Educational Article

Potential of Induced Pluripotent Stem Cells as a therapy in untreatable ocular diseases

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ABSTRACT

Human induced pluripotent stem cells (iPSCs) originate from human somatic cells by introducing certain transcription factors. They can then divide indefinitely being able to differentiate into every cell type. Recently, various ocular cells, including corneal epithelial-like cells, retinal pigment epithelium (RPE) cells, photoreceptors, and retinal ganglion cells, have all been successfully derived from iPSCs. Transplanting the iPSCs in animals is very promising. The first clinical trial on humans started in 2013. More work and research has to be done to ideally promote iPSCs integration into the host tissue, to prevent tumor growth, and to develop functionality of the transplanted cells.

Key words: Stem Cells, RPE Cells, iPSCs, Photoreceptors, Retinal Ganglion Cells.

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INTRODUCTION

The retina is a complex multilayered neural tissue that converts light energy to electrical signals. These signals are relayed through the optic nerve to the occipital lobe of the brain, achieving the visual processing. Up todate any degeneration of any part of the retina is considered permanent. Age-related macular degeneration, retinitis pigmentosa, and glaucoma, are major causes of irreversible blindness worldwide. Currently there is increasing interest in repairing damaged tissues with pluripotent stem cells which can divide indefinitely and have the potential to generate multiple types of cells. These characteristics of stem cells offer the opportunity to repair virtually all types of tissues, including the retina, through cell replacement or transplantation.

The first pluripotent stem cells that can be induced to generate all types of cells including retinal neurons, are the human embryonic stem cells (hESCs)[1,2]. Stem cells can also be induced from autologous somatic cells. Takahashi and Yamanaka showed that pluripotent stem cells could be generated from mouse fibroblast cultures by adding four transcription factors: Oct3/4, Sox2, c-Myc, and Klf4[3]. Yamanaka and Gurdon by studying the mode of reprogramming mature cells into embryonic cells, won the Nobel Prize in 2012 by observing the fact that induced pluripotent stem cells from autologous somatic cells can eliminate the post-transplantation rejections issues thus resolving all ethical concerns surrounding the use of embryonic cells and having enormous therapeutic potential through tissue modeling[4,5,6].

There are many studies on immunogenicity, potential for

tumor formation and epigenetic aberrations of iPSCs. Miura et al. reported the importance of the type of tissue from which iPSCs are supposed to be originated in order to avoid teratoma formation after transplantation[7]. The iPSCs originated from mouse embryonic fibroblasts and transplanted into murine models, were considered safe,while those originated from adult fibroblasts are considered unsafe because of the insurgence of severe teratoma formation[8]. To date, despite the safety and integrity concerns, studies demonstrating the treatment efficacy in various disease models have made the further research of iPSCs transplantation very interesting and worthwhile.

Degenerative diseases such as age-related macular degeneration and glaucoma, are considered incurable. Utilization of iPSCs could be very promising in replacing corneal epithelial cells, RPE, photoreceptors, and RGCs in order to restore visual function. This is a review on recent developments of stem cell therapy for AMD, corneal dystrophy, and RGCs diseases.

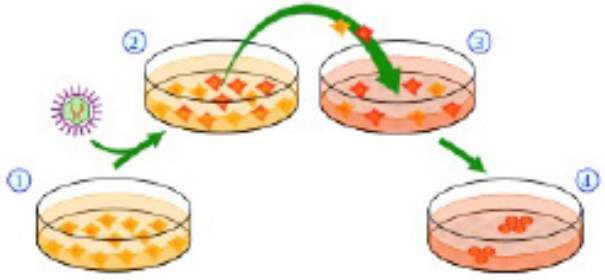

Figure 1: Schematic illustration of iPSC induction and reprogramming into ocular cells.

iPSCs derived Corneal Epithelial-Like Cells

To achieve optimal vision a transparent cornea is indispensable. Superficial corneal damages are self-limited[9,10]. But corneal ulceration and scarring are much more difficult to cure. So came up the idea of using iPSCs derivatives.

Corneal healing proceeds in a centripetal mode[11]. This is due to the adult stem cells derived from limbal cells[12]. In cases of corneal damages in one eye, autologous transplantation from the healthy eye by using limbal cells can be achieved[13]. But this is not possible in bilateral injuries. A valid alternative to this problem is to use corneal epithelial cells derived from full thickness biopsy limbal cells[14]. In this way we have unequivocal improvement of vision.

