·Review·

Development of gene and stem cell therapy for ocular neurodegeneration

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Abstract

• **Retinal degenerative diseases pose a serious threat to eye health, but there is currently no effective treatment available. Recent years have witnessed rapid development of several cutting-edge technologies, such as gene therapy, stem cell therapy, and tissue engineering. Due to the special features of ocular structure, some of these technologies have been translated into ophthalmological clinic practice with fruitful achievements, setting a good example for other fields. This paper reviews the development of the gene and stem cell therapies in ophthalmology.**

• **KEYWORDS:** stem cell; gene therapy; retina; translational medicine

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INTRODUCTION

As optics-related eye disorders and inflammatory ocular diseases can now be relatively well-controlled, retinal neurodegeneration is becoming a leading cause of irreversible blindness worldwide, posing a serious threat to the eye health of tens of millions of people. This includes retinitis pigmentosa (RP), age-related macular degeneration (AMD), glaucoma, *etc*. The pathological basis of the visual loss associated with these diseases mainly involves irreversible damage to retinal neurons [1], however, there is currently no effective treatment or cure for this.

In recent years, the rapid progress in basic scientific research sheds light on the treatment of these diseases, which mainly includes gene therapy, stem cell replacement therapy, sustained release drug delivery technology, tissue engineering, *etc*.

Due to the unique physiological and anatomical characteristics of eyes, ophthalmology has been a pioneer field in the utilization of translational medicine research. Firstly, owing to structures like blood-retina barrier, eye is a relatively independent organ, and delivery of drugs, foreign genes or cells is less likely to produce immune response, indicating some degree of immune privilege. Secondly, the volume of eyeball tissue is relatively small, with a constant and small number of cells, and thus the required dosage of drugs, genes or cells for treatment is relatively low, which may be associated with fewer adverse effects. Thirdly, as an external organ of human body, eye is relatively easy to operate on; since the dioptric media of eyes is transparent, most procedures on eye tissues are visible (or visible *via* minimally invasive methods). Moreover, the visual functions and the physiological characteristics of eyes are relatively easy to observe and analyze; most structures and tissues of eyes can be directly observed by using equipment such as: slit lamp microscope, ultrasonic device, fundus camera and so on; real-time recording and objective evaluation of the changes in visual functions are also feasible by using visual electrophysiology, fundus fluorescein angiography, optical coherence tomography (OCT) and other techniques. Finally, because each person has a pair of eyes, the unique feature of self-comparability of eyes makes it possible to assess the efficacy of therapies in clinical studies.

Translational Research of Gene Therapy in Ophthalmology The completion of the human genome project and the widespread application of sequencing techniques have led to a new revolution in the understanding of the nature of diseases, and have also offered a new hope for molecular phenotyping , biological diagnosis and gene therapy of diseases.

With an increasingly comprehensive understanding of eye diseases, more and more pathogenic genes are being discovered. To date, a total of 242 pathogenic genes and thousands of mutation loci associated with hereditary retinal diseases have been identified [2]. Chinese researchers have made some progress in studies of families with congenital cataract as well as genetic studies of Leber congenital amaurosis (LCA), RP, AMD, glaucoma, exfoliation syndrome, *etc*[3-6]. Our team firstly conducted a genome-wide

association study including 1854 primary angle-closure glaucoma (PACG) cases and 9608 controls across 5 sample collections in Asia; and then replication experiments were conducted in 1917 PACG cases and 8943 controls collected from a further 6 sample collections. Three new susceptibility loci were identified, *i.e. PLEKHA7, COL11A1, and rs1015213*, which might affect the development and regulation of trabecular meshwork, iris, and ciliary body, and thereby result in glaucoma [7]. The discovery of these loci opens up the possibilities of novel therapeutic strategies and early identification of at-risk populations.

Gene therapy has undergone considerable development during past decades. The first approved clinical trial of gene therapy initiated in 1990; and then in 2012. Alipogene tiparvovec became the first gene therapy drug approved for clinical application by the European Commission. According to the data provided by *The Journal of Gene Medicine*, a total of 2088 gene therapy clinical trials, involving 1973 subjects, have been or are being performed worldwide [8], of which 28 trials (1.3%) address ocular diseases, 10 for AMD, 8 for LCA, 5 for RP, and 5 for other conditions (Table 1). Treatment strategies mainly include gene replacement therapy, cytokine therapy and optogenetic therapy.

