Tempering hope with realism: induced pluripotent stem cells in regenerative medicine


Abstract

- Since their discovery in 2007, human induced pluripotent stem (iPS) cells have been widely championed as the future for regenerative medicine.
- By differentiating iPS cells into specialised cells for transplantation, it may be possible to replace diseased cells and “cure” patients of various chronic degenerative conditions, including Parkinson’s disease.
- Such putative iPS cell-based therapies would avoid the need to procure transplantable cells from (and in the process, destroy) human embryos, and could be administered to patients without immunosuppressive therapy.
- In reality, however, the optimism surrounding iPS cell research has outpaced progress to date and likely emanates, in part, from the “moral panic” surrounding human embryo research.
- At such an early stage of development, questions remain about whether iPS cells are strictly equivalent to human embryonic stem cells as a source of transplantable cells. The Heerey Committee considered this point in its recent review of the Commonwealth legislation governing embryo research and human cloning and its Report (tabled in federal Parliament in July 2011) recommended that embryonic stem cell research should continue in Australia.
- In addition, there are many ethical issues which will need to be considered when enrolling vulnerable patients in trials of iPS cell therapy given the uncertain benefits and risks involved.
- In the rush to embrace iPS cell therapy, there is a real risk that the public may overrate the benefits and expect imminent translation to the clinic.
Until recently, treatment for chronic degenerative conditions was largely to reduce symptoms and improving physiological functioning in the hope of gaining some (often limited) increase in life expectancy. For example, pharmacological agents and surgical procedures may relieve the symptoms of Parkinson’s disease – muscle rigidity, tremor, bradykinesia, and postural instability – caused by the loss of nigrostriatal dopaminergic neurons in the midbrain region, but are only effective in the early stages of disease progression and may induce serious adverse effects.  

In the last decade, however, the advent of “regenerative medicine” has raised hope that normal structure and function may be restored in these intractable conditions, by harnessing pluripotent stem cells – cells that can be converted into all cell types of the human body – to produce specialised cells and replace diseased cells in vivo. For instance, dopaminergic neurons could be generated from pluripotent stem cells for transplantation in patients with Parkinson’s disease. Until recently, however, pluripotent stem cells could only be derived from human embryos that are destroyed when harvesting the embryonic stem cells. Although these embryos are left over from infertility treatment and would otherwise be destroyed, their use for research remains ethically contentious and is governed by tight statutory constraints.

In 2007, it was discovered that somatic cells (e.g. fibroblasts obtained from patient skin biopsies) could be reprogrammed into human pluripotent stem cells – called iPS cells – without using, or destroying, human embryos. Certain genes are inserted into somatic cells to revert them to an embryonic stem cell-like state. Unlike stem cells derived from human embryos, iPS cells are derived from patients’ own cells so are more likely to be transplantable without the risk of immune rejection. For these reasons, iPS cell therapy has been championed as a superior, “ethical” alternative to embryonic stem cell-based approaches.

While iPS cell research holds significant promise, its purported benefits may have been overstated by certain religious groups opposed to research involving human embryos, by stem cell scientists keen to embrace a technology free of the regulations governing human embryo research, and by a patient community willing to believe the rhetoric of scientific “advance”. For example, Australians for Ethical Stem Cell Research have labelled iPS cells as “functionally identical” to human embryonic stem cells but “ethically uncontentious: it does not use women’s eggs and does not create and destroy human embryos”. Prominent scientists in iPS cell research have said that “the promise of regenerative medicine could soon be met” and “we could be there in five years time for diseases that are well understood, like Parkinson’s.” The public have seemingly accepted this rhetoric. The majority (188 out of 264) of the public submissions to the Heerey Committee, which recently reviewed the Commonwealth legislation governing embryo research and human cloning, “did not support the use of human embryos for research” and supported the use of iPS cells instead. And in a recent survey of 1000 Americans, 61% of respondents asserted that all public funding for embryonic stem cell research should cease and be redirected towards iPS cell research. Increasingly, patients are asking clinicians about stem cell therapies, with a growing number of Australians attracted by overseas clinics offering unsubstantiated stem cell “therapies” (lacking evidence of efficacy or safety) for various chronic diseases – termed “stem cell tourism”.

However, the public optimism surrounding cell replacement therapies (including with iPS cells) disregards the inevitable delays in effective translation to the clinic. Here we consider Parkinson’s disease – one of the key candidate diseases for regenerative medicine. While iPS cell therapy has been proposed as a potential “cure” for Parkinson’s disease (Figure 1 – located at the end of this document) involving the autologous transplantation of midbrain
dopaminergic neurons which may integrate into the host brain and restore motor function (as demonstrated in animal studies), many barriers remain to be overcome before this procedure can be adopted in the clinic. Some of these barriers are particularly salient in Parkinson’s disease, like obtaining consent and assessing the cognitive status of patients, but they are also relevant to other diseases targeted by iPS cell therapy.

