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Induced Pluripotent Stem Cell Therapies for Geographic Atrophy of Age-Related Macular Degeneration

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Abstract

There is currently no FDA-approved therapy for treating patients with geographic atrophy (GA), a late stage of age-related macular degeneration (AMD). Cell transplantation has the potential to restore vision in these patients. This review discusses how recent advancement in induced pluripotent stem (iPS) cells provides a promising therapy for GA treatment. Recent advances in stem cell biology have demonstrated that it is possible to derive iPS cells from human somatic cells by introducing reprogramming factors. Human retinal pigment epithelium (RPE) cells and photoreceptors can be derived from iPS cells by defined factors. Studies show that transplanting these cells can stabilize or recover vision in animal models. However, cell derivation protocols and transplantation procedures still need to be optimized. Much validation has to be done before clinical-grade, patient-derived iPS can be applied for human therapy. For now, RPE cells and photoreceptors derived from patient-specific iPS cells can serve as a valuable tool in elucidating the mechanism of pathogenesis and drug discovery for GA.

Keywords

cell replacement; disease models; photoreceptors; retinal pigment epithelium

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in elderly populations in the developed world. Typical symptoms include decreased central vision, central scotoma, and metamorphopsia. Vision loss is attributed to advanced AMD resulting from geographic atrophy (GA, also called advanced “dry” AMD), apoptosis of the retinal pigment epithelium (RPE) and overlying photoreceptors, or from choroidal neovascularization (CNV, also called “wet” AMD), the invasion of RPE or retina by abnormal blood vessels.¹

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Significant advances in the understanding of CNV pathogenesis have led to several FDA-approved therapies for wet AMD, including anti-VEGF agents, photodynamic and laser therapy for wet AMD.^{2–4} In contrast, GA pathogenesis is still nebulous and there is no FDA-approved therapy for the one million people in the United States who already have GA and the millions more who are at risk.⁵ The main pathological changes of GA are apoptosis of RPE cells and photoreceptors. As of today, there is no treatment available to repair damaged foveal RPE cells or photoreceptor cells. Therefore, replacement of these damaged cells with healthy cells is considered to be a promising therapy for GA in the future.⁶

The eye is considered to be one of the ideal organs for cell transplantation due to its specific structural characteristics. First, the clear optical media facilitates intravitreal injection or subretinal injection of cells. Second, imaging techniques, such as scanning laser ophthalmoscope and optical coherence tomography, are allowed for the assessment of transplanted cells. Third, treatments are available to ablate the cells by laser if they over-proliferate.^{7–10}

Many cells have been tried for transplantation to replace the lost RPE cells and photoreceptors, including fetal or adult cells,^{6, 11, 12} embryonic stem (ES) cells,^{13–18} other stem cells,^{19–25} and autologous RPE or iris pigment epithelium (IPE) cells.^{6, 26–32} However, the future use of these cells may be hindered by obstacles, such as limited cell source, ethical debate, and immunological issues. In 2006, Shinya Yamanaka's³³ group reported for the first time that mouse fibroblasts can be induced to ES-like cells and named them “induced pluripotent stem (iPS) cells.” Since then, RPE cells and photoreceptors have been generated from human iPS cells and have been transplanted in many animal disease models.^{34–38} In this article, we will summarize the recent advancement in iPS cell technology and discuss its potential for treating patients with GA. The review will also highlight the limitations that need to be overcome and plausible strategies for the application.