Gene replacement therapy The gene therapy for LCA has been regarded as a good example in the field of translational medicine research. LCA is considered as one of the most serious hereditary retinal diseases. Along with the widespread application of genetic studies, 14 pathogenic genes associated with LCA have been discovered [9]. *RPE65* was conferred retinal pigment epithelium (RPE) -specific expression, and the protein it encoded involved in the metabolic circulation of optical signals [10]. And then, researchers designed a vector-based technology to deliver *RPE65* into the host RPE cells and let its functions perform, which has led to some degree of visual recovery in LCA models of both small and big animals [11,12]. In 2008, ophthalmologists from different groups successfully administered a low-dose gene therapy for LCA patients in phase I clinical trials (NCT00643747; NCT00516477; NCT00481546; clinicaltrails.gov), and significant improvements in visual acuity were observed in patients, these novel findings ushered in a new era of therapies for gene-related eye diseases [13,14]; to date, patients who received the gene therapy have been followed-up for up to 3y and their visual functions are still being maintained [15]. These researchers formed an international study group of LCA gene therapy to assess the safety and efficacy of higher dose injection and multi-site injection or multiple injections in multi-center, large-scale, clinical phase III trials (NCT00999609; clinicaltrails.gov).

RP has a complex genetic background, and a large number of potential pathogenic genes have been discovered. Of these, *MERTK* gene replacement therapy has entered the clinical trails. The Royal College of Surgeons (RCS) rat is commonly used as an animal model in RP studies, which carries a mutated *MERTK* gene [16]. The mutation of the *MERTK* gene results in defective RPE phagocytosis, making the RPE unable to timely eliminate photoreceptor outer segment (POS), which may lead to secondary apoptosis of photoreceptor cells and subsequently retinal degeneration. A phase I clinical trial (NCT01482195; clinicaltrails.gov) utilizing an AAV2 vector with an RPE-specific promoter driving *MERTK* has been initiated in Saudi Arabia. At this point in time, three patients have been treated with no adverse events recorded[17].

Besides, some other gene replacement therapies have also been introduced into ophthalmological clinical trials, including *MYO7A* gene therapy for Usher syndrome and *ABCA4* gene therapy for Stargardt macular dystrophy. Due to the relatively large size of both genes, which is beyond the conventional packaging capacity of AAV vectors, equine infectious anemia virus (EIAV) vectors are employed to transducer retinal cells, and functional restoration has been successfully achieved in animal models (mice with *MYO7A* or *ABCA4* mutation) [18,19]. This has led to phase I clinical trials initiated by Oxford Biomedica UK utilizing the EIAV platform to deliver *MYO7A* or *ABCA4* to patients with Usher syndrome or Stargardt disease (NCT01505062; NCT01367444; clinicaltrails.gov).

Cytokine therapy The application of anti-vascular endothelial growth factor (VEGF) is considered as a major contribution to ophthalmological practice. The final common pathological process of visual loss caused by many ocular fundus diseases is retinal and/or choroidal neovascularization. Basic medical studies revealed that VEGF was one of the main regulatory molecules involved in the process of vascularization and angiogenesis[20]. And then, cytology and animal experiments confirmed that preventing VEGF from binding to its receptors (VEGFR1 and VEGFR2) on the endothelial cell surface could inactivate endogenous VEGF, suppress endothelial cell mitosis, decrease vascular permeability, and thereby effectively inhibit neovascularization [21]. Currently, there are two FDA-approved drugs available for clinical use, *i.e.* bevacizumab and ranibizumab. But both drugs require long-term and repeated intravitreal injections and invariably involve the cumulative risk of repeated intraocular procedures and significant financial burden. Soluble FLT-1 (sFLT1) is a portion of the VEGFR1 that competitively binds to VEGF to inhibit neovascularization [22]. Ocular neovascularization was proved to be inhibited in animal

