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REVIEW

Stem cell therapies for age-related macular degeneration: the past, present, and future

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Abstract: In the developed world, age-related macular degeneration (AMD) is one of the major causes of irreversible blindness in the elderly. Although management of neovascular AMD (wet AMD) has dramatically progressed, there is still no effective treatment for nonneovascular AMD (dry AMD), which is characterized by retinal pigment epithelial (RPE) cell death (or dysfunction) and microenvironmental disruption in the retina. Therefore, RPE replacement and microenvironmental regulation represent viable treatments for dry AMD. Recent advances in cell biology have demonstrated that RPE cells can be easily generated from several cell types (pluripotent stem cells, multipotent stem cells, or even somatic cells) by spontaneous differentiation, coculturing, defined factors or cell reprogramming, respectively. Additionally, in vivo studies also showed that the restoration of visual function could be obtained by transplanting functional RPE cells into the subretinal space of recipient. More importantly, clinical trials approved by the US government have shown promising prospects in RPE transplantation. However, key issues such as implantation techniques, immune rejection, and xeno-free techniques are still needed to be further investigated. This review will summarize recent advances in cell transplantation for dry AMD. The obstacles and prospects in this field will also be discussed.

Keywords: stem cell, age-related macular degeneration, retinal pigment epithelium, cell reprogramming, clinical trial

Background

In the Western world, age-related macular degeneration (AMD) is one of the leading causes of blindness in the elderly. The incidence rate of AMD has continued to increase in the past decades.[1–4] According to the presence or absence of choroidal neovascularization, advanced AMD can be generally classified into two types: dry AMD and wet AMD. Wet AMD could be controlled by drugs that target the vascular endothelial growth factor (VEGF), photodynamic therapy, laser photocoagulation, and vitrectomy at different phases. Dry AMD, which is primarily attributed to the accumulation of reactive oxygen species and lipid peroxide, can evoke chronic inflammations in the retina and lead to apoptosis of the retinal pigment epithelial (RPE) cells, and finally damages the photoreceptors.[5] Currently, no treatments can reverse dry AMD, regardless of the fact that dietary supplementation with defined vitamins and antioxidants has been shown to alleviate progression.[6] Therefore, RPE replacement and retinal microenvironmental regulation represent potential new approaches for dry AMD.

Functional RPE cells could be generated from stem cells or somatic cells by spontaneous differentiation,[7–16] coculturing,[17] defined factors,[18–22] or cell reprogramming.[23] Source of RPE cells for transplantation seems to be unlimited. More importantly, a clinical trial approved by the US government has shown promising prospects in RPE transplantation.[24] However, xeno-free techniques,[11,12] implantation techniques, immune rejection,[25–27] and the safety issues are still under debate.

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In addition, mesenchymal stem cells (MSCs) have various biological effects,[28] such as immunoregulation, antiapoptosis of neurons, and neurotrophin secretion. In vivo studies also have suggested that MSCs could recover and regulate the retinal microenvironment in different models of retinal degeneration.[29,30] Moreover, MSCs are also ideal vehicles in cell engineering. Gene-modified MSCs always have specific functions and could be utilized in AMD treatments.[31–34]

This review will focus on the following aspects: 1) RPE transplantation and 2) stem cell-based retinal microenvironmental regulation.

RPE transplantation

Healthy and vigorous RPE cells are ideal donors for transplantation, and pre-AMD is a viable therapeutic target. According to the cell source, they could be divided into 1) autologous RPE cells, 2) stem cell-derived RPE cells, and 3) reprogrammed RPE cells.

Autologous RPE cells

As the diseased RPE is a major component of dry AMD, several attempts have been made to replace the aged RPE cells located at the macula. Macular translocation surgery is conducted by the detachment and rotation of neural retina from the diseased macular RPE layer to another healthy place.[35–37] After up to 5 years of follow-up, three Snellen lines of improvement in best corrected visual acuity were obtained in some patients.[38–40] However, high complication rates were noticed, such as macular edema, retinal detachment, double vision, and cataract formation.[38–40] Nonetheless, successes in macular translocation demonstrated that 1) healthy RPE cells were located in the diseased retina and 2) these healthy RPE cells could restore the visual function in AMD patients.

