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Research review paper

Pluripotent stem cell-derived hepatocyte-like cells

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ABSTRACT

Liver disease is an important clinical problem, impacting over 30 million Americans and over 600 million people worldwide. It is the 12th leading cause of death in the United States and the 16th worldwide. Due to a paucity of donor organs, several thousand Americans die yearly while waiting for liver transplantation. Unfortunately, alternative tissue sources such as fetal hepatocytes and hepatic cell lines are unreliable, difficult to reproduce, and do not fully recapitulate hepatocyte phenotype and functions. As a consequence, alternative cell sources that do not have these limitations have been sought. Human embryonic stem (hES) cell- and induced pluripotent stem (iPS) cell-derived hepatocyte-like cells may enable cell based therapeutics, the study of the mechanisms of human disease and human development, and provide a platform for screening the efficacy and toxicity of pharmaceuticals. iPS cells can be differentiated in a step-wise fashion with high efficiency and reproducibility into hepatocyte-like cells that exhibit morphologic and phenotypic characteristics of hepatocytes. In addition, iPS-derived hepatocyte-like cells (iHLCs) possess some functional hepatic activity as they secrete urea, alpha-1-antitrypsin, and albumin. However, the combined phenotypic and functional traits exhibited by iHLCs resemble a relatively immature hepatic phenotype that more closely resembles that of fetal hepatocytes rather than adult hepatocytes. Specifically, iHLCs express fetal markers such as alpha-fetoprotein and lack key mature hepatocyte functions, as reflected by drastically reduced activity (~0.1%) of important detoxification enzymes (i.e. CYP2A6, CYP3A4). These key differences between iHLCs and primary adult human hepatocytes have limited the use of stem cells as a renewable source of functional adult hepatocytes for in vitro and in vivo applications. Unfortunately, the developmental pathways that control hepatocyte maturation from a fetal into an adult hepatocyte are poorly understood, which has hampered the field in its efforts to induce further maturation of iPS-derived hepatic lineage cells. This review analyzes recent developments in the derivation of hepatocyte-like cells, and proposes important points to consider and assays to perform during their characterization. In the future, we envision that iHLCs will be used as in vitro models of human disease, and in the longer term, provide an alternative cell source for drug testing and clinical therapy.

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1. Introduction

Chronic liver disease is a significant cause of morbidity and mortality, impacting over 600 million people worldwide (Gonzalez and Keeffe, 2011). As a result, the number of people living with end stage liver disease is increasing, and over 1 million people die each year from acute and chronic liver disease across the globe (Gonzalez and Keeffe, 2011). Liver transplantation is currently the only definitive and curative treatment for acute and chronic liver failure (Starzl and Fung, 2010). First accomplished in 1967 by Thomas Starzl, liver transplantation has been an unquestioned clinical success; however, the demand for liver transplantation has significantly outstripped the supply of donor organs (Perera et al., 2009; Starzl and Fung, 2010; Starzl et al., 1968). As a consequence, multiple attempts to expand the availability of donor organs have been employed: opt-out organ donation programs, the use of suboptimal donor organs (deceased cardiac donors or steatotic (fatty) livers), split donor transplantation, and living donor liver transplantation (Perera et al., 2009).

The search for alternatives to whole organ transplantation has been focused on expanding the availability of replacement liver tissue, such as developing cell-based therapies that include hepatocyte transplantation, engineered hepatic tissue constructs, and the bioartificial liver (Chen et al., 2011; Dhawan et al., 2010; Fox and Roy-Chowdhury, 2004; Fox et al., 1998; Nyberg et al., 1993). In particular, hepatocyte transplantation has been performed clinically for more than 15 years, primarily in the setting of acute liver failure and inherited liver metabolic disorders. A general problem facing hepatocyte transplantation is the limited repopulation capacity of engrafted cells, although in the case of some metabolic disorders, replacement of just 2–5% of the liver parenchyma with normal hepatocytes may be sufficient to improve liver function significantly. For example, Fox et al. reported the successful treatment of a 10-year-old with one such metabolic disorder, termed Crigler–Najjar disease, who was experiencing recurrent episodes of brain injury resulting from elevated bilirubin. The patient was shown to respond well to infusion of 7.5×10^9 hepatocytes, based on an improvement in metabolic function and reduced need for phototherapy (Fox et al., 1998). However, hepatocyte transplantation has not been widely adopted, due to a variety of technical reasons including the inability to monitor graft health and frequent signs of rejection (Dhawan et al., 2010). Moreover, these clinical treatments require scarce human liver tissue as a cell source of the transplanted hepatocytes.