Table 1 Clinical trails: gene therapy

No.	Trial ID	Phase	conditons	Intervention	Gene types	Vector	PI
L1	NCT00643747	I/II-12	LCA	rAAV 2/2.hRPE65p.hRPE65	*RPE65*	rAAV2	Ali RR (University College, London)
L 2	NCT00516477	I-9	LCA	AAV2-hRPE65v2-101	*RPE65*	rAAV2	Maguire AM (Children's Hospital of Philadelphia)
L 3	NCT00481546	I-9	LCA	rAAV2-CBSB-hRPE65	*RPE65*	rAAV2	Jacobson SJ (University of Pennsylvania)
L 4	NCT00749957	I/II-12	LCA	rAAV2-CB-hRPE65	*RPE65*	rAAV2	Stout JT (Applied Genetic Technologies Corp)
L 5	NCT00821340	I-10	LCA	rAAV2-hRPE65	*RPE65*	rAAV2	Banin E (Hadassah Medical Organization)
L 6	NCT00999609	III	LCA	rAAV2-hRPE65	*RPE65*	rAAV2	Maguire AM (Children's Hospital of Philadelphia)
L 7	NCT01208389	I/II	LCA	AAV2-hRPE65v2	*RPE65*	rAAV2	Maguire AM (Children's Hospital of Philadelphia)
L 8	NCT01496040	I/II	LCA	rAAV2/4.hRPE65	*RPE65*	rAAV2	Michel WEBER (Nantes University Hospital)
A1	NCT01024998	I	AMD	AAV2-sFLT01	*sFlt-1*	AAV2	Genzyme, a Sanofi Company
A2	NCT01494805	I/II	AMD	rAAV.sFlt-1	*sFlt-1*	rAAV	Ian Constable, (Lions Eye Institute)
A3	NCT01301443	I	AMD	AAV2-sFLT01	*sFlt-1*	AAV2	Peter Campochiaro, (Johns Hopkins University Hospital)
A4	NCT01367444	I/II	AMD/ Stargardt	StarGen	*ABCA4*	EIAV	Oxford BioMedica. Inc
A5	NCT00109499	I	AMD	AdGVPEDF.11D	*PEDF*	replication deficient adenovirus	GenVec. Inc
A6	US-1061	I	AMD	Endostatin Angiostatin	Endostatin Angiostatin	Lentivirus	Peter Campochiaro, (Johns Hopkins University Hospital)
A7-8	US-X001/X002	I	AMD	Cand5 (siRNA against VEGF)	siRNA	siRNA	Acuity Pharmaceuticals USA
A9-10	US-X007	II	AMD	siRNA-027(siRNA against VEGF R1)	siRNA-027	siRNA	Allergen Pharmaceuticals USA
R1	NCT01482195	I	RP	rAAV2-VMD2-hMERTK	*MERTK*	rAAV	Fowzan S Alkuraya, (King Faisal Specialist Hospital)
R2	NCT01505062	I/II	RP/Usher	UshStat	*MYO7A*	EIAV	Oxford BioMedica. Inc
R3-5	US-575/US-795 /US-796	II	RP	CNTF	Cytokine	Naked/Plasmid DNA	Sieving PA (National Institutes of Health USA)
1	US-539	I/II	Corneal Scarring	dnG1 Cyclin	Cell cycle	Retrovirus	Song JC (Keck School of Medicine,University of Southern California,USA)
2	US-589	I	Glaucoma	p21 WAF-1/Cip1	Cell cycle	Adenovirus	Kaufman PL (University of Wisconsin-Madison Medical School Madison, USA)
3	NCT01461213	I/II	Choroideremia	rAAV2.REP1	*REP1*	rAAV2	Robert E MacLaren, (University of Oxford)
4	US-X003	II	DME	Bevasiranib/Cand5	siRNA	siRNA	Acuity Pharmac-euticals USA
5	CN-0025	I/II	LHON	rAAV2-ND4	*ND4*	rAAV2	Lin B,(Tongji Medical college)

LCA: Leber congenital amaurosis; AMD: Age-related macular degeneration; RP: Retinitis pigmentosa; DME: Diabetic macular edema; LHON: Leber hereditary optic neuropathy; (r)AAV: (recombined) Adeno-associated virus vector; sFlt-1: Soluble vascular endothelial growth factor receptor 1; AdGVPEDF.11D: Replication deficient (E1, E3 and E4 deleted) adenovirus vector containing the gene for the pigment epithelium-derived factor (PEDF) protein; VEGF: Vascular endothelial growth factor; siRNA: Short-interfering RNA; CNTF: Ciliary neurotrophic factor; dnG1: Matrix-targeted retroviral vector bearing a dominant negative cyclin G1 construct; REP1: Rab-escort protein 1; ND4: NADH dehydrogenase subunit 4; EIAV: Equine infectious anemia virus.

