identifying conditions that increase the yield of definitive endoderm (DE) cells to enhance the efficiency of hepatic differentiation further.

These optimized parameters will be applied to the generation of hiPSC-derived liver organoids, with the long-term goal of establishing aging liver models. Such models could provide valuable insights into age-related hepatic dysfunction and support drug screening for age-associated liver diseases.

Conclusion

Our study identified two key parameters significantly enhancing the hepatic differentiation of RUES2 hiPSCs in a 48-well plate. First, seeding densities between 100K and 120K cells per well yielded the most consistent differentiation outcomes, as confirmed by cell morphology and hepatic marker expression.

Second, adding 5μM IWR-1 on Day 6 notably increased HNF4α gene expression, suggesting a critical window for Wnt pathway modulation. These findings provide a refined protocol for improving differentiation efficiency and functional maturation of hiPSC-derived hepatic cells.

References

1. Mallanna SK, Duncan SA. Differentiation of hepatocytes from pluripotent stem cells. Curr Protoc Stem Cell Biol. 2013 Sep 20;26:1G.4.1-1G.4.13. doi: 10.1002/9780470151808.sc01g04s26. PMID: 24510789; PMCID: PMC3920294.
2. Gupta S, Sharma A, Rajakannu M, Bisevac J, Rela M, Verma RS. Small Molecule-Mediated Stage-Specific Reprogramming of MSCs to Hepatocyte-Like Cells and Hepatic Tissue for Liver Injury Treatment. Stem Cell Rev Rep. 2024 Nov;20(8):2215-2235. doi: 10.1007/s12015-024-10771-x. Epub 2024 Sep 11. PMID: 39259445; PMCID: PMC11554881.