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iPSC-Derived Cell Types Relevant to Atherothrombosis

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Modeling Inborn Errors of Hepatic Metabolism Using Induced Pluripotent Stem Cells

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Abstract—Inborn errors of hepatic metabolism are because of deficiencies commonly within a single enzyme as a consequence of heritable mutations in the genome. Individually such diseases are rare, but collectively they are common. Advances in genome-wide association studies and DNA sequencing have helped researchers identify the underlying genetic basis of such diseases. Unfortunately, cellular and animal models that accurately recapitulate these inborn errors of hepatic metabolism in the laboratory have been lacking. Recently, investigators have exploited molecular techniques to generate induced pluripotent stem cells from patients' somatic cells. Induced pluripotent stem cells can differentiate into a wide variety of cell types, including hepatocytes, thereby offering an innovative approach to unravel the mechanisms underlying inborn errors of hepatic metabolism. Moreover, such cell models could potentially provide a platform for the discovery of therapeutics. In this mini-review, we present a brief overview of the state-of-the-art in using pluripotent stem cells for such studies.

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Key Words: embryonic stem cells ■ genome-wide association study ■ hypercholesterolemia type II ■ liver ■ mutation

Inborn Errors of Hepatic Metabolism

Inborn errors of hepatic metabolism are a class of heterogeneous, rare diseases that affect the activity of the liver. Most commonly, they are caused by mutations in a single enzyme or transport protein that has a crucial role in one of the many metabolic processes that are performed by hepatocytes.[1] The outcome of such mutations depends on the pathway affected; however, most lie within 2 broad categories. In 1 category, the mutations result in structural damage to the liver and some cases also impact peripheral tissues. In the second group, mutations affect a pathway in the liver, yet the liver itself is relatively healthy while peripheral organs are affected as a secondary consequence.[1]

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The estimated incidence of inborn errors of hepatic metabolism is 1:1000, and they contribute significantly to the requirement for liver transplantation particularly in children.[2,3] The shortage of donor livers necessitates a need for alternative therapies. Some alternatives to liver transplantation have been suggested. Proposed treatments include cell transplant therapy, including transplantation of healthy hepatocytes,[4] ex vivo gene-corrected hepatocytes,[5] or stem cell–derived hepatocytes.[6–8] Gene therapy is also a possibility, and despite technical challenges, several new approaches seem promising.[9,10]

Pluripotent Stem Cells as a Powerful Tool for Disease Modeling

Human induced pluripotent stem cells (iPSCs) have emerged as a powerful tool for modeling diseases with a genetic basis (Figure). The concept is that somatic cells can be reprogrammed into cells that resemble embryonic stem cells by forced expression of proteins that regulate the pluripotent state. The earliest reports of reprogramming used the transcription factors Oct4, Sox2, Klf4, and c-Myc.[11,12] Once pluripotent stem cells are available, they can be induced to differentiate into the cell type of choice, most commonly by the sequential addition of growth factors that mimic embryonic development. Several investigators have defined protocols that can generate cells with hepatocyte characteristics from pluripotent stem cells.[13–20] When iPSCs are generated from patients with an inborn error of hepatic metabolism, investigators can, therefore, use the iPSCs as a source of hepatocyte-like cells to model the patient's liver disease in culture.

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Nonstandard Abbreviations and Acronyms	
AAT	α-1-antitrypsin
ATD	α-1-antitrypsin deficiency
iPSC	induced pluripotent stem cell

The generation of iPSCs from patients has become routine, and a growing list of genetic diseases that affect a diverse array of cell types have been successfully modeled.[21–24] This list includes several inborn errors of hepatic function. Liver diseases that have been successfully modeled using an iPSC approach include α-1-antitrypsin deficiency (ATD),[8,25–28] glycogen storage disease,[28,29] tyrosinemia type I,[28,29] familial hypercholesterolemia,[6,28,30] Tangier disease,[31] Alpers disease,[32] Crigler–Najjar syndrome,[29] and Wilson disease[7] (Table). Interestingly, patients with ATD show a variation in the extent of liver disease associated with the mutation. Tafaleng et al[26] demonstrated that aspects of this variation could be recapitulated in iPSC-derived hepatocytes from different patients with ATD. Moreover, transcriptome analyses of iPSC-derived hepatocytes from a large cohort of patients with ATD revealed changes in expression of clusters of genes that could provide insight into the pathophysiology of ATD.[27] These findings imply that iPSCs could be used to predict the severity of disease and help physicians deliver appropriate therapeutic strategies.[26,27] In addition to hepatocytes, cells with cholangiocyte characteristics have also been produced from iPSCs, and this advance has allowed researchers to model Alagille syndrome, cystic fibrosis, and polycystic liver disease.[34,35]

