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Induced pluripotent stem cell therapies for retinal disease

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Abstract

Purpose of review: This review will discuss how recent advances with induced pluripotent stem (iPS) cells have brought the science of stem cell biology much closer to clinical application for patients with retinal degeneration.

Recent findings: The ability to generate embryonic stem (ES) cells by reprogramming DNA taken from adult cells was demonstrated by the cloning of Dolly the sheep by somatic cell nuclear transfer over ten years ago. Recently it has been shown that adult cells can be reprogrammed directly, without the need for a surrogate oocyte through the generation of induced pluripotent stem (iPS) cells. The method of reprogramming has since been optimised to avoid the use of retroviruses, making the process considerably safer. Last year human iPS cells were isolated from an 80 year old patient with neurodegenerative disease and differentiated into neurons in vitro.

Summary: For stem cell therapies, the retina has the optimal combination of ease of surgical access, combined with an ability to observe transplanted cells directly through the clear ocular media. The question now is which retinal diseases are most appropriate targets for clinical trials using iPS cell approaches.

Keywords

retinitis pigmentosa; transplantation; iPS cell; photoreceptor; clinical trials

Introduction

The retina, as part of the central nervous system (CNS), is made up of neurons which degenerate progressively throughout life. Like elsewhere in the CNS, retinal neurons in the mammalian class of vertebrates have little ability to regenerate or repair after injury – a problem that has perplexed experimental neurologists for well over a century [1]. In recent decades, however, it has become apparent that the mammalian visual pathway still exhibits considerable plasticity. In rodents, adult retinal ganglion cells can regenerate along a peripheral nerve graft to form functional synapses in the tectum [2–4] and during late

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development, ganglion cells can regenerate and navigate through the optic chiasm in a marsupial mammal [5]. These observations and others confirm that the adult visual pathway is capable of reforming new synapses and may have a capacity to guide navigation of regenerating axons in certain circumstances. In other words, if regenerating neurons are presented to the adult diseased CNS and the correct conditions are met, there is no scientific reason to presume that the host CNS will not accept new synapses with the capability to restore function. Hence the onus for CNS repair shifts to the donor cell and the problem becomes one of obtaining large enough numbers of these cells at the correct developmental stage to optimise any functional benefit following transplantation into the diseased CNS. Until recently this was the limiting step for any clinical applications since embryonic stem cells have ethical restrictions and would in any case most likely be rejected after neuronal differentiation without immune suppression. The generation and subsequent neuronal differentiation of induced pluripotent stem (iPS) cells, however, has removed many of these limitations and it is appropriate now for clinicians to begin considering which diseases might be applicable for early clinical trials.

The photoreceptor layer of the outer retina may be one CNS region ideally suited to cell transplantation studies. The subretinal space is a surgical cleavage plane that opens with minimal trauma following an injection of fluid (a form of retinal detachment) and physically traps transplanted cells against the tissue plane into which they must integrate - for full review, see [6]. The clear optical media facilitate the assessment of cells anatomically with imaging techniques such as optical coherence tomography (OCT) and vision testing can provide a precise functional assessment. Most importantly, several groups have demonstrated successful transplantation of photoreceptors into the degenerating mouse retina, showing synaptic connections between grafted cells and host retina [7,8] and functional improvements in vision [9,10]. Although most studies have shown that the immediate post-mitotic developmental stage of the photoreceptor may be critical [8,9,10], there is also evidence that more mature cells may survive and form synapses after transplantation [11]. Approaching the donor developmental stage question from the other direction, Lamba and colleagues [12] used defined culture media to differentiate human embryonic stem (ES) cells to the point where they expressed photoreceptor precursor transcription factors, similar to the stages identified as optimal in the mouse. In finding that these cells could also integrate into the degenerating mouse retina and restore visual function, they provided direct evidence that the previously identified developmental donor stage may translate to human cells [12].