models upon subretinal AAV2 carrying full-length sFLT1 and treatment was also shown to be safe in mice and nonhuman primates [23]. Recently, a phase I clinical trial has been launched to investigate intravitreal AAV2-sFLT01 for treating neovascular AMD (NCT01301443; clinicaltrails. gov). Aflibercept (VEGF Traq-Eye), another soluble receptor molecule that binds VEGF, has been recently approved for the treatment of exudative AMD. Phase III trials showed that intravitreal aflibercept produces visual improvement and decrease in macular thickness comparable with monthly ranibizumab [24].This year, Phase IV trials showed that Aflibercept treatment maintained mean visual acuity improvements in wet AMD patients, and led to significant anatomic improvement (NCT01543568; clinicaltrails.gov)[25].

Retinal neurogliocyte could secrete an endogenous neural factor-ciliary neurotrophic factor (CNTF), which might play a protective role for retinal neurons *via* several pathways[26]. Researchers further carried out a study to confirm the safety and protective effects of CNTF in animal models of photoreceptor cell apoptosis, and results showed that animals in the treatment group had an increased thicknesses of the outer nuclear layer and decreased damage of photoreceptor cells, which laid a foundation for CNTF entering into clinical practice [27]. However, researchers found that the traditional administration routes (intravitreal injection or subretinal injection) were really invasive and repeated procedures were always required, which could result in decreased tolerance of patients, unstable therapeutic effects, and probably a series of complications as well. Therefore, by integrating tissue

engineering with cell engineering techniques, the Neurotech company developed an encapsulated cell technology (ECT) and manufactured a sustained-release delivery system NT-501 for continuous and controllable release of CNTF, which further promoted the clinical application of CNTF[28]. A pilot clinical trial showed that NT-501 could produce a biological effect on patients with macular degeneration, mainly manifested as an increased thickness of the photoreceptor layer, which reflected the neuroprotective effect (NCT00447954; clinicaltrails.gov)[29]. Rather than repairing the mutation of a specific gene, these cytokines mainly act *via* providing nutritional support to all retinal neurons to inhibit cell apoptosis. And thus, the most recent clinical trials have expanded the indications of NT-501 to include RP and glaucoma (NCT01530659; NCT01408472; clinicaltrails.gov).

Optogenetics Optogenetics is a fast-growing, inter-disciplinary bioengineering technology that uses a combination of techniques from optics, genetics and electrophysiology. Its main rationale is to utilize genetic techniques to insert light-sensitive genes into particular types of nerve cells for the expression of specific ion channels. Specifically for retinal neurons, optogenetic therapy is capable of conveying light sensitivity to more neurons and impeling theses cells to deliver light signals into visual function-related brain areas.

Recent studies identified a type of intrinsically photoreceptive ganglion cells across the entire retina, whose primary role was to regulate non-image-forming visual functions, such as circadian rhythm and pupillary light reflex. These findings were named by the leading magazine Science as one of the top ten scientific breakthroughs of 2002[30,31]. This type of cells contains a new opsin-melanopsin, a photopigment which activates endogenous light sensitivity. Based on the rationale of gene therapy, researchers inserted *melanopsin* gene into the third-order retinal neurons-ganglion cells in mice lacking rods and cones, which restored the photoperceptivity of these blind mice whose leading cause of blindness was the death of the primary neurons (rods and cones)[32]. This trial provided a basis for the application of melanopsin in clinical treatment.

The same strategy also facilitated the application of *channelopsin-2* gene. Researchers inserted the vision forming gene *channelopsin-2*, a gene found in green algae, into the ganglion cells of RCS rats, which successfully promoted the repair of the rats' visual functions[33]. Now encouraging progresses have been made in primate models[34].

Such a strategy has broken away from the traditional pattern in which a specific pathogenic gene must be targeted in gene therapy. It seems that this therapeutic strategy is universally applicable, which is of great clinical and commercial value, especially for those patients with significant apoptosis of rods and cones in advanced retinopathy.