Using iPSCs derived from patients has advanced our ability to model liver disease in culture; however, the fact that individual inborn errors of hepatic metabolism are exceedingly rare (Table) means that having access to patients becomes a limiting factor. Recently, new techniques in genome engineering allow the introduction of specific mutations into the genome of iPSCs.[36] Transcription activator-like effector nucleases, zinc finger nucleases, and CRISPR/Cas9 can all be exploited to create insertions and deletions (indels) at defined genomic locations as a consequence of double-strand break repair. Moreover, the introduction of double-strand breaks by any method can be coupled with homology-directed recombination using DNA fragments or single-stranded DNA oligonucleotides to introduce known allelic variations into specific sites in the genome. The consequence of these technological advances means that specific nucleotide changes can be engineered into any iPSC line to recapitulate the mutations causing any inborn error of hepatic metabolism no matter how rare.[37]

Several examples of the power of genome engineering in iPSCs to advance our understanding and potential treatment of liver disease exist. Transcription activator-like effector nucleases were used to correct a mutation in the *NPC1* gene in iPSCs from a patient with a Niemann–Pick type C disease.[33] Similarly, transcription activator-like effector nucleases and zinc fingers were used to correct a mutation in iPSCs from an AAT (α-1-antitrypsin) deficiency patient and hepatocyte-like cells derived from gene-corrected iPSCs prevented the abnormal accumulation of misfolded AAT protein.[8,25] Finally, 1 study used genome editing in pluripotent stem cells to mutate 15 different genes that were associated with a variety of diseases, including dyslipidemia, insulin resistance, hypoglycemia, lipodystrophy, and hepatitis C infection.[38] One of the mutations targeted the *SORT1* gene (encoding sortilin) that was thought to regulate the level of low-density lipoprotein (LDL) cholesterol in the blood, based on genome-wide association studies.[39] When the *SORT1*$^{-/-}$ iPSCs were differentiated to hepatocyte-like cells, they were found to dramatically elevate the production of apolipoprotein B protein, which is the central component of LDL cholesterol.[40]