These vigorous scientific observations over the last two decades have therefore provided us with a framework and guiding principles to understand the mechanism whereby the retina might be repaired by photoreceptor transplantation. Despite this, the clinical applications of retinal stem cell treatments have been limited by the absence of a practical and/or ethically acceptable means of obtaining large numbers of human cells at the correct developmental stage. The recent development of human iPS cells, however, and specifically their induced differentiation into cells with human photoreceptor phenotype [13], has now provided us with the opportunity for embryo-free autologous transplantation. This brings the research into the arena of the clinician.

Induced pluripotent stem (iPS) cells

With the exception of the gametes, it is clear that virtually every nucleated cell in the body of any given individual has an identical genomic DNA structure. What identifies a particular cell over any other of the approximately 220 different cell types in the body is which of the genes within this DNA is switched on and which is off. This process occurs step wise throughout development, so that initially, genes regulating growth and cell division are

active, while later on, genes regulating specific cell functions are switched on and the former switched off. In practical terms this is regulated by DNA binding proteins known as histones (particularly histone H3, which is activated either to close or open DNA in specific regions), or by direct methylation of the DNA promoter sequences upstream of specific genes—reviewed in detail elsewhere [14]. These epigenetic changes to chromosomal DNA essentially regulate the access of transcription factor proteins to specific genes and hence provide the necessary molecular switches.

The successful creation of Dolly the sheep over ten years ago by somatic cell nuclear transfer (SCNT) proved for the first time that the DNA of adult cells could be reprogrammed – in other words, the histone modification and DNA methylation could be reversed back to the ES cell pattern by undefined factors within the cytoplasm of a donor oocyte [15]. Ten years later, using a modified nuclear labelling technique, the process of SCNT was achieved in primates [16]. With the exception of mitochondrial DNA, the end-stage differentiated cells created by SCNT would be genetically identical to the cell obtained from the donor animal and could therefore in theory be transplanted back into the original animal as functional cells without risk of rejection. Therapeutic applications of SCNT are however still limited by the practical and ethical difficulties of obtaining human oocytes. It was not until 2006 that a series of four transcription factors (Oct4, Sox2, Klf4 and c-Myc) capable of reprogramming DNA without need for an oocyte were identified by Takahashi and Yamanaka [17]. Subsequently the reprogrammed cells were referred to as ‘induced’ pluripotent stem (iPS) cells (see Figure 1).

Proof that a single cell has been reprogrammed to the point where it is truly pluripotent (as an ES cell) can be achieved if that cell subsequently differentiates into a normal and fully functional organism. Pluripotent cells contribute to every cell type of the embryo but this excludes the extraembryonic tissues, so after induction iPS cells need to be injected into a blastocyst to support their embryonic development and any mixing with residual ES cells will generate a chimera. For iPS cells, this proof was shown the following year when the two-week iPS cell reprogramming protocol was optimised by selecting cells expressing the Nanog pluripotent gene, leading to viable chimeras [18] and also mice made up entirely from iPS cells [19]. Subsequent technical advances in cloning have enabled the generation of iPS cells from human fibroblasts [20] and without the c-Myc gene, which significantly reduces the risk of tumour formation although with a lower efficiency [21]. Most recently the method of iPS cell cloning has been optimised to avoid retroviruses by using episomal vectors, which do not become incorporated into the genomic DNA and would therefore generate cells free of viral and transgene sequences altogether [22]. Human iPS cells have now been cloned from patients with neurological diseases such as Parkinson's disease, muscular dystrophy and Huntingdon's disease [23]. IPS cells cloned from skin cells of a patient with amyotrophic lateral sclerosis have been differentiated into motor neurons, the cell type requiring replacement in this disease [24]. Given the real progress with generating iPS cells from patients, the question now relates to how these cells might be transformed into photoreceptor cells or precursors, so that experimental clinical trials can begin to replicate the laboratory work.

Generation of photoreceptors from iPS cells

The generation of photoreceptor cells from ES cells was first described by the Takahashi group through a series of elaborate steps. Initially ES cells were induced to express retinal progenitor cell transcription factor markers, Pax-6 and Rx, by culture and exposure to Wnt and Nodal antagonists (which are required for gastrulation [25]) and an activin/serum combination to induce the retinal cell phenotype [26]. Subsequently the protocol was optimised by the addition of retinoic acid and taurine to direct differentiation towards

photoreceptors and included human ES cells [27, 28]. Finally the ES cell protocols have recently been replicated in iPS cells, generating both human photoreceptor and retinal pigment epithelium (RPE) phenotypes [13].

