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Induced pluripotent stem cells: From Nobel Prizes to clinical applications

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Summary

Advances in basic hepatology have been constrained for many years by the inability to culture primary hepatocytes *in vitro*, until just over five years ago when the scientific playing field was changed beyond recognition with the demonstration that human skin fibroblasts could be reprogrammed to resemble embryonic cells. The reprogrammed cells, known as induced pluripotent stem cells (iPSCs), were then shown to have the capacity to re-differentiate into almost any human cell type, including hepatocytes. The unlimited number and isogenic nature of the cells that can be generated from tiny fragments of tissue have massive implications for the study of human liver diseases *in vitro*. Of more immediate clinical importance were recent data demonstrating precision gene therapy on patient specific iPSCs, which opens up the real and exciting possibility of autologous hepatocyte transplantation as a substitute for allogeneic whole liver transplantation, which has been an effective approach to end-stage liver disease, but one that has now been outstripped by demand. In this review, we describe the historical development, current technology and potential clinical applications of induced pluripotency, concluding with a perspective on possible future directions in this dynamic field.

History of iPSCs

For many, the story of iPSCs began almost 60 years ago. Seminal experiments in the early 1960s carried out by Briggs and King in Philadelphia and Gurdon in Cambridge, described transplantation of donor tadpole intestinal cell nuclei into recipient enucleated frog eggs [1]. Transplanted eggs grew into living tadpoles genetically identical to the donor nucleus, leading to the hypothesis that the host egg cytoplasm had somehow converted the transplanted nucleus into a cell capable of forming an entire healthy adult frog (Fig. 1). It was concluded that an unknown myriad of factors present in the egg cytoplasm must have 'reprogrammed' the phenotype of a fully differentiated adult cell back into a totipotent one-cell-stage embryo. This experimental procedure was given the term 'somatic cell nuclear transfer' and by the mid 1990s had been applied to several mammalian species, most famously resulting in the cloning of 'Dolly' the sheep [2]. These experiments suggested Gurdon's earlier hypothesis that the pliability of adult cells secondary to epigenetic (non-genetic) factors was not only applicable in simple organisms such as the frog, but also held true for complex mammals such as sheep. Indeed the possibility that these principles may be at play in human cells formed the ideological basis for Yamanaka's landmark study in 2006. Yamanaka reasoned that enforced expression of a limited number of genes, critical to the maintenance of pluripotency in embryonic stem cells (ESCs), might enable direct reprogramming of somatic cells without the need for nuclear transfer [3]. By a process of stepwise elimination, just four transcription factor genes (*Oct3/4* (*Pou5f1*), *Sox2*, *Klf4*, and *Myc*) were eventually identified as sufficient to give rise to the newly formed pluripotent cells and the term "induced pluripotent stem cells" (iPSCs) was born (Fig. 1). These landmark advances in basic cell biology were celebrated by the recent award of the Nobel Prize for Physiology and Medicine to Professors Gurdon and Yamanaka.

Key Points

- Shinya Yamanaka and Sir John Gurdon were recently awarded the nobel prize for their work on induced pluripotent stem cells (iPSCs)
- iPSCs have the capacity to generate unlimited quantities of any cell type in the human body
- Since their discovery five years ago, numerous studies suggest this new technology could soon be used to generate novel, patient specific *in vitro* disease models and transplantation products
- One of the first disease areas to benefit from this exciting new resource is likely to be Hepatology
- To realize this clinical promise, several key challenges surrounding the reproducibility and epigenetic/genetic stability of the products will first need to be addressed

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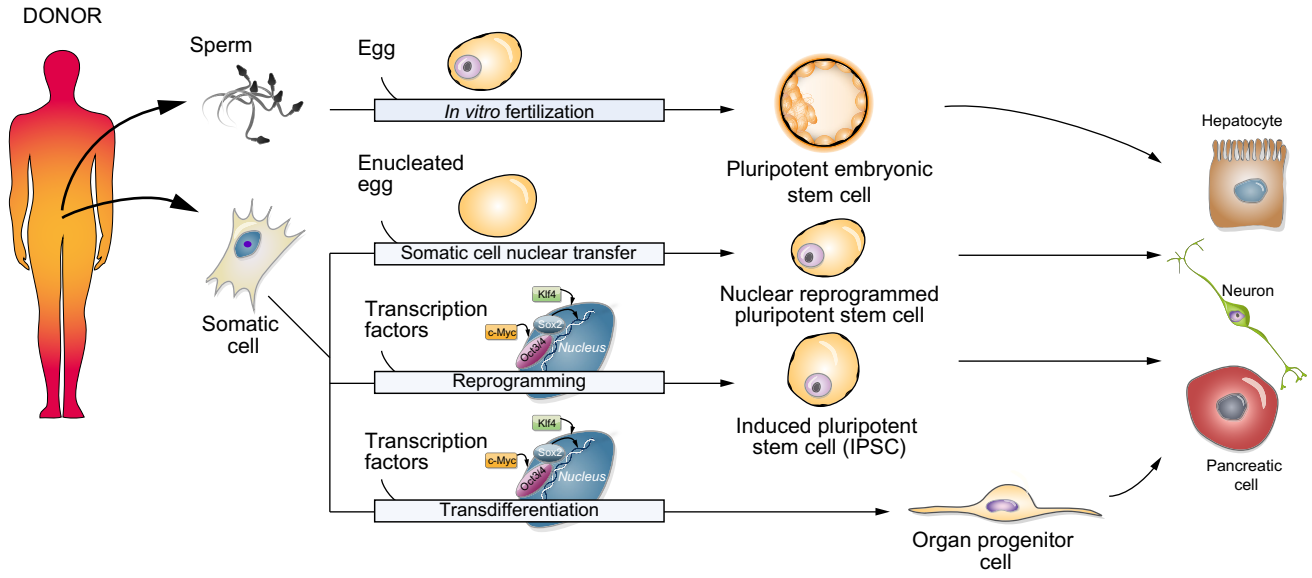

