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REVIEW

Hepatocyte-like cells differentiated from human induced pluripotent stem cells: Relevance to cellular therapies

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Abstract Maturation of induced pluripotent stem cells (hiPSCs) to hepatocyte-like cells (HLCs) has been proposed to address the shortage of human hepatocytes for therapeutic applications. The purpose of this study was to evaluate hiPSCs, HLCs and hepatocytes, all of human origin, in terms of performance metrics of relevance to cell therapies. hiPSCs were differentiated to HLCs *in vitro* using an established four-stage approach. We observed that hiPSCs had low oxygen consumption and possessed small, immature mitochondria located around the nucleus. With maturation to HLCs, mitochondria showed characteristic changes in morphology, ultrastructure, and gene expression. These changes in mitochondria included elongated morphology, swollen cristae, dense matrices, cytoplasmic migration, increased expression of mitochondrial DNA transcription and replication-related genes, and increased oxygen consumption. Following differentiation, HLCs expressed characteristic hepatocyte proteins including albumin and hepatocyte nuclear factor 4-alpha, and intrinsic functions including cytochrome P450 metabolism. But HLCs also expressed high levels of alpha fetoprotein, suggesting a persistent immature phenotype or inability to turn off early stage genes.

Abbreviations: AFP, alpha fetoprotein; Alb, albumin; ATP5g1, mitochondrial F0 complex, subunit C1 (subunit 9), ATP synthase 5 subunit e; ATP8, ATP synthase 8; BAL, bioartificial liver; BMP4, bone morphogenetic protein 4; CYP, cytochrome P450; CYP2C19, CYP Family 2, Subfamily C, polypeptide 19; CYP3A4, CYP Family 3, Subfamily A, polypeptide 4; ESC, embryonic stem cell; FGF2, fibroblast growth factor 2; GATA4, GATA-binding protein 4; HCF, human cardiac fibroblast; HGF, hepatocyte growth factor; hiPSC, human induced pluripotent stem cell; HLC, hepatocyte-like cell; HNF4α, hepatocyte nuclear factor 4-alpha; iPSC, induced pluripotent stem cell; MtDNA, mitochondrial DNA; ND1, NADH dehydrogenase subunit 1; ND5, NADH dehydrogenase subunit 5; OCT4, octamer-binding transcription factor 4; PAS, Periodic Acid-Schiff; QRT-PCR, Quantitative Reverse Transcription Polymerase Chain Reaction; QPCR, Quantitative Polymerase Chain Reaction; TEM, transmission electron microscopy.

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Furthermore, the levels of albumin production, urea production, cytochrome P450 activity, and mitochondrial function of HLCs were significantly lower than primary human hepatocytes.
Conclusion- hiPSCs offer an unlimited source of human HLCs. However, reduced functionality of HLCs compared to primary human hepatocytes limits their usefulness in clinical practice. Novel techniques are needed to complete differentiation of hiPSCs to mature hepatocytes.

Contents

Introduction

Liver transplantation is a successful treatment for patients with end-stage liver disease. However, transplantable donor livers are in short supply. Hepatocyte transplantation and bioartificial liver (BAL) devices have been proposed as therapeutic alternatives to the shortage of transplantable livers. BAL is an extracorporeal supportive therapy developed to bridge patients with liver failure to liver transplantation or to recovery of the native liver. Hepatocyte transplantation is best suited for patients with metabolic liver disease for which smaller number of cells (<10% of liver mass) may be curative. Both BAL and hepatocyte transplantation are cellular therapies that avoid use of a whole liver.

Though once controversial, it is now well accepted that hepatocytes can be derived from progenitor cells which include pluripotent stem cells, either embryonic or native to the liver or in blood (Basma et al., 2009; Wang et al., 2011). Furthermore, techniques now exist for production of human induced pluripotent stem cells (hiPSCs) from somatic cells (Yu et al., 2007). Therefore, in theory, hiPSCs could provide an unlimited source of human hepatocytes for BAL therapy and cell transplantation. Preliminary reports indicate that human hepatocyte-like cells (HLCs) can be derived from hiPSCs under *in vitro* conditions (Si-Tayeb et al., 2010). These findings are exciting since they suggest the possibility of producing HLCs from the patient's own cells and cell transplantation without immunosuppression.

The HLCs derived from hiPSCs express characteristic hepatocyte proteins including alpha-1-antitrypsin, albumin (Alb), and hepatocyte nuclear factor 4-alpha (HNF4α). They also display intrinsic hepatocyte functions including cytochrome P450 (CYP) metabolism. The efficiency of induced pluripotent stem cells (iPSCs) directed-differentiation into HLCs is variable. Some protocols describe over 80% differentiation efficiency, but none yet achieve complete differentiation of hiPSCs into hepatocytes. Transplantation of undifferentiated iPSCs in immunodeficient recipients results in the formation of teratomas. However, the risks and benefits of transplantation of iPSCs into immunocompetent recipients are poorly studied. Reports of BAL therapy using HLCs derived from hiPSCs do not yet exist.