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Mini-review

Human induced pluripotent stem cells derived hepatocytes: rising promise for disease modeling, drug development and cell therapy

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ABSTRACT

Recent advances in the study of human hepatocytes derived from induced pluripotent stem cells (iPSC) represent new promises for liver disease study and drug discovery. Human hepatocytes or hepatocyte-like cells differentiated from iPSC recapitulate many functional properties of primary human hepatocytes and have been demonstrated as a powerful and efficient tool to model human liver metabolic diseases and facilitate drug development process. In this review, we summarize the recent progress in this field and discuss the future perspective of the application of human iPSC derived hepatocytes.

INTRODUCTION

Since the first discovery of induced pluripotent stem cells (iPSC) in 2006 (Takahashi and Yamanaka, 2006), the study of iPSC technology has advanced through several exciting moments: from mouse iPSC to human iPSC, from fibroblasts to a variety of cell origins, from retrovirus-driven iPSC to integration-free iPSC, and from healthy iPSC to patient-specific iPSC harboring disease-related genetic backgrounds (Robinton and Daley, 2012; Zhang et al., 2012). The generation of patient-specific iPSC together with directed differentiation provides an invaluable tool for modeling inherited human diseases *in vitro*, including many human liver syndromes.

The liver is the single largest gland in adult human body and performs both endocrine and exocrine functions. Liver is the organ in charge of many important life functions, including food digestion, glycogen storage, control of metabolism, drug detoxification and hormone production (Si-Tayeb et al., 2010a). Since liver is so vital to life, malfunction or failure of the organ often results in high rates of morbidity and mortality. Hepatocytes are the major parenchymal cells of human liver, accounting up to 70%–80% of total liver cell mass. While being the major structural units of liver body, hepatocytes are also the functional units for both liver metabolism and regeneration (Si-Tayeb et al., 2010a). Because of its tremendous potential of application in regenerative medicine and drug discovery, an unlimited source of functional hepatocytes is of great interest. Nevertheless, the current inability to culture and expand primary human hepatocytes *in vitro* with unimpaired physiological functions represents a major obstacle in the field. Directed cell differentiation from embryonic stem cells (ESC) provides a nice alternative to obtain hepatocytes *in vitro*. However, the utilization of human ESC in both research and clinic is embarrassed badly by ethical and legal issues. Therefore, the rise of human iPSC derived hepatocytes (iPSC-hepatocytes) presents a new hope for the liver study.

As sharing most common features to human ESC, human iPSC are proved to be a suitable source to regenerate hepatocytes, and a variety of strategies have been developed in the past few years (Busletta et al., 2011; Chistiakov and Chistiakov, 2012). Song et al. (2009) described the first successful hepatic differentiation of human iPSC, closely followed by several other reports (Liu et al., 2010; Rashid et al.,

2010; Si-Tayeb et al., 2010b; Sullivan et al., 2010). Similarly to the strategies developed in ESC differentiation, most reported studies of directed hepatic differentiation of human iPSC adopt multi-step protocols to mimic the liver embryogenesis by sequentially adding endodermal and hepatic inductive cytokines and small molecules. These methods commonly result in iPSC-hepatocytes appearing many key attributes of primary human hepatocytes. However, since to date none of the reports claimed the generation of cells which completely recapitulate all features of primary human hepatocytes, a term hepatocyte-like cells (HLC) is also commonly used to describe their identity. The human iPSC-hepatocytes exhibit similar gene expression profile as primary hepatocytes, secret hepatic functional proteins and metabolize chemicals *in vitro*. On the other hand, the iPSC-hepatocytes frequently secrete less albumin and exhibit lower levels of P450 enzyme activities compared to primary hepatocytes. Meanwhile, the persistent expression of alpha-fetoprotein (a fetal stage hepatocyte marker) implies an immature or fetal-stage status. More importantly, iPSC- hepatocytes have demonstrated a capacity to pass stringent *in vivo* test as engrafting and maturing in animal models, as an ideal proof for their identity and functionality (Si-Tayeb et al., 2010b; Chen et al., 2011). The advantages of using iPSC-hepatocytes over their ESC counterparts in both basic and clinical studies are obvious: (1) the generation of iPSC from patient bearing inherited pathological background provides a more objective platform for disease modeling and drug development; (2) the cell reprogramming using patient own cells makes iPSC-hepatocytes a preferred source for autologous cell therapy; (3) the somatic origin of iPSC avoids controversies surrounding the use of human embryonic cells and erases many ethical concerns in application. Taking together, the human iPSC-hepatocytes represent a promising tool in many aspects of research in liver disease and regenerative medicine.