Translational Research of Stem Cell Therapy in Ophthalmology Despite the dramatic development of gene therapy in ophthalmology, there are still concerns. A report by the study group of LCA gene therapy noted that, although *RPE65* gene replacement could improve visual functions, which appeared to be maintained up to at least 3y, it did not prevent the apoptosis of rods and cones[35]. This indicates that we need to combine another type of genes that may inhibit cell apoptosis for the delivery, or resort to cell replacement therapy which may restore visual functions by replacing the defective neurons with functional cells.

The ability of stem cells to extensively self-renew and variable degrees of differentiation capacities offers new promise for cell replacement therapy. Despite the deepening study, only a few have been translated into the clinical trails. The application of stem cell technologies in ophthalmology has obtained substantial development in recent years, providing useful insights to the translational research in other disciplines.

We performed a search in "clinicaltrails.gov" using the keywords of "stem cell" and "ocular", and 22 items were retrieved. Aside from corneal disease, the indications most frequently addressed by stem cell therapy clinical trials included AMD (27%, n=6) and RP (14%, n=3), probably because for both diseases the damaged cells that need to be replaced are located at the outer retina, and long or bi-directional nerve fiber projections are not required. In addition, the most frequently used cell types in stem cell therapy clinical trials included bone marrow-derived mesenchymal stem cells (BMSC) (41%, n=9) and embryonic stem cells (ESC; 27%, n=6).

Stem cells and retinal pigment epithelium Located at the outermost layer of retina, RPE cells are in charge of providing nutritional support for the neural retina and phagocytize POS and toxic debris. The pathogenesis of AMD and some types of RP involves RPE damage, which may further result in photoreceptor cell loss and hence visual loss. Clinical studies using macular translocation[36] or autologous RPE transplantation[37] indicated that such procedures were associated with many adverse events and complications, though certain therapeutic effects were obtained; moreover, the autologous RPE cells used for transplantation also carry genetic defects, unable to function as healthy RPE cells. Therefore, stem cells are regarded as a more favorable source of donor cells.

ESC possess a nearly unlimited self-renewal or propagation capacity as well as the potential to differentiate in vitro into specialized cell types. Numbers of research have been done on the focus of ECS derived differentiation of RPE and RPE replacement therapy[38]. Researchers found that induction by

the stromal cell line PA6 could generate pigmented epithelial cells from primate ESC, and help express the early eye field transcription factors, Pax6 and RPE-specific genes, *RPE65* and *MERT* [39]. When human ESC (HESC) were allowed to overgrow on the mouse embryonic fibroblasts which were inactivated and basic fibroblast growth factors (bFGF) that had pivotal roles in maintaining pluripotent stem cells were removed, HESC might spontaneously differentiate into some pigmented cells over 4-5wk. After further sorting and expansion, these pigmented cells might express many RPE-specific transcription factors, such as MITF, OTX2, PAX6, *etc* [40]. More recently, with a deeper understanding of eye development-related signaling pathways, recombinant proteins or small molecular compounds were used to regulate relevant pathways, so that RPE might be directly induced from HESC[41,42]. Its main rationale involves stepwise treatment with inducers based on the mode of eye development. Firstly, inhibitors of the WNT and Nodal pathways were used based on a serum-free-embryoid body-suspension culture condition, which could induce directed differentiation of ESC toward retinal progenitor cells (RPC); and then, after adherent culture of embryoid bodies and interventions to activate RPE development-related pathways, RPE progenitor cells could be induced and expression of early markers like RX, MITF and PAX6 might be detected at approximately 40d; mature RPE cells having the capacity to secrete pigments could be generated at approximately 60d[41].

These RPE like cells induced by ESC are not only comparable to adult RPE cells in terms of morphology and expression of marker proteins, but also have similar biological functions. An *in vitro* experiment demonstrated that the HESC-RPE cells were capable of secreting relevant cytokines, including pigment epithelium-derived factor (PEDF) and VEGF; and meanwhile, they also possessed the important function of RPE - phagocytosis of photoreceptor OS [43]. After subretinal transplantation into RP rat models (RCS), the HESC-RPE cells had the capacity to support photoreceptor survival and preserve visual function without provoking any overt pathological responses in the host retina; moreover, such therapeutic effects were remained for long term[44,45].

