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Hepatocytes from Induced Pluripotent Stem Cells: A Giant Leap Forward for Hepatology

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With an estimated 467,000 new cases per year worldwide, cirrhosis remains the fourth most common cause of death in the United States. Except for complete liver transplants, which are only available to a few, to date, there is no medical treatment available. Clearly, abrogation of end-stage liver disease is of great clinical significance. In this issue of Hepatology, two investigations reveal significant and seminal strides to solving the problem of liver replacement therapies.

Hopes for curing diseases with poor prognosis such as cirrhosis, diabetes, heart disease, Parkinson's, and various spinal cord afflictions were raised in 1998 with the discovery of human embryonic stem cells (hESCs).[1] In the 12 years since, an explosion of research has elevated hESCs, and stem cell biology as a whole, to a completely independent and elite field of research. Discovery after discovery of new genes, biochemical and molecular pathways, and ingenious ideas and theories about how cells make their decisions to remain pluripotent or differentiate have all been at the forefront of this relatively young field. The guiding principle behind investigating hESCs is the fact that they can differentiate into all three germ layers: ectoderm, mesoderm, and definitive endoderm. As a result, the ultimate goal driving hESC biology, and much of stem cell biology, has been their eventual use in the clinic as stem cell therapies.[2–5] In many respects, ESCs have indeed lived up to their billing by reversing signs of paralysis, virtually curing diabetes, and significantly reversing infarcted heart muscle…of course, that is if you are a rodent.[6–8] Unfortunately for humans, the past 12 years has brought about more questions concerning ESC efficacy, safety, and bioethics than cures. In fact, after more than a decade of research, only one trial has been approved by the Food and Drug Administration (FDA) for assessing hESCs in patients. However, this study, slated to have begun in August of 2009 by the Geron Corporation, was designed to only test the safety of these cells and is now on an indefinite hold by request of the FDA.

To date, the questions surrounding hESCs have not been answered enough to say that hESCs will be used clinically in the near future. Arguably, a major hurdle for hESC research has been concerns surrounding bioethics. Because hESCs must be obtained by destroying human embryos, many political and religious entities around the world have, either rightly

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or wrongly, hindered hESC research. Interestingly, though, a case could be made that these bioethical concerns have driven stem cell research toward a more “ethical” route for curing disease, a prime example being the intensification of research on adult stem cells.[9–12] Indeed, many articles on adult stem cells have embedded somewhere in their introductions and/or discussions a distinct explanation why the adult stem cell system being studied circumvents the bioethics problem. However, with the exception of bone marrow transplants, adult stem cells, to date, still have their problems, which, similar to hESCs, have also kept them out of the clinic for use as stem cell therapies.

Hence, human ingenuity has led to profound discovery that somatic cells could be induced to become pluripotent by simply adding four genes. Induced pluripotent stem cells (iPS cells) were first generated by two research teams led by Drs. Yamanaka and Thomson, respectively, who pioneered and generated stem cells from human skin through ectopic expression of four genes (Oct3/4, Sox2, c-Myc, and Klf4, or Oct3/4, Sox2, Nanog, and Lin28).[13–15] Since their discovery, improvements have been made in generating iPS cells including the ability to remove the inducing genes,[16] the addition of only one or two genes in certain cell types,[17,18] and generation of iPS cells by chemical induction.[19,20]

In each case, no matter the inductive route, human iPS cells have been shown to mimic hESCs in virtually all aspects of pluripotency and differentiation. These iPS cells are pluripotent because they can form all three germ layers. Moreover, mouse iPS cells have been repeatedly shown to make chimeric mice, contribute to the germ line, and generate pups.[21] However, to date, most of the in vitro investigations into iPS cell differentiation have focused on mesodermal-derived cardiomyocyte and ectodermal-derived neuronal lineages—that is, until now. In this issue, two independent laboratories reveal, for the first time, complete derivation of iPS cells into endodermal-derived hepatocytes (Sullivan et al.[22] and Si-Tayeb et al.[23]). While the elegance of each study enables them to stand alone, when taken together, they, in essence, delineate the true potential of iPS cells for the field of hepatology. The data clearly reveal that iPS cells can become fully functional liver cells.

Both articles demonstrate that iPS cell–derived hepatocytes express distinct hepatocyte markers; however, and perhaps more importantly, both also show definitive function of their hepatocytes in vitro and in vivo. The magnitude of these investigations will probably be felt straight away because they represent a seminal advancement in current hepatocyte cell-culture technology. A constant problem experienced by many who try to culture hepatocytes is that current protocols generally revolve around the need for consistent derivation and culture of primary hepatocytes, which have the reputation for being difficult to cultivate, are generally scarce, and are usually rather heterogeneous once in culture. Hepatocyte cell lines do not seem to fare any better because primary cultures de-differentiate and neither is true to their original hepatocyte nature.

On the other hand, hepatocytes derived from iPS cells do appear to be true to their nature as shown by “proof of concept” experiments from Sullivan et al.[22] Their assessment of P450 components cytochrome P450 1A2 (CYP1A2) and CYP3A4 are convincing among each iPS cell–derived line. Also notable is their test of iPS cells from both genders and different races. Their finding that race and gender are not factors for generating functional hepatocytes from iPS cells adds an exclamation point onto their findings.

A number of unique highlights are also found in the work from Stephen Duncan’s laboratory. Most notable is the explicit attention in establishing a protocol for generating specific endodermal cell types including definitive endoderm, specified hepatic cells, hepatoblasts, and hepatocytes. Existing protocols for generating hepatocytes from hESCs and adult stem cells have generally included steps where ill-defined components are added

to the culture medium. Here, Si-Tayeb et al.[23] describe how they eliminate the use of serum, the feeder cell layer, the formation of embryoid bodies, and undefined reagents. Such detail enables anyone with an interest in this field a simple, straightforward approach for generating hepatocytes.

Also highlighted is their evidence demonstrating the evolutionary importance of this differentiation process; results from both mouse and human iPS cells aptly parallel each other. However, probably their most impressive feature is the approach used to test the efficacy of the hepatocytes derived from iPS cells. By using tetraploid complementation in a mouse model, they demonstrate that iPS cells could follow the hepatocyte developmental pathway in vivo, and all liver cell types were represented in the iPS cell–derived embryos. Although the tetraploid complementation approach has been used by a number of investigators to circumvent embryonic lethality in knockout mouse models,[24,25] this was one of the first studies to utilize it as a functional assay showing the fate of a certain cell type (i.e., iPS cells). In essence, Duncan's group cemented the fact that iPS cells can be used in every respect to ESCs for liver regeneration and for studying pathogens as the cause of hepatocyte and liver dysfunction.

Looking ahead, the showing development of hepatocytes from iPS cells could potentially revolutionize hepatology with respect to the study of hepatitis B and C viruses, alcohol-induced cirrhosis, and congenital liver diseases. In vivo, iPS cell–derived hepatocytes will most likely advance the concept of tissue therapies particularly with respect to the autologous nature of these cells. It is true that iPS cells still have many remaining hurdles to overcome before they can enter the clinic, such as removal of the genes necessary to make them pluripotent and abolishing the chance of hepatocellular cancer stem cells and teratoma formation generation of a transplantable organ. Regardless, it is easy to appreciate how the work from these two laboratories provides hope for millions of people with certain types of liver disease. Indeed, hepatocytes from iPS cells represent a giant leap forward for hepatology.

Abbreviations

hESC	human embryonic stem cell
iPS cell	induced pluripotent stem cell

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