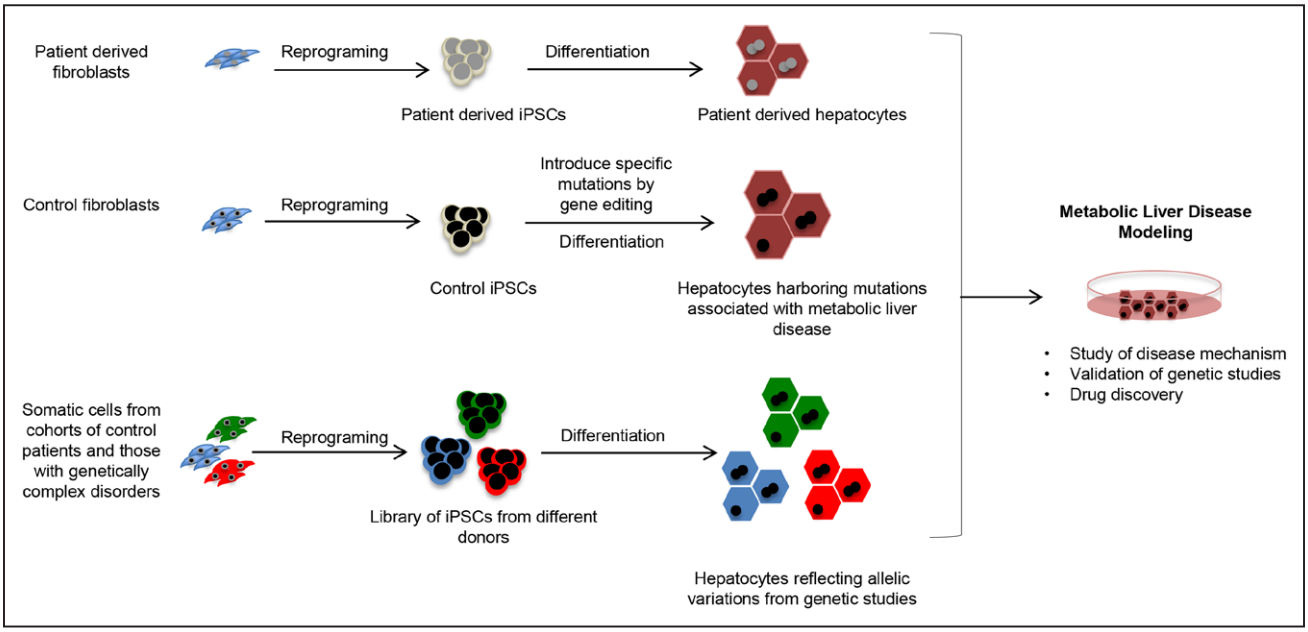

Figure. Human pluripotent stem cells as a model for metabolic liver disease. iPSC indicates induced pluripotent stem cell.

Table. Inherited Liver Diseases Modeled With iPSCs Technology

Liver Diseases	Incidence	Pathophysiology	Defective Protein	iPS-Based Disease Modeling	Ref
Alpha-1-antitrypsin deficiency (A1ATD)	Approximately 1:1600 to 1:2000 in the white population	Increasing circulating level of A1ATD, neonatal cholestasis, liver failure	Single gene defect, mutation in A1AT	Patient-derived iPSCs differentiated to hepatocytes	8,25–28
Glycogen storage diseases (GSD1A and GSD1B)	1:100 000	Hypoglycemia, neurological defect	G6PC, glucose-6-phosphatase catalytic subunit (GSD1A)	Patient-derived iPSCs differentiated to hepatocytes	28,29
			SLC37A4, glucose-6-phosphate transport protein 1 (GSD1B)		
Tangier disease, familial α-lipoprotein deficiency	50 cases worldwide	Severely reduced high-density lipoprotein	ATP-binding cassette transporter A1	Patient-derived iPSCs differentiated to hepatocytes	31
Tyrosinemia type I	1:100 000 to 1:120 000	Accumulation of toxic compound, hepatocyte and renal tubular cell death	Fumarylacetoacetate hydrolase	Patient-derived iPSC differentiated to hepatocytes	28,29
Familial hypercholesterolemia (FH)	HeFH (1:200 to 1:250)	High level of circulating LDL, coronary artery disease, and myocardial infarction	LDL receptor	Patient-derived iPSC differentiated to hepatocytes	6,28,30
	HoFH (1:160 000 to 1:250 000)				
Wilson disease	1:30 000	Accumulation of too much copper in liver, brain, and some other vital organs	ATP7B, copper-transporting ATPase 2	Patient-derived iPSCs differentiated to hepatocytes	7
Alper disease	1:1000	Neurodegenerative disease with occasional liver failure	POLG	Patient-derived iPSCs differentiated to hepatocytes	32
Crigler–Najjar syndrome	1:1000 000	Hyperbilirubinemia	UDP-glucuronosyltransferase 1-A	Patient-derived iPSCs differentiated to hepatocytes	29
Niemann–Pick type C	1:150 000	Lysosomal storage disease	Progressive neurological disease, occasional hepatomegaly	TALEN-mutated iPSCs	33

HeFH indicates heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; iPSC, induced pluripotent stem cell; LDL low-density lipoprotein; and POLG, DNA polymerase gamma.

Using iPSC-Derived Hepatocytes to Model Complex Genetic Variations Associated With Liver Disease

Genetic studies using large cohorts of patients have identified genetic alterations linked to various diseases. Differences in genomic sequence revealed through genome-wide association studies and quantitative trait locus analyses have been found to contribute to many polygenic diseases. Despite advances in genetic techniques, establishing the functional consequence of mutations remains challenging. The Next Generation Genetic Association Studies Consortium was therefore formed to determine whether iPSCs could be used to investigate the impact of genetic variation on cell function. This effort required the generation of thousands of iPSC lines from control patients and those with genetically complex disorders.[41,42] Two of the groups within the consortium focused on using iPSC-derived hepatocytes.[41,43] Both groups established that hepatocytes produced from iPSCs could successfully address the role of genetic variations on the regulation of blood lipid levels. Warren et al[41] demonstrated that the 1p13 rs12740374 single nucleotide polymorphism affected the expression of key genes that control lipid accumulation, and Pashos et al[43] identified the functional impact of numerous variants on lipid levels. Although working with scores of iPSC lines is challenging, these studies validate that complex phenotypes can be probed in vitro and highlight the advantages of having indefatigable access to target cell types.

