Hematopoietic Development of iPSC Derived Human Liver Organoids

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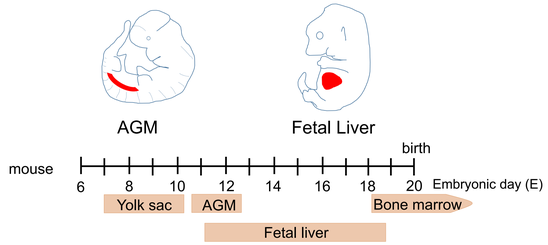
Background

The liver is one of the largest organs, which allows for essential metabolic, excretory, and endocrine functions. In addition to producing bile, the liver functions include breaking down dietary compounds, detoxification, regulation of glucose levels, and control of blood homeostasis by secreting serum proteins, such as Albumin. Hepatocytes account for approximately 70% of the mass of the adult organ. During development, definitive hematopoietic stem cells (HSCs) first appear in the aorta/gonad/mesonephros (AGM) region, and later migrate to the fetal liver prior to undergoing hematopoiesis in the bone marrow. Hemangioblasts are the primary common precursors for hematopoietic and endothelial cells. With the successful cultivation of functional human liver organoids via induced pluripotent stem cells (iPSCs), we give rise to a system that allows for simultaneous differentiation of both hematopoietic and hepatic cells similar to what is seen during development. This innovative model will potentially address the severe donor organ shortage and lower the high medical costs incurred by the increasing numbers of waiting patients.

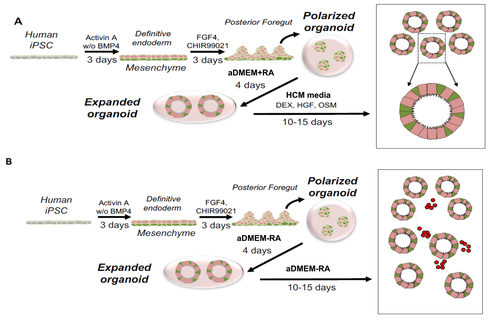


Aim

- **The creation of a novel culture system containing both hepatic and hematopoietic lineage cells to model developing fetal liver**

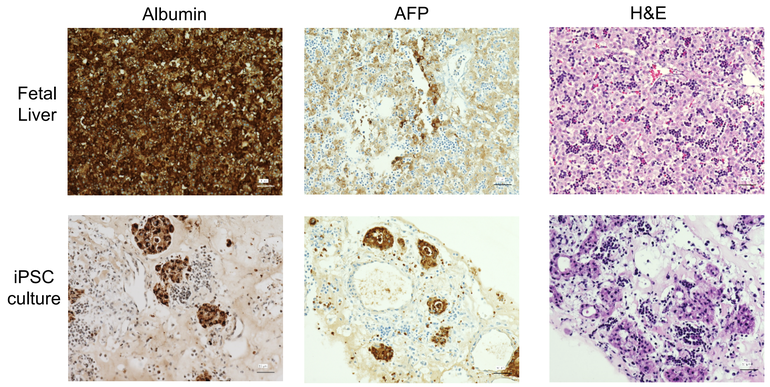
Methods

Human iPSCs (TkDA cell-line) were differentiated on laminin coated plates into endoderm by treatment of Activin and BMP, then treated with FGF4 and CHIR to further differentiate into posterior foregut. The cells were embedded into Matrigel droplets and cultured in Advanced DMEM. Droplet media was collected for ELISA to measure Albumin concentrations. The droplets were collected for histology and RNA isolation to test for AFP, ALB, and HBG1 genes.