HUMAN IPSC DERIVED HEPATOCYTES FOR DISEASE MODELING

One great advantage of iPSC technology is that it allows the generation of human cells in the context of specific genetic identity. Recently, a number of studies have reported the generation of patient-specific iPSC from individuals with inherited liver disease. Rashid et al. (2010) reported the first success in modeling inborn liver metabolic disorders using human iPSC. The authors obtained skin fibroblasts from patients diagnosed with α1-antitrypsin deficiency, familiar hypercholesterolemia and glycogen storage disease type 1a, which represent three different mechanisms of liver disease respectively: defective protein secretion, lack of cell surface receptor and lack of a critical intracellular enzyme (Greenbaum, 2010). Patient iPSC lines were established followed by a three-step hepatic differentiation protocol to generate dis-

ease-specific hepatocytes. Remarkably, the iPSC derived hepatocytes exhibited the characteristic abnormalities in culture which recapitulate key pathological features of the diseases (Greenbaum, 2010; Rashid et al., 2010). The significance of this report is profound as it is the first proof-of-principle study demonstrating the feasibility of modeling hepatic diseases in which the pathological defects are only seen in terminally differentiated adult hepatocytes (late-onset disease). Later on, Zhang et al. (2011) reported modeling of the Wilson's disease, another type of liver metabolic disease, using patient iPSC. The iPSC-hepatocytes differentiated from Wilson's disease patient iPSC demonstrated abnormal cytoplasmic localization of mutated ATP7B protein and defective copper transportation. Moreover, the authors showed that these functional defects could be reversed by lentivirus mediated gene therapy or chaperon drug treatment (Zhang et al., 2011). Interestingly, the first iPSC model of human liver infectious disease has also been reported recently. Schwartz and the colleagues reported that human iPSC-hepatocytes could support the entire life cycle of hepatitis C virus (HCV) including inflammatory responses to the viral infection (Schwartz et al., 2012). This study did not only offer a novel infectious disease model to study HCV pathogenesis, but also enabled future research regarding how host genetics could impact viral infection using personalized iPSC models.

Though tremendous efforts have been spent on generating iPSC from numerous human diseases, effective disease modeling is not always achievable since differentiated cells might fail to recapitulate the pathological defects *in vitro* (Robinton and Daley, 2012). Therefore, it is quite cheerful to see the inherited hepatic disorders can be effectively modeled using iPSC, which implies that the subtle and myriad intracellular communications required for the development of pathogenic defects are not prevented by the considerable cellular stresses during cell reprogramming and directed differentiation procedures. On the other hand, it has been evidenced that iPSC may retain an "epigenetic memory" of the cells they originated from (Robinton and Daley, 2012). For some non-inherited liver diseases, it might be difficult for the non-hepatic originated iPSC to faithfully reproduce the cellular defects. In this regard, it is suggested that hepatic-sourced iPSC may have advantages in modeling certain acquired diseases like cirrhosis and hepatocellular carcinoma (Chun et al., 2010). Indeed, primary human hepatocytes derived iPSC have been reported and readily differentiated into hepatocytes, though the authors did not observe dramatically enhanced efficiency of hepatic differentiation (Liu et al., 2010). Therefore, it will be interesting to see if these hepatocytes-iPSC derived hepatocytes offer any advantage in certain disease modeling or drug development related application, although obtaining the patient hepatocytes itself may present a challenging task.