**Scientific and clinical challenges**

Unlike embryonic stem cells which are harvested directly from human embryos, iPS cell technology requires reprogramming somatic cells towards pluripotency. Initially, retroviral vectors were used to deliver the pluripotency-inducing transgenes into the somatic cells. However, as retroviral vectors may randomly integrate into the host genome and trigger the expression of cancer-promoting genes, scientists have investigated alternative techniques like removing transgenes from genomic integration sites once reprogramming is complete, delivering the transgenes via non-integrating DNA microcircles, and exposing somatic cells to a cocktail of small molecules – approaches which have yielded varying reprogramming efficiencies. As iPS cell research advances, techniques to produce iPS cells will probably become safer and more efficient. However, this also means that reprogramming methods are at risk of rapidly becoming obsolete and generating the impetus to initiate clinical trials may be difficult. Indeed the field of reprogramming is moving so quickly that according to a recent study, a patient’s somatic cells may be directly reprogrammed into replacement cells of the desired phenotype (e.g. neurons) without the pluripotent intermediary.

A challenge that applies to both iPS cell- and embryonic stem cell-based therapies is deriving clinical-grade dopaminergic neurons. Exposing pluripotent stem cells to specific growth factors successfully induces differentiation into dopaminergic neurons but leaves behind contaminating non-dopaminergic cell types in the culture. Also, to generate the correct type of dopaminergic neuron (i.e. of midbrain phenotype, as implicated in Parkinson’s disease), it may be necessary to include an additional cellular feeder layer or over-express certain genes important for early midbrain development, but it is unclear whether these strategies, which were tested on embryonic stem cells, work with iPS cells. Subtle differences between iPS cells and embryonic stem cells have been reported at the molecular level, as iPS cells may be imprinted with information tracing them back to their cell of origin. Even after continuous passaging in vitro to attenuate these epigenetic anomalies, iPS cells may still have reduced stability, homogeneity and ability to differentiate into all cell types as compared to embryonic stem cells (which possess a more fully “naïve” ground state of pluripotency). Since grafts containing a low proportion of dopaminergic neurons may cause side effects like dyskinesia (sudden switches between mobility and immobility), attaining high purity yields of midbrain dopaminergic neurons is clearly an important prerequisite to first-in-human trials.

Another limitation of iPS cell therapy is that deriving immunologically-matched grafts for each patient would require generating, expanding and testing multiple iPS cell clones before obtaining one suitable for transplantation – a time-consuming and costly process. Contrary to the assumption that autologous iPS cell therapy eliminates the risk of immune rejection, a recent study showed that abnormal surface antigen expression in iPS-cell derived grafts induces a T cell-mediated immune response and tissue damage when transplanted in genetically identical recipients. Therefore, as with embryonic stem cell-based therapies, scientists would need to assess the stability and immunogenicity of each iPS cell clone (for which reliable and efficient assays are currently lacking) before transplantation. Due to these practical limitations, any future application of iPS cells, except for the select few who can
afford personalised therapy, may involve public stem cell banks with limited iPS cell lines representing sufficient genetic diversity to be compatible with the majority of the population. This raises important ethical concerns like resource allocation, donor consent and equity of biological access.20

The absence of reliable animal models of Parkinson’s disease poses an additional barrier to translational research on iPS cell therapy. Although a translational gap exists whenever moving from pre-clinical to clinical testing of any medical intervention, in Parkinson’s disease research, the animal models are notoriously unreliable in predicting the patient response as they are toxin-based and exhibit acute dopaminergic depletions that fail to reflect the broader phenotype associated with the disease.21 For example, a novel class of drugs (monoamine uptake inhibitors) that had yielded promising results in toxin-based primate models produced limited functional improvement and many side effects when tested in patients with Parkinson’s disease.22 Thus, animal models may have limited value in predicting the safety and efficacy of iPS cell therapy for patients.

Assuming iPS cell research is translated to the clinic, patients receiving therapy will require long-term monitoring due to the potential risk of serious, irreversible harm. As with embryonic stem cell-based therapies, grafts contaminated with undifferentiated cells may cause tumour formation. This was tragically illustrated when a patient with ataxia telangiectasia developed a multifocal brain tumour after travelling overseas to receive neural stem cell “therapy” from an unlicensed Russian clinic.23 In response to the real risk of tumour formation, scientists have developed molecular imaging technologies to monitor grafts post-transplantation, but these currently lack the spatial resolution and sensitivity to be adopted in the clinic.24 Another strategy is inserting “suicide genes” to render cell lines susceptible to specific drugs that can be administered if the graft undergoes malignant transformation. However, this approach is untested in humans and the administered drugs may have non-specific toxicity.25