Considering the need for allogeneic grafts to treat bilateral corneal epithelium deficiency, Homma et al. studied transplantation of ESC-derived epithelial progenitors[15]. In this case immunosuppressive therapy is completely necessary. The early development of iPSC-derived cells using two molecule inhibitors was recently studied by Mikhailova et al.[17] Modulating intracellular pathways to differentiate ESCs or iPSCs, could lead to produce neuroectoderm[18] or surface ectoderm (corneal epithelium). Thus these studies are very promising showing the possibility of using iPSC-derived cells in autologus treatment of corneal diseases.

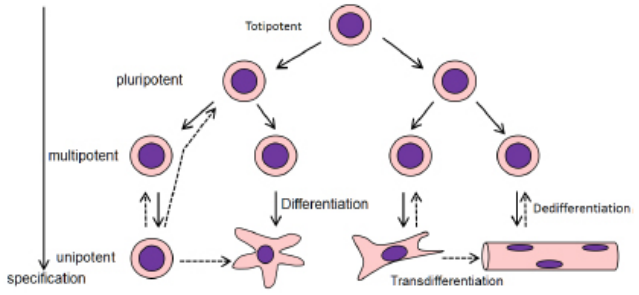

Figure 2: Schematic illustration of corneal epithelial cell differentiation from ESCs or iPSCs.

Photoreceptor Degeneration Therapy using iPSC-Derived RPE

Retinal degeneration is untreatable and irreversible up today, indifferently to the time of onset. It may occur in younger patients such as in Stargardt's disease, or in eldery patients with macular degeneration. The possibility of repairing the degenerated cells with iPSCs is astonishing.

Rod and cone photoreceptors work closely together with the RPE. Its main function is to transport nutrient and waste products, participate in vitamin A-rhodopsin conversion cycle, phagocyte outer segments of photoreceptors, and absorb scattered light. Photoreceptors are connected to retinal ganglion cells (RGCs) through bipolar cells. The cell bodies of RGCs reside in the ganglion cell layer, and bipolar cell nuclei reside in the inner nuclear layer. The axons of the RGCs meet to form the optic nerve.

Dysfunction of the RPE can be a disease-initiating event. There is the possibility of correlation with age-related macular degeneration[19] and retinitis pigmentosa[20]. RPE cell transplantation has thus been investigated both in animal models and in humans for a long time[21]. There are two possibilities of RPE cell transplantation either injected as a cell suspension or on a monolayer cell sheet. The membrane potential, ion transport, and secretion of vascular endothelial growth factor in RPE cells derived from iPSCs are all similar to native RPE[22]. Telomere shortening and rapid senescence were observed after several cell cycles, determining impaired function[23]. Only cells from the first passages could eventually be used to preserve correct functioning of RPE

cells[22]. Molecular markers can be used to determine the differentiation status of the iPSC derived RPE cells [24].

Replacing damaged Photoreceptor Cells

Damaged photoreceptor cells can be replaced, indifferently from RPE status. Replacement of photoreceptor cells is definitely difficult and challenging because new neurons have to form and develop appropriate neuronal connectivities. Precursor photoreceptor cells survive and improve visual function after subretinal transplantation[25]. Human ESCs can be used to differentiate into retinal neuron progenitors, which can then be ulteriorly differentiated into similar photoreceptor cells and RGCs[26-28]. Tumor formation was observed after transplanting early stage retinal progenitors. But using late stage retinal progenitors showed no efficient integration with the retina[27]. However injecting cells in the outer nuclear layer (ONL), determined the formation of new outer segments[26]. Using these cells in cases of Leber's congenital amaurosis an improvement in visual function was observed. However, the use of a small amount of integrated cells should be taken into account.

Photoreceptors generated from iPSCs preserve characteristics of membrane current, gene expression and intermembrane channels[29]. Nevertheless there are many questions regarding the generation of photoreceptors from iPSCs. These issues involve a highly homogeneous population of donor photoreceptors, adaptation of the host environment to allow survival and integration of the grafted cells, and immune suppression. This is the basis for iPSC-based retinal therapy. Lamba's group studied injection of iPSC-derived photoreceptors in wildtype mice[30]. A small amount of subretinally transplanted cells had migrated to the ONL three weeks after the subretinal transplantation. Similar integration into the ONL was observed in a degenerative swine model, after transplantation of swine iPSC-derived rods[30]. However by using electroretinography no improvement of retinal function was found. But this could be explained by the small amount of injected cells. These studies are promising in treatment of previously untreatable blinding diseases.