Proof that a differentiated iPS cell is truly a photoreceptor is difficult to achieve in vitro for several reasons. First in the normal state the photoreceptor resides in the densely packed outer nuclear layer of the outer retina, with inner segments bound to Mueller cells through tight junctions of the outer limiting membrane and outer segments shedding discs towards the RPE. This unique integrated structure leads to the photoreceptor adopting features secondary to the retinal environment, such as a single spherule synapse connected by a long cytoplasmic fibre to the nucleus that may not exist in isolated photoreceptors in vitro. Second, the functional test of photosensitivity is not exclusive to photoreceptors as it becomes a property of any neuronal cell that happens to express melanopsin [29]. Finally the differentiation of photoreceptors in vivo is also regulated by direct contact to other surrounding cells which cannot easily be replicated in vitro. The RPE-photoreceptor interaction is critical for foveal photoreceptor development, as evidenced by foveal hypoplasia in albinism when melanin synthesis is defective in the RPE [30]. Similarly Mueller cells connect to photoreceptors through zona adherens junctions mediated by the CRB1 protein and disruption of this junction leads to a disorganised outer retina with photoreceptor degeneration [31]. Hence in late stages at least, photoreceptor development will be guided by interactions with surrounding retinal tissues. This may explain why photoreceptor features such as a cilium and outer segment discs have not yet been identified following ES or iPS cell differentiation [27]. Nevertheless full differentiation may not be necessary, indeed it may be counter-productive, if the precursor [8-10] rather than fully mature photoreceptor [11] is the optimal cell for transplantation.

When considering therapeutic strategies by translation of basic science, it is also critically important to assess the problem from the clinical perspective. In the case of photoreceptor transplantation, three clinical observations should be considered in the design of the first clinical trials. In the first instance it is widely accepted that photoreceptors will not survive without a functional underlying RPE layer [32]. Thus any iPS cell transplantation strategy to treat diseases that affect both layers, such as age-related macular degeneration (AMD), should aim to replace the RPE cells (see below) prior to photoreceptor transplantation. Second, it is also well known that cone photoreceptors cannot survive without rods. Hence classic retinitis pigmentosa due to defects in rod-specific genes such as RHODOPSIN and NRL presents initially with rod loss, but inevitably cone loss follows shortly after, leading to complete loss of sight [33]. Transplantation of iPS cell-derived cones into the totally degenerate retina is therefore a flawed strategy, because there is every reason to believe that the transplanted cones will degenerate by the very same rod-related mechanism, even if they are perfectly differentiated and optimally connected. The strategy should therefore include a mix of both rods and cones, or alternatively a combined gene therapy approach to re-express rod-related proteins to sustain transplanted cones in the subretinal space [34]. Finally many patients with retinitis pigmentosa lose photoreceptors throughout life but the time course can be slow, implying that the half lives of photoreceptors affected by milder genetic mutations may be many years. Autologous photoreceptors generated by iPS cells isolated from an affected patient will also retain the genetic mutation, however, although gene defects retained in iPS cells can be repaired at the reprogramming stage [35], this may not actually be necessary if the degeneration is slow enough. This would simplify the cloning steps, facilitating the generation of greater numbers of pluripotent cells, which is currently the rate-limiting step in the whole process. Hence looking at the problem from the clinical perspective, an initial trial might be designed to assess the ability of autologous iPS-derived rods in preventing cone loss after transplantation into patients with chronic rod-cone dystrophies.