Epidemiology of Geographic Atrophy

AMD is a leading cause of legal blindness in persons 65 years of age or older. Currently, it is estimated that 1.75 million individuals suffer from this disease in the United States and about 7 million are reported to be “at risk.”³⁹ GA accounts for a third of AMD and is responsible for about 20% of the legal blindness related to AMD. For people age 75 and above, the prevalence of GA is about 3.5%.⁴⁰ In people 85 years of age or older, GA was found to be present in about 25% of eyes with a visual acuity of 20/200 or poorer.⁴¹ The 15-year cumulative incidence of GA in persons 43 to 86 years of age with signs of early AMD is 14%.⁴²

Pathology of Geographic Atrophy

The RPE is a neuroepithelium-derived, cellular monolayer that lies on Bruch's membrane between the photoreceptor outer-segments and the choriocapillaris. Its apical portion faces the outer segments of photoreceptors, and its basolateral surface interacts with the choriocapillaris. Together with the selectively permeable Bruch's membrane (BM), it serves as the metabolic gatekeeper between the photoreceptors and the choriocapillaris. Daily, the RPE phagocytose a portion of the photoreceptors' outer segments.⁴³

The RPE and BM suffer cumulative damage over a lifetime. GA is believed to be caused by RPE atrophy, which consequently leads to subsequent damage of the photoreceptors and the underlying choriocapillaris. The first visible change of GA is drusen formation. Drusen is thought to be accumulations of vast amounts of lipofuscin. The pathogenesis of drusen is not completely understood; however, oxidative stress and/or blue light damage along with age-

related decline in lysosomal enzyme function, antioxidant levels or melanin function, and toxic RNA have been implicated.^{44–47}

RPE cell dysfunction can be seen in pigment irregularities with either hyper- or hypopigmentation, which often presents together with drusen. As the disease progresses, drusen might disappear and RPE cell atrophy becomes more significant.^{48, 49}

Treatment of Geographic Atrophy

Promising treatments for wet AMD have been developed and subsequently approved by the FDA to inhibit the processes involved in disease progression and vision loss. In contrast, until recently, no comparable treatment is available for GA patients to prevent or reverse visual loss. Since the main pathologic change of GA is RPE atrophy and later photoreceptor loss, healthy RPE transplantation at the early stage or RPE and photoreceptors transplantation at the advanced stage may prevent development of geographic atrophy.⁵⁰ In addition, transplantation may possibly repair the degeneration and recover macular function due to GA.

RPE Transplantation

Various cell types have been used in RPE cell replacement, including immortalized human RPE cell lines,⁵¹ human embryonic stem (hES) cells-derived RPE cells,^{13–16,52} fetal and adult RPE cells,^{11,12} and some non-RPE cells.^{24, 26, 53, 54} Human ES cells are totipotent cells arising from the eight-cell morula stage that can undergo unlimited self-renewal and differentiate into any adult cell type. By using defined culture conditions, hES cells can differentiate into RPE, and therefore can serve as a source of RPE for transplantation.

Studies have shown that transplanting hES cell-derived RPE cells into the subretinal space of animal models can protect the photoreceptors from death and preserve visual function. However, these animals need daily doses of immunosuppressant injections which can be cumbersome for future human therapy. Currently, there is one instance of stem cell-based treatment for retinal disease (Stargardt's Macular Dystrophy) under pre-clinical trial.¹⁶ Fresh fetal and adult RPE cells have been employed to replace lost RPE in patients with GA, but there are limitations in this approach since there is considerable variability in the quality of the obtained RPE.^{55, 56} More importantly, the RPE cells from hES cells, fetal or other adult material are not autologous, and transplantation of these allogenic RPE cells could result in rapid and pronounced graft rejection. In addition to issues related to transplant rejection, the use of hES cells is still ethically debated.

One way to circumvent long-term systemic immunosuppression following RPE transplantation is to use autologous cells for implantation. These include RPE cells or a RPE-choroid sheet from the peripheral part of the same eye. Many centers have tried to translocate RPE cells or RPE-Bruch's into the fovea of AMD patients after CNV membrane removal. The results were that the visual acuity can be enhanced or maintained for a certain period of time. Unfortunately, trauma from surgery resulted in other complications, including hemorrhage, proliferative vitreoretinopathy formation, graft dislocation, and recurrent neovascularization membrane.^{7,28–30,57} Another problem of this one time strategy is that it is difficult to get grafts of sufficient size and RPE and Bruch's membrane of satisfactory quality.⁵⁸

IPE from the same eye has also been used for transplantation. Reasons for employing IPE include: same embryogenesis as RPE, ability to phagocytose outer segments, and easy to be harvested.^{26, 59} Prevention of further loss of vision or some degree of improvement has been reported by IPE implantation.^{26, 31, 60, 61} However, the lack of crucial enzymes involved in the retinoid visual cycle may affect its further use in the replacement of RPE.⁶² Another

disadvantage of autologous transplantation of RPE and IPE is that the transplants may have the same gene defect as the host.

