Overcoming clinical hurdles for autologous pluripotent stem cell-based therapies

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Abstract

Introduction

Human induced pluripotent stem cells can now be derived by using factor-mediated reprogramming to revert an individual's somatic cells, such as skin cells, back into a pluripotent epigenetic state. These autologous cells hold immense potential for cell-based therapeutics. Not only are they capable of forming any cell type in the human body, but they are also immunologically matched to the patient. Such cells thus have a variety of possible applications in the treatment of a wide range of diseases and injuries, including those associated with age-related tissue degeneration. Autologous cells also hold great promise for use in other areas, including in vitro research, developmental biology, drug screening and potential future reproductive applications, such as the development of infertility treatments. However, there are five obstacles which thus far have prevented the safe clinical application of personalised human induced pluripotent stem cell therapeutic derivatives. These are (1) the high levels of genetic instability observed in human induced pluripotent stem cells; (2) reports of aberrant epigenetic reprogramming or epigenetic memory or both; (3) post-transplantation issues surrounding efficacy of the transplanted cells, including low levels of survival, functionality, reports that human induced pluripotent stem cell derivatives are foetal in nature, and reports of immuno-genicity gene expression or immune rejection (or both) in perfectly matched hosts; (4) issues surrounding post-transplantation safety, including an increased risk of neoplasms from transplanted cells; and (5) issues surrounding the feasibility of human induced pluripotent stem cell-based therapeutics, including the time, cost and regulatory hurdles and the difficulties associated with consistently generating a therapeutic cellular product on an individualised basis. This review conducts a detailed examination of these hurdles and evaluates the feasibility of various possible solutions.

Conclusion

Induced pluripotent stem cells possess numerous possible applications, such as cell-based therapeutics for the treatment of a wide range of diseases, injuries and age-related tissue degeneration, in addition to in vitro research, developmental biology, drug screening and potential future reproductive applications, such as possible treatments for infertility. In this review, we have examined the five hurdles to be overcome in safely moving towards personalised human induced pluripotent stem cell-based theapeutics.

Introduction

John Gurdon first discovered that a differentiated somatic cell could be reprogrammed back into a pluripotent state capable of generating an entirely new organism, in this case the African clawed frog Xenopus laevis1,2. However, this somatic cell nuclear transfer (SCNT) procedure proved extremely inefficient; over 700 nuclear transfers were performed and only approximately 1% directly resulted in swimming tadpoles3. Despite these difficulties, this represented the first demonstration of a differentiated cell's remarkable ability to revert backwards in development when directed by exogenous factors, such as (in this case) the enucleated frog egg. The scientific concept that vertebrate oocytes contain factors capable of reprogramming somatic cell nuclei into a pluripotent epigenetic state was then extended from amphibians to mammals in 1997, when the first mammal cloned from an adult somatic cell was born4. This was followed by the first derivation of pluripotent stem cells (PSCs) from oocyte-reprogrammed murine somatic cells in 20005, non-human primate somatic cells in 20076 and human somatic cells in 20137. Notwithstanding this success, the primary disadvantage of the SCNT reprogramming approach is the significantly limited supply of human oocytes for SCNT-based research because of practical, legal and ethical constraints. In 2006, Shinya Yamanaka and colleagues discovered that mouse and, subsequently, human somatic cells could be directly induced into a pluripotent epigenetic state with the use of exogenously delivered reprogramming factors8,9, which generated ‘induced pluripotent stem cells’ (iPSCs). This discovery represented a fortunate development for the advancement of autologous PSC-based therapies generated by using exogenously delivered factors. It provided a feasible alternative to

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Figure 1: Scientific concept behind human induced pluripotent stem cell (hiPSC)-based therapeutics. The long-term goal of the hiPSC-based therapeutic field is to use hiPSCs and their derivatives to develop personalised cellular therapies for treating diseases, injuries, age-related tissue degeneration and infertility. The underlying concept is that a patient’s own skin cells could be epigenetically reprogrammed back into pluripotent stem cells. Typically, a minimally invasive skin punch biopsy is obtained from the patient and provides a reliable source of autologous cells with extensive proliferation potential. These primary dermal fibroblasts then are reprogrammed into hiPSCs and can be differentiated into any therapeutic cell type and then autologously transplanted back into the original individual, without the risk of immune rejection.