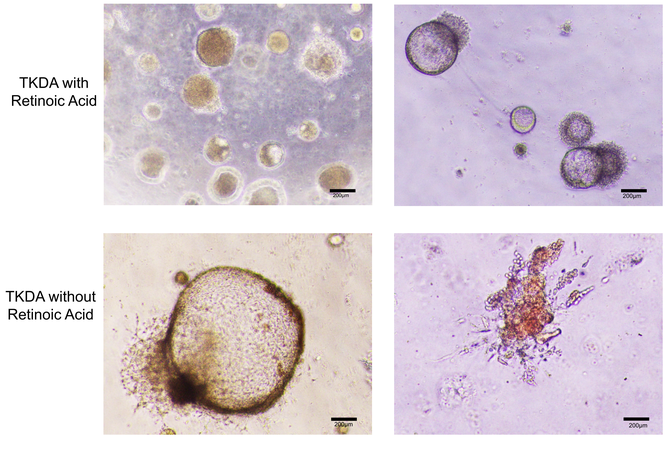
Results

iPSC liver organoid culture resembles fetal liver histologically

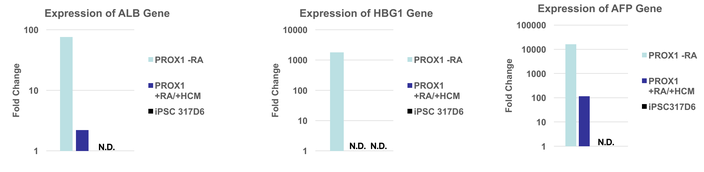


Comparison of TKDA iPSC Differentiation Using Different Protocols

The original protocol (A) calls for addition of Retinoic Acid the media. The amended protocol (B) used here does not include Retinoic Acid in the media and allows for red blood cell formation.



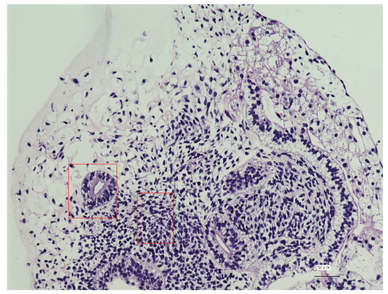
RNA Isolation (Day 19)

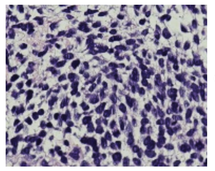


- *Alpha-fetoprotein (AFP) is found in early fetal liver development*
- *Albumin (ALB) is the primary protein in adult liver*
- *Hemoglobin-gamma I (HBG1) is expressed in the fetal liver during development*

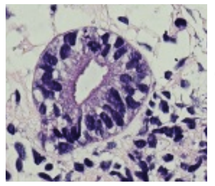
Results

H&E



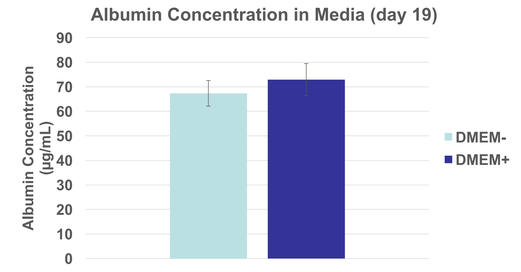


Blood cells surrounding organoid with an abundance in nuclei.



Organoid with hepatocytes showing greater amounts of cytoplasm.

Albumin ELISA



While removal of retinoic acid allows for blood cell formation, it does not significantly decrease the hepatic functions as measured here by albumin production.

Conclusions and future perspectives

This new model has given rise to a system that mirrors the development of a fetal liver, allowing for an environment that favors the growth of a mixed population of cells. By using iPSCs created from the patient's own cells, the risks of liver inflammation and drug toxicity are decreased and immunosuppressive therapy is no longer required, thus lowering the high medical costs incurred. The development of multipotent hematopoietic stem cells will serve to aid patients in need of blood transfusions when in need, as well as bone marrow transplants to potentially treat diseases like leukemia, aplastic anemia, and other immune system or genetic diseases.

Estimated U.S. Average 2017 Billed Charges Per Transplant

Transplant	30 Days Pre-Transplant	Procurement	Hospital Transplant Admission	Physician During Transplant Admission	180 Days Post-Transplant Discharge	OP Immuno-Suppressants & Other RX	Total
Bone Marrow-Autologous	$61,500	$15,300	$226,300	$10,700	$81,300	$14,500	$409,600
Liver	$41,400	$94,100	$463,200	$56,100	$126,900	$30,800	$812,500

http://www.milliman.com/uploadedFiles/insight/2017/2017-Transplant-Report.pdf

Acknowledgements

- CCHMC Research Pathology Core
- Funding Source: New York Stem Cell Foundation (Takanori Takebe)