Photoreceptor Transplantation

Protocols for differentiating photoreceptors from ES cells have been extensively optimized by Osakada et al.^{14, 63} and Lamba et al.^{18, 56} in recent years that allow ES cell-derived photoreceptors to be a possible cell source for transplantation.¹⁸ Other donor cells include fetal retinal cells or fetal retinal tissue.⁶⁴ After transplantation, the dissociated cells or whole neuroretina sheets can differentiate, survive, and improve some extent of visual function.^{65, 66} However, the major disadvantages of ES and fetal cells are ethical restrictions and immune rejection following implantation.

In addition to ES and fetal cells, neural stem cells from brain, retinal stem cells from the developing retina and adult stem cells from bone marrow have also been explored as possible replacements for diseased photoreceptors. Major limitations of these cell sources as potential transplantable cells include limited cell quantity, difficulty in cell harvesting, and difficulty to differentiate the cells into viable photoreceptors. These limitations may influence their potential use as transplantable cells.

Induced Pluripotent Stem Cells and its Recent Development

Induced pluripotent stem cells were first introduced in 2006 by Takahashi and Yamanaka in their groundbreaking paper.³³ They screened 24 potential candidates that were thought to be essential for maintaining pluripotency and self-renewal of hES cells, and found that ectopic expression of a combination of just four transcription factors: octamer 3/4 (Oct4), SRY box-containing gene 2 (Sox2), Kruppel-like factor 4 (Klf4), and c-Myc, (abbreviated as OSKM) was sufficient to reprogram murine fibroblasts to an embryonic-like state using a retroviral system.³³ In the following year, the same group successfully generated human iPS by introducing human orthologs of the four transcription factor-encoding genes into human fibroblasts.⁶⁷ Concurrently, James Thomson and colleagues also reported that a slightly different combination of genes comprising POU5F1 (OCT4), SOX2, NANOG, and LIN28A (LIN28) (OSLN) can transform human fibroblasts into iPS cells using lentiviral system.⁶⁸ Studies from these two groups reinforce the importance of stem cell biology in regenerative medicine.

Induced pluripotent stem cells are morphologically identical to embryonic cells, display similar gene expression profiles and phenotypic markers, can self-renew, and retain the potential to be differentiated into all cell types in the body.^{33, 67, 68} Unlike ES cells, iPS cells are derived from terminally differentiated cells, such as skin fibroblasts or B lymphocytes, without the need of destroying embryos or oocytes as in ES cell derivation. Therefore, applying human iPS cells for future stem cell-based therapy will be more acceptable than human ES cells given that there are no ethical and political issues involved.

The application of iPS cells is more widespread than ES cells since the cells can be generated from the patients themselves. Generation of patient-specific pluripotent cells presents more advantages than merely providing a source of cells for transplantation. There is lower risk of allogeneic immune rejection since the transplanted iPS cells are derived from the patient to be treated. Furthermore, patient-derived iPS cells allow for the study and treatment of diseases, through the development of disease models and ultimately transfer of corrected gene autologous progenitors. This is particularly important for diseases that lack adequate in vitro or animal models, such as disorders affecting the brain and heart. Such disease/mutation-specific cell lines also offer an opportunity to map out the developmental course of complex medical conditions, such as diabetes and Parkinson's disease, in a manner

not possible through animal research alone or by observing patients. In fact, disease-specific iPS cell lines have already been created from patients suffering from amyotrophic lateral sclerosis,⁶⁹ juvenile onset type 1 diabetes mellitus,⁷⁰ Parkinson's disease, and spinal muscular atrophy.^{69, 70}