Discussion
The author has referenced some of his own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

Potential uses of human induced pluripotent stem cells (hiPSCs)
hiPSCs can be used for both in vitro and in vivo applications. In vitro applications include a wide range of possible in vitro research areas, such as ageing research, developmental biology, drug screening, toxicology and disease modelling; for reviews, please read. In vivo applications include the promising use of hiPSC derivatives for the treatment of diseases and injuries. However, in vivo applications also include potentially more controversial applications. One such example is the possible use of hiPSCs and their derivatives to rejuvenate human tissues and organs after age-related tissue degeneration, thereby increasing the quality and length of human life. Moreover, hiPSCs and their derivatives may be used for reproductive applications, such as treating infertility. Such infertility treatments could be achieved through the generation of germ cells with or without gene correction (genetic manipulations that would pass to all subsequent generations) or possibly through the generation of ‘cloned’ individuals through embryological manipulations like tetraploid complementation, the profound bioethical considerations of which are beyond the scope of this article.

Advantages of hiPSC derivatives for autologous cellular therapies
The advantages of hiPSC derivatives for autologous cellular therapies are the following: (1) iPSCs are pluripotent and thus are theoretically capable of forming any therapeutic cell type; (2) iPSCs possess unlimited proliferation capacity (i.e.,...
they are immortal), facilitating genetic manipulation, such as is necessitated in the case of patients with genetic disorders;(3) iPSC derivation does not involve the destruction of human embryos and therefore is not subject to the same ethical considerations as embryonic stem cell (ESC)-based research; and (4) iPSCs can be derived from a patient’s own somatic cells (Figure 1), placing iPSC derivatives at a reduced risk of immune rejection and eliminating the cost, inconvenience and negative health consequences associated with long-term immuno suppression. The capacity for murine iPSC derivatives to restore physiological function has already been established in mouse models of heart disease, sickle cell anaemia, Parkinson’s disease and haemophilia A, providing pre-clinical data in support of the underlying promise of these cells. However, despite the obvious advantages and successes of previous animal studies, a number of remaining hurdles must be overcome before the promise of hiPSCs for regenerative medicine can be safely realised.

Current hurdles in advancing personalised pluripotent stem cells (PSCs)

There are five hurdles to be overcome in safely advancing PSCs derivatives into personalised human therapeutics. These hurdles are (1) high levels of genetic instability, (2) aberrant epigenetic reprogramming, (3) issues surrounding post-transplantation efficacy, (4) issues surrounding post-transplantation safety, and (5) issues surrounding feasibility.

High levels of genetic instability

Recent studies have demonstrated a predisposition to genetic instability within in vitro cultured PSCs. Specifically, both human ESCs and hiPSCs demonstrate inherent genetic instability during in vitro culture. This is a concern for the therapeutic use of these cells, as the frequently observed genetic (karyotypic) abnormalities in human PSCs are characteristic of malignant tumours. Moreover, even differentiated astrocytes, derived from karyotypically normal human PSCs, have demonstrated characteristics analogous to malignant tumours. One extremely common genetic aberration is the duplication of chromosome 12. This chromosomal duplication is often associated with a proliferative growth advantage and potential tumorigenesis, rendering these cells unacceptable for personalised cellular therapeutics. One potential solution to this genomic instability obstacle is the optimisation of culture conditions to promote genomic stability. The candidate supplements required for addition to the culture media will be dependent upon the underlying causes of the genomic stability. It is thus necessary to determine whether the instability is caused by oxidative or replicative stress or both, with chemicals such as antioxidants and culture under lower oxygen levels, offering potential solutions for the ‘oxidative stress/genomic instability hypothesis’, and exogenous nucleoside supplementation, offering solutions for the ‘replicative stress/genomic instability hypothesis’.