Generation of RPE from iPS cells

Given the prevalence of AMD and increase in the elderly population, it is not surprising that much ophthalmological interest focuses on the use of iPS cells to regenerate the RPE. However the scientific argument for an iPS cell approach is not as clear cut as for the neurosensory retina. This is because AMD is a focal disease affecting the macula and the surrounding retinal tissue remains relatively healthy, allowing autologous transplantation of the adult tissue without the need for stem cells (similar to an autologous skin graft). This proof of concept has been demonstrated in clinical studies showing an improvement in vision following autologous grafts of the RPE and choroid in exudative AMD [36-37], geographic atrophy [38] and even a case of inherited eye disease [39]. The argument for an iPS cell approach is that the surgery is likely to be simpler and certainly one of the main drawbacks of autologous transplantation relates to the complications, such as haemorrhage or uncontrolled proliferation of RPE cells arising from the large donor site in peripheral retina [37].

Human ES cells have been shown to differentiate relatively easily into RPE cells, possibly because these arise quite early in development [40] and similar techniques can be applied to iPS cells to regenerate RPE cells in vitro [13,41]. Here again however it is helpful to re-examine the problem from the clinical perspective, because AMD is also a disease of Bruch's membrane and there is no evidence that this is generated together with RPE cells after iPS cell cloning. One possible explanation for this is that the two structures are embryologically distinct; with RPE cells arising from the neuroectoderm of the eye cup and Bruch's membrane developing from the invaginating endoderm of the choriocapillaris [42]. It is likely that both layers would need to be replaced because healthy RPE cells do not grow well on damaged or aged Bruch's membrane in vitro [43]. In a previous clinical study, relatively healthy RPE cells harvested from the nasal retina were transplanted onto the diseased Bruch's membrane under the macula of patients with AMD who had recently developed neovascular complications [44]. In that study the transplanted RPE cells were unable to restore a functional monolayer on the diseased tissue, but residual cells left at the donor site were able to proliferate and cover the surgically exposed Bruch's membrane unaffected by AMD in the nasal retina [44]. Hence the clinical observations have provided excellent proof of concept that RPE replacement in AMD will require restoration of Bruch's membrane and this remains one of the limiting factors for translation of this particular field of iPS cell research.

Generation of ganglion cells from iPS cells

There are many examples of generating human neuronal cells from iPS cells [24, 45] and a preliminary report of generating ganglion cells from ES cells [46]. No doubt generation of ganglion cells from human iPS cells will soon follow, however, the problem with repairing the optic nerve is not so much one of obtaining the correct donor cells, but reconnecting these cells appropriately to central targets [47]. For diseases such as glaucoma or traumatic optic nerve injury, reconstruction of the optic nerve by regeneration of ganglion cells is arguably the only way to restore sight and the challenges here are very similar to neuronal regeneration elsewhere in the CNS. The ganglion cells would need to regenerate axons across relatively long distances and through the heavily myelinated optic nerve, which is a known impediment to axon growth [48]. Finally even if long distance regrowth is possible and proximal connections in the retina can form with bipolar/amacrine cells, it is not clear whether or not the ganglion cells would be able to reform an appropriate retinotopic map in the lateral geniculate nucleus. The challenges for ganglion cell transplantation to be successful are therefore huge in comparison to the relatively straightforward photoreceptor paradigm.

As an alternative, however, it may be possible to use iPS cells to sustain degenerating ganglion cells by inhibition of apoptosis. Recently oligodendrocyte precursor cells [49] and Mueller cell-derived stem cells [50] have slowed optic nerve degeneration by integrating into the degenerating nerve fibre layer in the rat glaucoma model. A similar strategy of slowing degeneration, by supporting the inner retina through an indirect trophic effect, may be the most logical application of iPS cells in clinical trials for glaucoma.

Conclusion

The development of iPS cells over the last three years has overcome many potential drawbacks of exploratory clinical trials because therapeutic cells can now be obtained directly from the patient. It is difficult to see how this will not revolutionise our treatments for degenerative disease over the next century. Some problems remain, such as risk of teratoma formation and the successful conversion rate of reprogrammed cells is still relatively low at less than 1%. Nevertheless the ability to image transplanted cells directly through the clear optical media and subsequently ablate them painlessly with laser (if proliferation is seen) may make the retina the safest site for the first iPS cell clinical trials.

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Figure 1.
Embryonic or induced pluripotent stem cell routes to photoreceptor genesis