Using iPSCs as a Platform to Identify Pharmaceuticals for the Treatment of Inborn Errors of Hepatic Metabolism

Researchers' ability to generate iPSCs from patients with inborn errors of hepatic metabolism and to use them to produce cells with hepatocyte characteristics in culture offers the possibility of using the cells for drug discovery.[44]

Choi et al[25] exploited iPSC-derived hepatocytes from a patient with ATD to screen a library of drugs that could potentially have off-label benefits for the treatment of ATD. In this study, they identified drugs that could promote autophagy that enhanced clearance of the misfolded AAT protein from the hepatocytes.[25] The researchers screened the ≈3000

compounds from the Johns Hopkins Drug Library and identified 5 that reduced the accumulation of misfolded AAT by iPSC-derived hepatocytes. Of these drugs, carbamazepine that is used to treat epilepsy has been shown to clear misfolded AAT protein in both mice and iPSC-derived hepatocytes by autophagy.[45]

Recently, Cayo et al[30] used iPSC-derived hepatocytes to model homozygous familial hypercholesterolemia. Hepatocytes with compound heterozygous mutations in the LDL receptor revealed a block in LDL uptake, an inability to respond to statin treatment, and elevated apolipoprotein B levels in the medium of the familial hypercholesterolemia cells compared with controls.[30] A screen of a 2500 compound drug library revealed that cardiac glycosides, such as digoxin, were capable of reducing the level of apolipoprotein B in the medium of cultured familial hypercholesterolemia iPSC-derived hepatocytes.[46] Cardiac glycosides are used to treat heart failure by inhibiting the sodium–potassium pump and have been used for >200 years.[47] Importantly, analyses of patient electronic medical records and mice with humanized livers revealed that digoxin could also lower LDL produced by human livers. These findings demonstrated that iPSCs could be used as an efficient platform to discover novel therapies for inborn errors of hepatic metabolism that could be used effectively in humans.

Closing Remarks

The efficiency of using CRISPR/Cas9 coupled with homology-directed repair is likely to substantially increase as new approaches are described, and existing techniques are optimized.[37] With advances in next-generation sequencing, new allelic variations of uncertain significance are being identified that correlate with liver disease. Combining genome editing with the generation of hepatocyte-like cells from iPSCs could provide models to test the functional significance of such variants. Also, the availability of large cohorts of patient-derived iPSCs could advance our understanding of complex polygenic liver diseases. Although the potential of using iPSCs as a culture model of rare liver diseases is exciting, some caveats and limitations must be acknowledged. Most iPSC lines are clonal, and as such, each line may have specific characteristics associated with epigenetic and genetic alterations that potentially affect differentiation.[48] Clonal variability in differentiation potential is not restricted to iPSCs and has also been reported for embryonic stem cells.[49] Such variability can confound interpretation especially if the impact of a mutation on cell function is subtle. Under such circumstances, the inclusion of appropriate controls and potentially the use of multiple iPSC lines are essential. It is also important to realize that hepatocyte function varies depending on its relative position within the liver lobule.[50] Although the production of hepatocyte-like cells is highly efficient, the resulting cells are not identical to hepatocytes that are freshly isolated from a donor liver.[16,43,51] Differences include the expression of CYP450 enzymes, which could impact the identification of drugs that require metabolic activation and could also complicate drug toxicity studies. Efforts to improve the generation of fully functional hepatocytes from iPSCs include the differentiation of cells using small molecules,[52] culture as organoids and in other 3-dimensional formats,[53] coculture with supporting cells,[54] differentiation of cells on advanced matrices,[55] and purification of mature hepatocyte using cell surface markers.[56] Although the full power of using genome editing and pluripotent stem cells to probe the mechanisms underlying inborn errors in hepatic metabolism is in its infancy, it has already begun to yield valuable insights.

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Highlights

- Hepatocytes from pluripotent stem cells provide a platform for modeling liver disease.
- Induced pluripotent stem cell–derived hepatocytes can also model complex genetic variations associated with liver disease.
- Induced pluripotent stem cells provide a platform to identify pharmaceuticals for the treatment of inborn errors of hepatic metabolism.

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