Based on the apparent success of hepatocyte transplantation combined with the challenges in sourcing appropriate donor cells, a strong focus has been placed on developing a safe and reliable method to expand the small number of available human hepatocytes. Indeed, the liver has been known for its capacity to regenerate since antiquity, as depicted by the story of Prometheus. Modern studies have shown that in vivo, human hepatocytes are capable of cellular proliferation based on the observed replacement of damaged hepatocytes following injury, or even during the daily turnover of the liver (Michalopoulos, 2007). However, in vitro, researchers have been unable to induce and/or support the cellular proliferation of human hepatocytes; rather, attempts to culture human hepatocytes have led to the loss of differentiated function rather than any increase in cell number (Castell et al., 2006; Kobayashi et al., 2000). Consequently, attempts to expand adult human hepatocytes have historically been unsuccessful as a target approach to achieving cellular therapy of the liver, although alternatives are under active investigation, including our recent screen that identified small molecules that support up to 10-fold expansion of adult human hepatocytes in vitro (Shan et al., 2013). Other approaches include utilizing cell lines derived from hepatocellular carcinoma, or generated through SV40 or Large T antigen transformation, both of which have enabled the expansion and creation of in vitro model systems (Ito et al., 2009). However, these cell lines poorly recapitulate primary hepatocyte functions such as detoxification enzyme activities and thus show poor prediction of clinical outcomes such as drug toxicity(Gerets et al., 2012; Wilkening et al., 2003).

2. Hepatocyte differentiation from pluripotent stem cells

Several alternative sources have been proposed as options to circumvent the limited supply of human hepatocytes, including using human fetal tissue or even xenogeneic material, but both paths have been sidelined due to a variety of ethical, sourcing, and safety issues (Yu et al., 2012). While still prone to some ethical and safety challenges, pluripotent stem cell-based therapies overcome many of the drawbacks that challenge other cell lines and fetal tissue, and thus are considered by many as an ideal alternative source of human hepatocytes (Dalgetty et al., 2009; Espejel et al., 2010). Human pluripotent stem cells include embryonic stem (hES) cells, first isolated from human blastocysts by James Thomson and colleagues (Thomson et al., 1998), as well as the more recently described induced pluripotent stem (iPS) cells first generated by Yamanaka and colleagues following the forced expression of a panel of transcription factors in adult-derived cells (Takahashi and Yamanaka, 2006; Takahashi et al., 2007). These cell lines are defined as pluripotent in that they can self-renew in culture, maintain genetic stability, and differentiate into cell lineages of all three germ layers including endodermal hepatocyte-like cells (HLCs) (Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Thomson et al., 1998). Importantly, iPS cells can be derived from adult tissue in a reliable manner and have been shown to differentiate efficiently into hepatocyte-like cells (Si-Tayeb et al., 2010; Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Touboul et al., 2010). However, unlike relatively simple in vitro models designed to reproduce embryonic differentiation, in vivo development advances through a much more complex, structured and highly organized series of patterning and differentiation events in which cell–extracellular matrix and cell–cell interactions are tightly controlled and play an important role (Deutsch et al., 2001; Matsumoto et al., 2001; Wandzioch and Zaret, 2009; Zaret and Grompe, 2008). Consequently, the hepatocyte-like cells generated from pluripotent stem cells in culture exhibit many morphologic and phenotypic characteristics of primary adult human hepatocytes. However, the examination of their functional traits has been more limited, and many signs suggest that only partial differentiation has been attained, as discussed below.

Despite the challenges inherent in performing developmental studies in an in vitro setting, and the roadblocks that remain regarding the current capacity to treat patients with human hepatocytes from any available derivation source, the importance of being able to develop experimental models to study human disease states cannot be overstated. To date, many genome-wide association screens (GWAS) have identified a variety of genetic variants associated with human liver disease (Ott et al., 2011). However, many of these variants represent novel loci whose contribution to liver disease is entirely unknown. Linking GWAS findings to biologic mechanisms has been an ongoing challenge in the genetics community. In most studies, mouse models have been employed; however, the usefulness of mouse models is unclear given its low-throughput nature and the physiologic and metabolic

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