Regarding the success of these and other culture condition optimisation approaches for the promotion of genomic stability in hiPSCs and their derivatives, it would be prudent to screen each and every iPSC clone for genetic aberrations prior to any therapeutic applications.

Aberrant epigenetic reprogramming

Studies comparing the DNA methylomes of mouse and human iPSCs with their respective species-specific ESCs revealed that many iPSC lines retain aberrant iPSC-specific differential methylation patterns. This phenomenon is referred to as ‘epigenetic memory’. The epigenetic memory of iPSCs was observed to impair the differentiation capacity of the iPSCs, with iPSCs demonstrating a reduced capacity to differentiate into cells from lineages different from that of the donor cell type. There is significant evidence to suggest that, if not all, iPSC lines can gradually resolve some, if not most, of their transcriptional and epigenetic differences with ESCs over time via increased passaging. However, it has also been observed that a subset of iPSC lines continue to retain epigenetic memory, even after extended passaging. A recent study discovered that all of the examined iPSC lines (9 out of 9) demonstrated incomplete hydroxy methylation levels in multiple subtelomeric regions when compared with control human ESC lines (4 out of 4). It has yet to be established how significant a hurdle this residual epigenetic memory and aberrant epigenetic reprogramming will prove to be for future autologous cellular therapeutics. However, it may be prudent to err on the side of caution and continue to investigate novel approaches to augment the epigenetic reprogramming process, including use of chromatin-modifying chemicals, such as sodium butyrate, 5-azacytidine and valproic acid. These represent chromatin-modifying chemicals which have been previously used to (1) augment epigenetic reprogramming to pluripotency; (2) increase the inclusion of factors that promote developmental competence, such as Glis1, L-Myc and Tbx3; and (3) test the ‘CORF-augmented reprogramming hypothesis’. This hypothesis proposes that the addition of putative candidate oocyte reprogramming factors (CORFs), such as ARID2, AS1, ASF1B, DPPA3, ING3, MSL3, H1FOO, KDM6B and/or others, may be capable of opening up the somatic heterochromatin (including subtelomeric regions) and increase access for epigenetic reprogramming factors, such as the demethylation...
enzyme TET3 (which is present in the oocyte)\textsuperscript{43}. This, in turn, may augment the nuclear reprogramming process.

**Issues surrounding post-transplantation efficacy**

Post-transplantation efficacy is determined by (1) an efficient and reliable *in vitro* differentiation protocol into the target therapeutic cell type; (2) sufficient post-transplantation integration, maturation and survival; and (3) maintained functionality to induce a therapeutically detectable effect. Efficient and reliable differentiation protocols have been developed for motor neurons\textsuperscript{46}, dopaminergic neurons\textsuperscript{47}, cardiomyocytes\textsuperscript{48}, hematopoietic precursors\textsuperscript{49}\textsuperscript{50} and hematopoietic precursors\textsuperscript{51}. However, recent research by William Lowry and colleagues\textsuperscript{52} strongly suggests that iPSC and ESC derivatives are foetal in nature\textsuperscript{49}. The ‘*in vivo* development recapitulation/maturation hypothesis’ proposes that the differentiation of iPSCs, into mature adult cells, can be obtained through *in vitro* recapitulation of *in vivo* developmental processes. Indeed, the derivation of every functional tissue in adult ‘all-iPSC’ mice serves to highlight the remarkable developmental capacity of certain, albeit rare, mammalian iPSC lines under optimal (*in vivo*) differentiation conditions\textsuperscript{53}. One possible solution to the low levels of survival, integration and maturation of cells post-transplantation is the ‘cell delivery optimisation/pro-survival hypothesis’. This hypothesis proposes that cell delivery approaches could be optimised with pro-survival factors, such as anti-inflammatory and free radical scavengers, tissue engineering strategies, co-transplantation with carrier cells, preconditioning the cells (for example, heat-shock) genetic modification to enhance engraftment and survival or a combination of these factors\textsuperscript{54,55}.