Even if iPS cell therapy is shown to be safe, it cannot be assumed that it will be beneficial. As Parkinson’s disease is a multi-system disorder, symptoms like dementia and autonomic dysfunction which are caused by pathology in non-dopaminergic systems (and may be equally disabling as the motor symptoms) may not be improved by dopaminergic grafts alone.26 (The caveat is that a proposed treatment may not need to resolve all disease pathology to provide significant benefit and therefore merit use.) Also, the striatum has been targeted as the primary site of graft placement in virtually all previous animal studies, but this one-size-fits-all approach may be unsuitable for patients since the extent of dopaminergic depletion varies between individuals and may involve extra-striatal regions.26 Thirdly, grafted neurons may not fully integrate into the host neuronal circuitry, resulting in minimal functional improvement.27 Finally, the transplanted neurons may be susceptible to degeneration within the toxic environment of the diseased host brain.28 Although cotransplanting neurons with neuroprotective molecules may hinder disease progression in the host brain and hence improve survival of the grafted cells, this approach remains unproven.29

**Ethical challenges**

The clinical testing of iPS cell therapy also raises significant ethical concerns given the substantial risks involved. First is the degree to which iPS cells must be shown to be safe in animal models before first-in-human studies are justified. Even where it is decided that human trials can begin, serious questions remain regarding subject recruitment, safety monitoring and consent. The high prevalence of dementia and state of dependency amongst
patients with Parkinson’s disease may limit their ability to make voluntary informed decisions about trial participation. The challenge of obtaining informed consent is compounded by the limited accuracy of the currently available clinical tests in assessing individual decision-making capacity. Alternative mechanisms will be required to increase participation of Parkinson’s disease patients in iPS cell therapy trials, including research advance directives and the identification of appropriate surrogate decision makers. The types of research to which surrogate decision makers may consent and the degree of risk to which they may subject the patient will also require clarification.

Furthermore, as with gene therapy trials, subject selection for clinical trials of iPS cell therapy is invariably problematic. Recruiting patients with mild Parkinson’s disease may be preferable as they are more likely to benefit from the procedure and have the capacity to provide informed consent. However, subjecting relatively “healthy” patients to the uncertain risks of stem cell therapy is controversial as their condition is stable and manageable by other available treatment options. From an ethical standpoint, late-stage Parkinson’s disease patients lacking alternative treatment options should perhaps be recruited. Yet these patients may be susceptible to unrealistic expectations of efficacy, be unable to provide informed consent (although surrogates perhaps could) and be least likely to benefit from iPS cell therapy due to an advanced and potentially irreversible state of disease.

Conclusion

iPS cell technology clearly has the potential to transform the field of regenerative medicine and may one day deliver personalised therapeutics for a broad spectrum of chronic degenerative conditions, not merely limited to Parkinson’s disease. Preliminary results from animal studies have been promising and there are compelling reasons for public optimism and support for continued research. Initially, the benefit of iPS cell technology for medicine may be realised in disease modelling and screening of promising new drugs. However, we must recognise that the scientific, clinical and ethical issues facing iPS cell therapy are equally complex as those presented by embryonic stem cell-based approaches and are likely to delay its translation from bench to bedside. iPS cell therapy may indeed prove beneficial in the future, but we must be careful that the rush to embrace its potential is tempered with realism and not just a manifestation of “moral panic” over human embryonic stem cell research.

References


Text box 1. Glossary of key terminology

- **Stem cells** - cells with the ability to divide indefinitely in culture and to give rise to specialised cells. Examples include adult somatic stem cells, embryonic stem cells and iPS cells.

- **Potency** - the capacity of a stem cell to differentiate into different cell types. Embryonic stem cells and iPS cells are pluripotent and can differentiate into cells from all lineages of the body.

- **Embryonic stem cell** – a type of pluripotent stem cell derived in the laboratory from the inner cell mass of a pre-implantation IVF embryo (blastocyst).

- **iPS cells** – a type of pluripotent stem cell created in the laboratory from a non-pluripotent cell, typically an adult somatic cell, by reactivating the expression of specific genes associated with pluripotency.

- **Autologous transplantation** - the transplantation of cells, tissues or organs which have been derived from a particular individual back into the same individual, with perfect immunological compatibility between donor and recipient.

- **Transgene** - a segment of genetic material (DNA) that is transferred from one organism to another organism.

- **Stem cell tourism** – where patients seek unproven stem cell-based treatments outside the accepted clinical trial framework. Many companies offering such “treatments” use aggressive online advertising strategies to recruit patients and charge considerable sums of money with little or no evidence of efficacy or safety.
Figure 1. Differentiation of patient-matched iPS cells into dopaminergic neurons for transplantation in Parkinson’s disease patients. (Reproduced in an abridged form from Kiskinis E, Eggan K. Progress toward the clinical application of patient-specific stem cells. J Clin Invest 2010; 120: 51-59.)