HUMAN IPSC DERIVED HEPATOCYTES FOR DRUG DEVELOPMENT

Another prospective area of using iPSC-hepatocytes is the drug development. Hepatotoxicity and cardiotoxicity are two principal causes of drug failure in preclinical development (Rubin, 2008). Isolated primary human hepatocytes are the current "gold standard" *in vitro* model for drug metabolism and toxicology studies as they express the full spectrum of drug metabolizing enzymes and transporters (Hewitt et al., 2007). However in practice, these safety evaluations rely heavily on the availability and quality of primary human hepatocytes, which often display batch-to-batch variations. Thereby, a stable source of hepatocytes in good quality is in urgent need to improve the reproducibility for pharmaceutical studies. In this regard, human iPSC derived hepatocytes would potentially satisfy the needs by providing a ready supply for reliable metabolism and toxicology studies of candidate compounds, as well as high throughput screening for lead compounds. An additional advantage of iPSC-hepatocytes for drug development is that it enables the establishment of a broad spectrum of hepatocyte lines which represent the potential genetic and epigenetic variations of human population. The use of this tool offers exciting opportunities to reduce the risk and cost associated with early-stage clinical trials and may lead to personalized drug administration. Specifically, iPSC-hepatocytes generated from individuals with different P450 polymorphisms would be of great value for drug metabolism study and toxicity prediction of new drugs (Asgari et al., 2010). Moreover, the utilizing of iPSC would offer opportunities to generate liver cells at different stages of maturation, as well as the potential to give rise to all the composite cells of the adult liver, which may provide extra advantages and substantially expand the scope of the traditional drug metabolism and toxicology studies (Baxter et al., 2010).

Though the future is promising and influence is profound, the real application of iPSC-hepatocytes still needs considerable amount of investigations. *In vitro* hepatocyte models have innate limitations in terms of preserving metabolic enzyme activities and maturity. In addition, despite of numerous protocols aiming at efficient differentiation of hepatocytes from human pluripotent stem cells, phenotypic stability and expansion capacity of derived hepatocytes are still major challenges for the application, as the cell purity and scale-up capability are both mandatory for practical use in pharmaceutical industry (Baxter et al., 2010). Even if the direct *in vitro* application of iPSC-hepatocytes in drug discovery is still in infant stage, a feasible alternative would be an *in vivo*, artificial human liver for drug testing (Yoshizato and Tateno, 2009). It has been reported that the livers of the humanized mice can be over 90% repopulated with human hepatocytes and express human phase I/II metabolic enzymes (Katoh et al., 2008). It will be exciting to see that this strategy, combined with iPSC-hepatocytes, may provide an ideal tool to investi-gate drug metabolism for human hepatocytes with a great range of genetic diversity. In either way, further development and investment of human iPSC-hepatocytes would enable better prediction of drug-induced hepatotoxicity during pre-clinical stages and more successful clinical trials, which may substantially benefit the current protracted, time- and cost-consuming drug development process.

HUMAN IPSC DERIVED HEPATOCYTES FOR CELL THERAPY

Human liver has an immense regenerative capacity. Under physiological conditions, the liver does not need any external cell source to undergo repair, as resting hepatocytes have the capacity to re-enter the cell-cycle efficiently after injury (Zaret and Grompe, 2008). However, in persistent injury condition, the sustained proliferative stress prematurely ages the hepatocytes and exhausts their ability to replicate. Allogeneic liver transplantation is the single conventional treatment for patients with end-stage liver diseases. Nevertheless, the shortage of donor organs together with other hurdles including operative damage, post-transplant rejection and high clinical cost are all serious problems hampering its application (Lorenzini et al., 2008). In recent years, hepatocytes based cell transplantation has been demonstrated as an experimental procedure which has been successfully applied in clinical trials for acute liver failure and chronic liver disease. Cheerful results have been seen from individual patient, especially from the treatment of liver metabolic disorders (Dhawan et al., 2010). Though it has been proven effective, one crucial bottleneck of this practice is the limited supply of allogeneic donor hepatocytes provided in good quality. Therefore, the latest advance of iPSC technology may provide a very promising source for the generation of truly isogenic grafts. To date, considering the unrelieved concerns regarding safety and efficacy, it hasn't yet been clinical trial using human iPSC derived hepatocytes for treating liver disease. However, it has been demonstrated in animal models that human iPSC-hepatocytes can efficiently engraft into mouse hepatic parenchyma and perform normal function *in vivo* (Si-Tayeb et al., 2010b). More recently, human iPSC-hepatocytes generated by a newly developed three-step protocol have been shown to rescue lethal fulminant hepatic failure in a non-obese diabetic severe combined immunodeficient mouse model, which comprehensively proved their functionality *in vivo* (Chen et al., 2011).