Based on these experimental findings, phase I clinical trials for Stargardt's disease and AMD using HESC-derived RPE cells were approved, which were sponsored by the US Advanced Cell Technology, Inc. (NCT01345006; NCT01344993; clinicaltrails.gov). Preliminary results were reported last year: during the first 4mo after transplantation, the HESC-RPE cells survived and integrated into the host RPE layer, no signs of hyperproliferation, tumorigenicity, or apparent rejection were identified, and visual function increases were also observed [46]. This is the first description in publications of HESC-derived cells transplanted into human patients, and is now regarded as a milestone in the clinical application of ESC, though longer-term follow-up data and a larger sample size are still required.

Most of these transplantation procedures are performed using subretinal injection of cell suspensions, which is often associated with difficulties in controlling the target location of transplanted cells and their integration into the host retina, a high propensity to form rose-like structures, as well as a low survival rate of transplanted cells. The emergence of three-dimensional polymer scaffolds may help to resolve these issues. At a higher induction efficiency than other methods, the HESC cells were cultured on the extracellular matrix matrigel to generate the HESC-derived RPE monolayer, which was then transplanted into the subretinal space of RCS rats. Following transplantation, visual improvement was achieved and the grafted cells survived for at least 1y, supporting the effectiveness of functional restoration [43]. In addition, Yaji *et al* [47] utilized temperature-responsive culture materials to non-invasively construct monolayer RPE cell sheets, providing a novel approach for constructing tissue-engineered retinal cell sheets.

Finally, a clinical trial was approved in which researchers from University College, London, UK seeded the differentiated HESC onto a polyester film to construct tissue-engineered RPE sheets for the treatment of macular degeneration (NCT01691261; clinicaltrails.gov); the trial is still ongoing.

Since ESC are derived from allogenic embryos, ethical challenges and allograft rejection concerns also exist. In this case, induced pluripotent stem (iPS) cell becomes an alternative source for RPE replacement therapy. By introducing 4 genes into adult cells, this reprogramming technique is used to obtain iPS cells that own the properties of ES cells [48]. As iPS cells can be made from autologous skin, hair follicle and even blood cells, and the harvested cells carry the same genetic information as the donor, this method has many benefits, including convenient source, lack of ethical concerns, and reduced risk of immune rejection. Recently, researchers have successfully differentiated human iPS cells into RPE-like cells, which could exhibit similar morphology of adult RPE cells, express numerous RPE-specific marker proteins, and function to phagocytize photoreceptor OS membranes [49,50]. It has also been demonstrated in animal studies that iPS cells can be differentiated into functional iPS-RPE and transplantation of these cells can protect photoreceptor cells and thereby improve visual function[51].

In the production of iPS cells, viral transfection and genetic intervention are often required which may limit their use in clinical practice. Advances in cellular reprogramming

techniques, such as the use of small molecules [52] and recombinant proteins[53], provide new insights for the clinical application of iPS cells. The first clinical trial using autologous iPS cells is approved by the Ministry of Health, Labour, and Welfare of Japan. This trial, led by Dr. Masayo Takahashi[54] of RIKEN Center for Developmental Biology of Japan, plans to use iPS-derived RPE cells to treat patients with AMD, making ophthalmology a pioneer in the utilization of iPS cells.

Stem cells and photoreceptor cells In the end stage of ocular degenerative disorders like RP and AMD, loss of photoreceptor cells is deemed to be inevitable. At this point in time, replacement of RPE cells can no longer restore visual functions, and thus replacement of photoreceptor cells may be required.