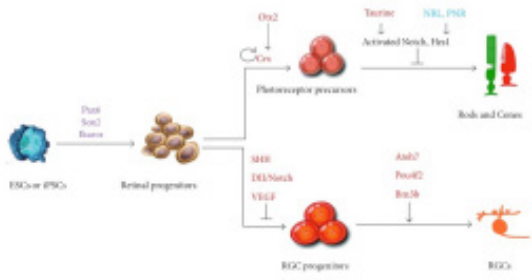

Figure 3: Schematic illustration of photoreceptor (rod and cone) or RGC production from ESCs or iPSCs

Replacing RGCs and repairing Optic Nerve

Replacement of retinal ganglion cells is necessary in glaucoma and optic nerve injury,because they are the major cell type affected in these conditions. The replacement of these cells has huge obstacles compared to the replacement of any other retinal cell types,because retinal ganglion cell fibers must extend through the optic nerve and connect with appropriate visual centers. Injection of retinal ganglion cells progenitors into the intravitreal cavity of rats showed migration and integration into different layers of the retina by a small number of cells. Trying to protect and regenerate the optic nerve, human iPSC-derived neural progenitors were injected into rats with optic nerve damage[31]. Observed cells integrated into the retina, thus leading to surviving optic nerve axons, and a significant increase in visual capacity.

The regeneration of three-dimensional optic vesicle-like structures with layered retinal neurons has recently been reported, by using mouse embryonic stem cells and human iPSCs[32,33]. In 28 weeks the development of retinal ganglion cells and other types of retinal cells, were observed but without optic nerve restructure. This eye cups culture system provides an optimum opportunity for studying the factors that control retinal ganglion cell pathology, regeneration, and development. Developments in iPSC field offer great opportunities for therapy development of previously incurable retinal diseases.

iPSC-derived cell transplantation

The innate capacity of iPSCs to divide indefinitely is extremely important in obtaining sufficient cell numbers for transplantation. But there is the probability of uncontrolled cell divisions, that could lead to the insurgence of ocular tumors. Thus research studies in animal models about the tumorigenicity of human stem cells is extremely important ,before human clinical studies can be performed [34]. Kanemura et al studied the injection of suspensions of human iPSC-derived RPE cells into the subcutaneous tissue of immunodeficient mice[35]. Also no tumor growth was observed by performing subretinal injection of human iPSC-derived RPE cells, up to 15 months after transplantation. Moreover no tumor formation was seen in rats injected with iPSC-derived RPE cells suspension subretinally[36]. Completion of clinical trials for subretinal transplantation of human ESC-derived RPE suspension in AMD or Stargardt's disease patients demonstrated the absence of adverse events up to 22 months[37,38]. Ongoing clinical trials for human ESC or iPSC-derived RPE cells, are hopeful for stem cell-based therapies without adverse effects like tumor growth.

Autologous cell replacement using iPSC-derived RPE in AMD therapy by injecting cell suspensions, probably does not provides long-term cell survival. The transplanted cells

were no longer detectable after 3 months[36]. But an improved visual response in the transplanted eye, demonstrated a possible protective effect. In another study iPSC-derived RPE suspension was transplanted into mice with retinitis pigmentosa[39]. By injecting small number of cells there was an improvement in electroretinography, with no tumor growth. But transplanting human iPSCs into a rod photoreceptor dystrophic mouse model showed integration and cell to cell contacts[40]. In this case RPE cells may be developed as a monolayer on an artificial scaffold in vitro. Currently there are researches on using or not the idea of the artificial scaffold to increase survival and therapeutic capacity of RPE monolayer in retinal diseases.

Conclusions

Many studies have been done or are still running on integrity, tumorigenicity and therapeutic uses of reprogrammed stem cells for neurodegenerative conditions in ophthalmic field. The increasingly improved quality of iPSC-derived RPE cells and photoreceptors, deriving corneal epithelium cells, RGCs is really encouraging. The astonishing in this new field is that these cells derive from adult somatic cells using several transcription factors, without the involvement of human embryonic tissues. Thus offering patients the opportunity to receive autologous cell therapy [41]. The complexity and the variations in reprogramming technology and protocols in cell ocular differentiation are so big, that the full potential of iPSC-based therapy is yet to be realized. However there are important issues to be solved. Such as the potential risk of tumor formation, and the long-term effects of reprogramming somatic cells. The technology of iPSCs offers individualized disease modeling and personalized autologous grafts for transplantation purposes. It opens a window in therapy of previously untreatable diseases.

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