Since the pathogenesis of GA is still largely unknown, creating a disease model of GA using iPS technology can be a valuable tool. RPE cells differentiated from normal and GA patient-derived iPS cells should have distinct differences with respect to their morphology, proliferation and/or apoptosis rate, RPE cell markers, and integration efficiency into rodent disease model. This in turn allows the patient-specific RPE cells to be used for elucidating the molecular mechanisms or factors that lead to cellular apoptosis, and even as a biological tool for drug discovery and toxicity screening for both in vitro and in animal models.

To employ iPS cells in cell replacement therapy, several issues need to be addressed before the full potential of iPS cells can be utilized. To date, iPS cells are more efficiently generated by transducing somatic cells with retroviruses or lentiviruses that integrate randomly into host genome. Although the integrated proviruses are silenced during iPS cells generation, there is potential for the reactivation of these viral transgenes, including potent oncogenes Myc and Klf4. Indeed, retrovirus transduction of Myc was reported to be reactivated and resulted in tumor formation in half of chimeric mice generation from iPS cells.^{71, 72} Furthermore, leaky expression of these transgenes may inhibit complete iPS cell differentiation and maturation, leading to greater risk of immature teratoma formation.⁷³

The use of retroviruses and lentiviruses in generating iPS cells poses another safety issue in that transgene integration leads to mutation within the host genome. In addition, the integrated provirus can alter expression of neighboring host genes, leading to oncogenesis as seen in the offsprings of SCID mice following transplantation of retroviral gene-modified hematopoietic stem cells.^{74, 75} Mapping studies of an iPS cellular genome also reveals retroviral integrations into sites that are known to induce tumors in mice.⁷⁶

Several approaches have been recently reported to eliminate the issue of genomic integration of the reprogramming genes. Various transgene-free and/or virus-free reprogramming techniques have been derived using Cre-recombinase excisable lentiviruses,⁷⁷ Epstein-Barr nuclear antigen 1-based episomal vectors,⁷⁸ piggyBac transposon expression vectors carrying a polycistronic transgene of Yamanaka 4 factors,⁷⁹ and conjugating cell-penetrating peptide with recombinant proteins of OSKM.⁸⁰ However, these methods are still facing issues such as low reprogramming efficiency, slow kinetics, cumbersome positive clone selection, and some degree of abnormal alternation of genomic activities. Nonetheless, recombinant protein-based technique still possesses the potential for generating virus-free and transgene-free human iPS cells for clinical use with future improvement in the reprogramming speed and efficiency. Other approaches have also been employed: the application of non-viral minicircle DNA, which allows longer ectopic expression with high transfection efficiencies but lower activation of exogenous silencing mechanisms,⁸¹ as well as addition of small molecules,^{82–86} small interfering RNAs,^{82, 87, 88} or micro RNAs⁸⁸ to the reprogramming cocktails to replace some of the reprogramming factors or increase the efficiency and kinetics.

Aside from the safety issues, there is still significant variability in the efficiency and kinetics of reprogramming somatic cells from the three germ layers. It seems that the differentiation status of the target cells influences the reprogramming frequency. For instance, hematopoietic stem cells are more efficiently reprogrammed than terminally differentiated B and T lymphocytes.⁸⁹ It also appears that iPS cells from different somatic cell origins have distinct differentiation properties, such as those derived from hepatocytes differentiate

poorly into neurospheres and are more likely to form teratomas.⁹⁰ These functional differences are currently difficult to resolve as individual iPS clones derived from a given target cell type using the same method may vary in their ability to differentiate into different lineages. Even the same reprogramming experiment yields iPS cell colonies that vary in their reprogrammed state and differentiation potential. Furthermore, numerous growth factors and animal cell feeder layers are needed to support the growth of even limited numbers of iPS cells. A reliable standard protocol is therefore required, not only for efficient generation of iPS cells but also for identification of *bona fide* iPS cells. The presence of a few aberrantly reprogrammed cells within a pool of differentiated iPS cell progenitors could increase the risk of immature teratoma development post-transplantation.