Fig. 1. Derivation of pluripotent stem cells. Pluripotent stem cells, possessing the ability to form any cell type of the human body, can be created using *in vitro* fertilisation (Steptoe and Edwards), somatic cell nuclear transfer (Gurdon), and transcription factor overexpression (Yamanaka).

How have iPSCs been used so far?

Disease modelling

Following the first human fibroblast derivation of iPSCs, investigators went on to show that the reprogramming protocol could be used in cells taken from patients with a wide range of Mendelian and complex genetic disease backgrounds [4]. These observations were then followed-up by studies proving that human iPSCs could be differentiated into adult-like somatic cells. A range of different cells with varying relevant functional properties have since been generated including motor and dopaminergic neurons, cardiac cells, pancreatic cells, adipocytes, blood cells, vascular cells, retinal progenitors, and liver cells [5]. Importantly, several laboratories have also now shown that such patient specific hIPSC-derived somatic cells can be used to generate exciting new *in vitro* disease models. Examples of diseases modelled in this way include LEOPARD syndrome, Long QT/Timothy syndromes, RETT syndrome, and Hutchinson–Gilford progeria [6]. Within the liver field, we recently demonstrated dermal fibroblasts obtained from patients suffering from five of the "inherited metabolic liver disorders" (α_1-antitrypsin deficiency, glycogen storage disease type 1a, familial hypercholesterolaemia, hereditary tyrosinaemia, and Crigler–Najjar syndrome) could be used to generate patient specific human iPSC lines [7]. Each of the human iPSC lines was then differentiated into hepatocyte-like cells using a novel three-step differentiation protocol in chemically defined conditions. The cells were assessed for *in vitro* function, ability to replicate key features of disease pathology, and response to targeted small molecules. Patient specific human iPSC-derived hepatocytes with *in vitro* function approximating adult human hepatocytes exhibited key pathological features of the diseases from which they were derived, most notably protein misfolding in the ER, deficient receptor-mediated extracellular lipid uptake and impaired enzyme dependent cellular metabolism. Whilst hIPSC-derived hepatocytes possess functionality approximating their primary hepatic counterparts, they are still far from being identical with respect to activities such as cytochrome P450 activity. This drawback may not present a problem for the modelling of inherited monogenetic disorders [7,8], but may stunt efforts to apply this technology for use with drug toxicology and viral hepatitis studies [9]. (The topic of human iPSC-based disease modelling is reviewed more extensively by Wu and Hochedlinger [10] and Robinton and Daley [6].)

Regenerative medicine

Human trials using iPSCs have not yet been started. This is not altogether surprising since when we consider that human ESC products, despite their longer history, are only now entering into clinical trials. A phase I study based on the use of human ESC-derived retinal pigment epithelium for macular degeneration, for example, recently demonstrated no adverse side effects associated with therapy [11]. Such advances suggest it will only be a matter of time before iPSC-based trials are also conducted.

The complex biological interactions that might account for positive results in regenerative medicine trials suggest to many that this type of therapy may not be easily translatable across different organ systems. Cell-based therapies for well-characterised monogenetic disorders that affect single adult cell types, however, will probably prove an easier and more realistic first target for such trials. As such, inherited liver disorders would appear to be one such credible first target. Several animal models have already demonstrated that transplanted wild type hepatocytes possess a selective survival advantage over genetically mutated host cells [12]. Though translation of such studies into humans has proven disappointing [13], this lack of clinical success is attributed to the scarcity of high-quality cells and continued requirement for life-long immunosuppression.

Using patient specific hiPSC-derived heaptocytes to treat monogenetically inherited liver disorders could overcome these problems. Importantly, correction of the underlying genetic abnormality in a manner fully compatible with clinical applications would firstly be required. Since the most widely validated