Another important advantage of iPSC over conventional cell therapy is the potential of repairing pathogenic mutations by gene targeting and correction (Liu et al., 2011, 2012). Yusa et al. (2011) reported the targeted gene correction of α1-antitrypsin deficiency in human iPSC. Utilizing a combined technology of zinc finger nucleases (ZFNs) and piggyBac, the authors efficiently corrected the pathogenic point mutation in patient iPSC. The normal metabolic function was restored in
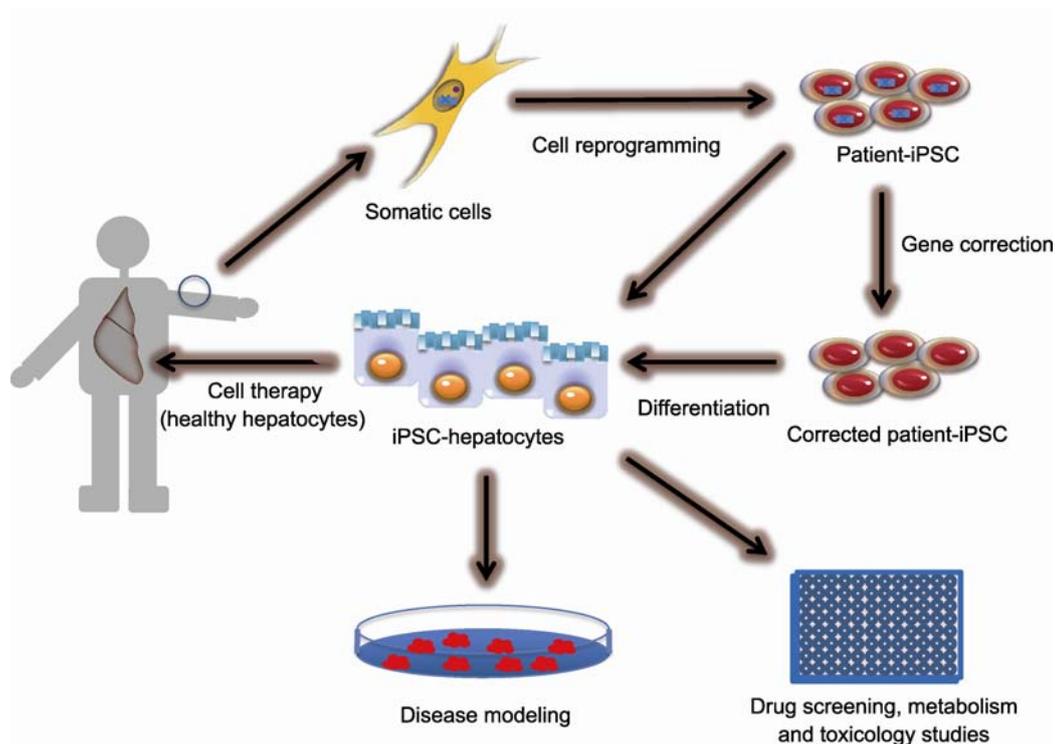
Figure 1. Summary of application potential of human iPSC derived hepatocytes. Human iPSC can be generated by cell reprogramming through virus or integration-free methods. Pathogenic mutation may be corrected by gene correction to generate disease-free iPSC. Hepatocytes differentiated from either disease-bearing or disease-free iPSC can be utilized for a wide range of applications upon desired purposes, including disease modeling, drug screening and metabolism/toxicology studies, as well as prospective cell therapy through transplantation.

hepatocytes derived from corrected iPSC, which demonstrated *in vivo* functionality (Yusa et al., 2011). This study proved a concept that inherited liver metabolic disorder can be cured through gene correction. Assuming that the safety concern of human iPSC will be eventually addressed, the combination of iPSC and gene targeting technology will truly illuminate a new future for autologous cell therapy.

CONCLUDING REMARKS

Recent progress in human iPSC technology and hepatocytes differentiation strategy has provided an enormous opportunity for basic and clinical studies of liver diseases and drug development (Fig. 1). It will be exciting to see one major step forward which is the moving of iPSC-hepatocytes from the dish into the clinic and pharmaceutical development. Nevertheless, before this significant step to be made, several non-neglectable issues need to be satisfied, which include: (1) generation of "safe" human iPSC; (2) establishment of reliable and efficient hepatocyte differentiation and isolation method; (3) *in vitro* maintenance and expansion of functional human hepatocytes; and (4) optimized engrafting and homing strategy for cell transplantation. Frankly speaking, neither one

is an easy task and the conquest of each step may prove to be expensive in both time and efforts. However, given the rapid and cheerful progress within this field, it is worthwhile to wait and expect the turnaround of iPSC-hepatocytes from the bench to the bedside.

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