To overcome the hurdles that hinder application of iPS cells in clinical therapy, techniques have to be improved to ensure proper selection of *bona fide* iPS cells and to increase the efficiency of the differentiation protocol that generates progenitors of defined quality and characteristics that are safe for the patient. To date, human ES cell lines have been used to generate a highly pure pool of neuronal and retinal progenitors that meet regulatory satisfaction and are in the process of being used to treat patients with spinal cord injury and congenital blindness, respectively.^{16, 37, 52} This is, however, yet to be achieved by iPS cells, as there is still lack of consensus on the derivation, culture, and differentiation methods. Variability of individual iPS cell lines in their differentiation makes testing and approval by regulators more difficult, and dampens the interest amongst pharmaceutical companies in creating personalized iPS cell products.

Careful consideration has to be taken when choosing an appropriate cell type for reprogramming future autologous patient-specific iPS cell production and clinical therapy. An ideal cell source to be isolated from patients for reprogramming should meet the criteria of easy accessibility with minimal risk procedures, availability in large quantities, relatively high reprogramming efficiency, and fast iPS cell derivation. As of now, iPS cells have been derived from skin fibroblasts, keratinocytes, CD34⁺ cells from peripheral blood, melanocytes, cord blood cells, adipose-derived stem cells, and human fetal neural stem cells,⁹¹ with each cell type possessing its own advantages and limitations in serving as an origin for iPS cells derivation.

Induced Pluripotent Stem Cell-derived Retinal Pigment Epithelium and Photoreceptors

With much success in deriving RPE cells and photoreceptors from ES cells,^{56, 63, 92} many research groups have applied what they learned from the differentiation protocols to that of iPS cells. Meyer et al.⁹³ has recently shown that iPS cells can be differentiated towards retinal cell types while Clegg's³⁸ and Coffey's³⁷ groups have both demonstrated that human iPS cells can be differentiated into RPE cells which display functional similarities to cultured fetal RPE and hES-RPE in vitro. Takahashi and colleagues have also established defined culture methods that successfully derived RPE cells and photoreceptors from both ES and iPS cells of mouse and human origins.^{14, 94} Recently, the group focused on employing small molecules instead of recombinant proteins to induce retinal cells from both human ES and iPS cells.⁹⁵ They hope to create iPS cells under safer conditions that are free of serum and animal derivatives that may lead to cross-species contamination for cell replacement therapy.

In order to identify and purify photoreceptors differentiated from human ES and iPS cells, Lamba and colleagues transduced the differentiating cells with lentivirus that drives GFP from the photoreceptor-specific, IRBP promoter.⁹⁶ When cells differentiated into photoreceptors, they expressed IRBP that led to GFP expression. This in turn allowed derived photoreceptors to be enriched using fluorescent activated cell sorting (FACS). The FACS purified iPS-derived photoreceptors were then transplanted to the subretinal of wild-

type mice and they showed good integration into the mouse retina with expression of photoreceptor markers. Taken together with their previous report, where the ES cell-derived photoreceptors integrated following transplantation and restored light response to Crx deficient mice,¹⁸ they have demonstrated the possibility of using stem cell approaches in retinal cell replacement therapy. The ability to purify cells from undifferentiated contaminants also allows a safer cell-based therapy for treating retinal degeneration.

Methods of Transplantation

Two different approaches have been used for RPE and photoreceptors transplantation: subretinally^{17,97,98} and intravitreally.²⁵ Compared with the subretinal procedure, intravitreal injection is easier from a procedure standpoint and creates less trauma to the eye. However, one issue with intravitreal injections is that the host retina may act as a physical barrier for the transplanted cells and impair their ability to migrate and integrate into the retinal tissue. Moreover, if the transplant is an RPE-photoreceptors-scaffold complex and not a cell suspension, the intravitreal injection cannot be employed because it is impossible for the “huge” complex to migrate through the inner retina and integrate into the BM and choroidal vasculature.