Of particular concern for future proposed iPSC-based patient-specific cellular therapeutics is that multiple independent groups have observed that what appears to be an above baseline immune response after transplantation of iPSCs, or iPSC-derivatives, into perfectly matched (autologous/syngeneic) hosts\textsuperscript{56-58}. It remains unclear whether this immune rejection is (1) associated with expression of immunogenicity genes, such as *HORMAD1* or *ZG16* or both\textsuperscript{59}, (2) linked to low developmental competency\textsuperscript{60}, or (3) linked primarily to residual transgene expression\textsuperscript{55,56}. In any event, the immunogenicity issue represents an important problem that needs to be resolved, ideally in the human system, in order to ensure that transplanted hiPSC derivatives are not rejected by the recipient’s immune system.

**Issues surrounding post-transplantation safety**

The key post-transplantation safety concern is that hiPSC derivatives will give rise to neoplasms, particularly teratomas and teratocarcinomas\textsuperscript{61}. In addition to the safety concerns traditionally associated with teratomas, there is the potential that other types of embryonal neoplasms, relating to specific tissue lineages, may arise after engraftment\textsuperscript{52}. These ‘monodermal’ teratomas have been observed after the transplantation of human PSC derivatives in rodents\textsuperscript{56}, thus highlighting the post-transplantation safety concerns. In addition to the genomic instability issues previously discussed in the context of post-transplantational safety concerns, there are three, potentially complementary, putative sources of post-iPSC transplantation teratomas: (1) contaminating undifferentiated cells, (2) transgenic expression of potentially oncogenic reprogramming factors, and (3) potentially of oncogenic insertional mutagenesis. Contaminating PSCs (as few as two cells) can generate teratomas\textsuperscript{62}, thus demonstrating the need to generate extremely pure populations of cells for therapeutic applications. This could theoretically be achieved by using positive selection to identify and purify the target cell\textsuperscript{63} or negative selection to identify and remove undifferentiated cells\textsuperscript{64} or both. Such selections would be applied in addition to the use of transgenic selection with tissue-specific promoters. This would allow fluorescent marker expression-based purification, using flow cytometry, or antibiotic resistance gene expression-based purification, using antibiotic selection\textsuperscript{65}. However, non-genetic methods, based upon tissue-specific metabolism, might provide safer and very efficient protocols\textsuperscript{66}. The concern regarding the potential for residual or reactivated transgene expression in the iPSC derivatives is based on the observation that reprogramming factors used to generate iPSCs are also associated with tumours and hyperplasia\textsuperscript{67,68}. In addition, chimeric mice generated from iPSCs containing the cMyc transgene have demonstrated a predisposition towards tumour formation\textsuperscript{69}, and random integration of viruses into tumour suppressor genes may increase the risk of neoplasms. However, multiple transgene-free and integration-free approaches have now proven effective in generating hiPSCs, and even if these cells are derived under research-grade conditions, they still may prove useful for therapeutic applications, after conversion to putative clinical-grade conditions\textsuperscript{70}. One potential solution, which could addres several aspects of post-transplantation safety, is to genetically modify iPSCs to express a suicide gene, such as herpessimplex virus 1 thymidine kinase (*HSV1tk*). This, remarkably, permits both positron emission tomography (PET)-based *in vivo* imaging (using the PET probe fluoro-3-hydroxymethyl-butyl-guanine, FHBG) of the transplanted iPSC derivatives\textsuperscript{66,67} and the ability to induce cell death (using the drug ganciclovir) in the event the transplanted cells result...
in tumour formation or other negative outcomes. Amphibia to mammals.