As the first stem cell transplanted into the retina of animal models, hippocampus-derived neural stem cells (NSC) were well integrated into the host retina after transplantation, but lack of the capacity to differentiate into retinal neurons[55,56]. And then, it was found that RPC cells, as early-born neurons in the retina, could be well differentiated into photoreceptor cells, possibly because RPC itself had a tendency to differentiate into retinal neurons. But unfortunately, little evidence of being able to integrate into the host retina was obtained in the grafted cells [57,58]. MacLaren *et al*[59] further revealed that if RPC cells were taken from the developing retina at a time coincident with the peak of rod and cone genesis, they could integrate into the outer nuclear layer of the retina, differentiate into photoreceptors, survive long-term, and improve visual functions, reflecting different integration capacities of the committed progenitor cells at different ontogenetic stages. Afterwards, Lamba *et al*[60] showed that after transplantation into the subretinal space of Crx mice (a model of LCA), the HESC-derived RPC cells integrated into the retina, differentiated into functional photoreceptors and restored light responses to the animals. However, it was also noted that only a small number of cells could migrate into the outer nuclear layer of the host retina, and the improvement in visual functions was somewhat limited. A recent study [61] reported that transplantation of photoreceptor precursors, taken from donor mice at postnatal day 4-8, obtained levels of photoreceptor integration that were 20-30-fold higher than previous studies; moreover, visual functions assessed by grating stimulation test and water maze test were significantly improved following transplantation, theoretically demonstrating the feasibility of cell replacement as a therapeutic strategy for restoring vision after photoreceptor damage. An ongoing clinical trial using human embryonic photoreceptor progenitor cells in the treatment of RP is being conducted by Southwest Hospital of The Third Military Medical University, China [62], and results are pending.

Stem cells and ganglion cells Retinal ganglion cell (RGC) injury is a common feature of many ophthalmic disorders including glaucoma, optic nerve contusion, and optic canal fracture, which may result in optic atrophy and visual loss. Despite numerous studies, the treatment of RGC injuries is still unsatisfactory. As for RGC replacement therapy, the transplanted cells need to not only be well integrated into the inner layers of the host retina, but also form RGC-specific long axons for their projections into relevant brain areas as well as accurate synaptic connections with visual neurons in the brain; all of these challenges remain to be resolved. Although many researchers have successfully induced RGC from ESC and iPS cells [63-65], the transplanted cells seemed unable to integrate into the host retina of animal models, and no evidence of projections into brain areas or visual improvement was observed[65].

Thus, the current stem cell therapy for RGC injuries mainly depends on various cytokines that stem cells secrete, which may help to improve the local microenvironment of the host retina and hence prevent RGC loss. Our team is considered as one of the pioneers in this field; we transferred brain derived neurotrophic factor (BDNF) into NSC, and intravitreal transplanted these genetically modified NSC into the rat models of optic nerve transaction, and results showed that these cells could secrete BDNF over the long term and reduce the apoptosis of RGC [66]. In another study [67], rat models of chronic ocular hypertension received an intravitreal transplant of BDNF-secreting BMSC, and results indicated that the BDNF-BMSC-transplanted eyes displayed a greater level of RGC preservation than the control group.

Challenges in clinical application of stem cells The following challenges need to be addressed before stem cell therapies can really be used in ophthalmological clinical practice.

Firstly, for the preparation, storage, transportation and induced differentiation of stem cells, standard operation procedures (SOPs) in compliance with Good Clinical Practice (GCP) must be established. Under the guidance of such SOPs, a stem cell bank for ESC or iPSCs may be set up for easier search of potential matches.

Secondly, as losses of RPE cells and photoreceptor cells often exist concurrently in end-stage AMD and RP, simultaneous transplant of both layers of cells may be required. The emerging technique of *in vitro* induction of three-dimensional optic cup morphogenesis [68,69], and the application of tissue engineering provide promise for such transplant.

Thirdly, for hereditary retinal diseases, the constructed autologous iPS cells still carry mutated genes, and thus these cells should first undergo *in-vitro* genetic modifications and then be used for *in-vivo* transplantation. Additionally, the

iPS cells that carry disease information may be used for *in-vitro* drug screening.

Finally, the phenomenon of retinal restructuring in end-stage diseases may alter the microenvironment throughout the retina and thereby the release of relevant cytokines and inflammatory factors. Thus, the impact of microenvironment on transplanted cells should be taken into consideration.

Prospects Both gene therapy and stem cell therapy are still within preliminary stages, although their translational research in ophthalmology has achieved some progresses. In this era of translational medicine, it can still be challenging to facilitate the translation of basic research findings into clinical application to benefit more patients. These ophthalmological studies mentioned above help to accelerate the clinical translation and application of gene therapy and stem cell therapy, making ophthalmology a probable breakthrough in the use of stem cells in clinical practice. This may bring about a revolution in medical science and even throughout the healthcare industry, shed light on the treatment of other so-called "incurable" diseases, and thereby make a profound, positive impact upon human health.

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