The subretinal space is the intended location for transplanted cells or tissues; therefore delivering them directly to the subretinal space seems logical. Indeed, researchers found that cells implanted into the subretinal space have better differentiation and integration into the host retina. However, the main issue with subretinal implantation is that the surgery is complex and more traumatic to the host eyes. Nevertheless, along with the rapid development of surgical techniques, delivering the cells or tissue sheets to the subretinal space is no longer a difficult task for an experienced surgeon, and the surgery-related trauma can be reduced. Although iPS cell technology harbors many advantages, the cell transplantation strategy for GA still needs to be optimized. First, the sequence for effective transplantation needs to be determined. The pathological changes of GA include the death of RPE cells and photoreceptors, both of which need to be repaired by transplantation. As photoreceptors cannot survive without the underlying healthy RPE, transplanting RPE cells and producing a healthy RPE layer should be the first step, then the photoreceptors' transplantation. The main drawback of this procedure is that the patient will have to go through at least two operations to complete this therapy. Otherwise, two kinds of cells must be delivered simultaneously in one procedure. This leads to two types of cell preparation for the transplantation: 1) mixing the two types of cells in suspension; or 2) growing them on a carrier complex, with a structure of photoreceptors, RPE cells, and biological carrier from the top to the bottom. Our hypothesis is that the latter may be better to restore the retina to its original structure of Bruch's membrane, RPE layer, and photoreceptors.

Next, the differential stage that is optimal for the transplantation needs to be resolved. In the normal state, the photoreceptor's inner segments are bound to Müller cells through tight junctions in the outer limiting membrane and connect with bipolar cells through synapses. If the fully differentiated photoreceptors are transplanted, it may be impossible for the cells to form these important connections with the surrounding tissue. Therefore, the photoreceptor precursors rather than the mature photoreceptors on a fully mature RPE layer³⁷ will be more ideal for transplantation. Finally, of significance, is the determination of the ratio of cones and rods in the photoreceptors mixture. Cones are the only photoreceptor cell type at the fovea and are responsible for the sharp visual acuity. The density of rods increases from the center of the macula to the periphery and reaches its greatest density at the point around 5 mm from fovea. Trying to establish this original structure during the transplant process is crucial for recovery of central vision.

CHALLENGES AND FUTURE DIRECTION

Induced pluripotent stem cells represent a valuable source for producing patient- and disease-specific adult cells for future clinical applications and drug development (Figure 1). In addition, these cells will bypass the ethical concerns related to ES cell derivation and potential issues of allogeneic immune rejection. However, several issues need to be resolved before advancing the application to clinical trials: 1) validation of the safety issues and improvement of iPS cells derivation and differentiation to targeted cells; 2) ideal cell combination for transplantation and methods and time-point for transplantation; 3) safety and efficacy evaluation in large animal models that are anatomically and physiologically closer to humans.

CONCLUSION

Rapid development of iPS cells from somatic cells allows generation of RPE and photoreceptors from patient-specific iPSCs. This development provides an important tool to understand the pathogenesis and opens an avenue for cutting-edge treatment of retinal degenerative diseases. The pathologic change of GA is relatively simple and limited, as compared with other retinal degenerative diseases, such as wet AMD and glaucoma, making it an ideal model for initial studies. GA may become the first disease to be treated by iPS-derived RPE cells and photoreceptors transplantation.

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FIGURE 1.

Application of iPS cell technology for GA treatment. iPS cell-derived, patient- or disease-specific RPE and photoreceptors can either be used for creating disease models for elucidating the mechanism of pathogenesis and drug discovery for GA or made into scaffold complex for cell replacement therapy.