**Issues surrounding feasibility**

Although hundreds of clinical trials are currently being performed using hematopoietic and mesenchymal stem cells, no clinical trial has yet been performed using hiPSCs. Two clinical trials have been performed using human ESCs. These trials, in particular, thus serve to highlight the difficulties facing the safe transition of human PSC-based therapeutics into clinical trials. Specifically, the phase 1 clinical study by Genn (Menlo Park, CA, USA) to use human ESC-derived oligodendrocytes to treat patients with spinal cord injury was discontinued for business reasons, and the long-term clinical benefits of the phase 1 clinical trial of Advanced Cell Technology (Marlborough, MA, USA) to treat macular degeneration are unknown. Both companies faced rigorous implementation of regulatory protocols by the Food and Drug Administration (FDA) before the trials were eventually approved, thus highlighting the FDA’s safety concerns that contaminated PSCs could possibly generate teratomas in the recipients. The safety measures proposed herein may prove helpful in reducing some of the regulatory burden for future PSC-based clinical trials. However, other feasibility considerations, including the amount of time taken to reprogram, characterise and differentiate the iPSC derivatives prior to clinical application, must also be taken into account. Most reprogramming and characterisation studies require several months, and certain differentiation protocols can take many additional months (for example, differentiation into retinal pigmented epithelium). The extended time required to reprogram, characterise and differentiate a therapeutic population of cells may prove a limitation to the suitability of this personalised iPSC derivative approach in certain cell-based treatments, particularly those in which transplantation to the patient is required within a short time frame. The time taken to reprogram a therapeutically useful iPSC colony is prolonged by the traditionally low iPSC reprogramming efficiency (<0.1%). However, technological advances, such as combinations of synthetic mRNAs and various small-molecule reprogramming enhancers, may sufficiently augment the reprogramming process to a level at which reprogramming efficiency is no longer a concern. In addition to time considerations, high financial costs are involved in performing this multi-step reprogramming, characterisation and differentiation approach, particularly when performed with the use of expensive, defined, good manufacturing practice-grade media under clinical-grade conditions. These factors thus may reduce the commercial feasibility of the approach. These time and financial considerations are further exacerbated when genetic modification of the iPSCs is also performed, such as when performing gene correction in the case of inherited genetic diseases, transgenic insertion to augment cellular function and/or the genetic modification of the iPSCs for in vivo tracking and suicide cell responses, as previously discussed. The overall feasibility of the iPSC-based therapeutic approach will be determined essentially by whether the technical, financial and temporal issues can be adequately resolved. With the extensive amount of research currently being conducted in the iPSC field, in addition to the ongoing scientific investigation of the mechanisms of nuclear reprogramming, it is plausible to consider that these feasibility issues will be adequately addressed in due course. However, when this will occur remains to be seen.

**Conclusion**

Defined factors can reprogram differentiated cells back into pluripotency. The discovery of iPSCs has provided a broad range of possible applications. Such potential in vivo applications include cell-based therapeutics to treat a wide range of diseases, injuries and age-related tissue degeneration. Moreover, iPSCs hold significant promise for in vitro research, developmental biology, drug screening and potential future reproductive applications, such as a possible treatment for infertility. In this review, we have examined the five hurdles to be overcome in safely moving personalised hiPSC therapeutic derivatives into the clinic and discussed various possible solutions.

**Abbreviations**

CORF, candidate oocyte reprogramming factor; ESC, embryonic stem cell; FDA, Food and Drug Administration; FHBG, fluoro-3-hydroxymethyl-butyryl-guanine; hiPSC, human induced pluripotent stem cell; iPSC, induced pluripotent stem cell; PCS, pluripotent stem cell; PET, positron emission tomography; SCNT, somatic cell nuclear transfer.

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**References**