Thereafter, autologous RPE transplantation as an alternative surgical approach was widely studied. It is accomplished by collecting healthy RPE cells in the peripheral retina and transplanting them into the subretinal space at the diseased macula.[41–45] The clinical outcomes are similar to those of the macular translocation: maintenance or slight elevations in visual acuity were reported in several trials after 3 or 4 years of follow-up.[41–44] Although autologous RPE transplantation has a relatively low rate of complication when compared with macular translocation, there are some remarkable drawbacks: 1) The initial harvesting of RPE cells from patients increases the length of the surgical procedure and the risk of postsurgery complications, such as cataract formation and retinal detachment. 2) No evidence could demonstrate that the transplanted RPE cells in suspension can first attach to the diseased Bruch's membrane and form the desired monolayer which is required for optimal RPE function. In contrast, these cells always clump into rosettes[46] or undergo anoikis,[47] a form of apoptosis specific to anchorage-dependent cells that are dissociated from their usual extracellular matrix. 3) The cells being harvested are the same age as the cells they are designed to be replaced. 4) Autologous RPE transplantation requires more than 60,000 viable RPE cells. It is quite difficult to collect enough cells to repopulate the entire macula adequately.

Stem cell-derived RPE cells

RPE cells from the patients might be insufficient for transplantation, and highly efficient protocols for generating functional RPE cells are eagerly required. Stem cells are able to differentiate into several cell types as well as self-renew. According to their potential, they can be generally classified into pluripotent stem cells (embryonic stem cells [ESCs] and induced pluripotent stem cells [iPSCs]) and multipotent stem cells (neural stem cells, MSCs, and so on). Recent studies have revealed that 1) functional RPE cells could be differentiated from pluripotent stem cells or multipotent stem cells by defined protocols and 2) visual function could be restored in vivo by transplantation of stem cell-derived RPE cells.

ESC-derived RPE cells

ESCs have extensive abilities to differentiate into all three germ layers. In the past decade, with the development of cell sciences, ESCs manifest extremely attractive prospects in the treatment of degenerative diseases. Several defined protocols were conducted to generate mature RPE cells from ESCs.

Spontaneous differentiation

Adherent culture

In natural conditions, ESCs can spontaneously differentiate into RPE-like cells by adherent culturing. This was originally reported by Kawasaki et al.[7] They found that about 8%±4% of pigmented cells could be generated from primate ESCs by coculturing with PA6 stromal cells. The ESC-derived RPE cells were hexagonal and contained significant amounts of pigment. They also expressed the mature markers of RPE cell: ZO-1, RPE65, CRALBP, and MerTK. Electron microscopy revealed that these cells had extensive microvilli and were able to phagocytose latex beads. After transplanting into the subretinal space of RCS (Royal College of Surgeons, London, UK) rats (a well-known model of RPE degeneration, which has a mutation in MerTK, characterized by loss

of phagocytic function of RPE cells), the grafted RPE cells increased the survival of host photoreceptors. Histologic analyses and behavioral tests further confirmed this.[8] This protocol has multiple advantages: 1) the techniques were relatively simple, and ESCs were only seeded onto the PA6 stromal cells feeder to form colonies; 2) neural differentiation of ESCs is efficient and speedy; and 3) no exogenous reagent was used. But harvested cells could be contaminated by PA6 stromal cells.

Human RPE cells also could be differentiated from human ESCs (hESCs) by similar ways: hESCs were seeded on an inactivated feeder and allowed to overgrow until confluent (approximately 2 weeks). Then, the basic fibroblast growth factor was removed from the medium, and the cells were allowed to differentiate spontaneously. After 4–5 weeks, pigmented foci could be observed. When these cells were transplanted into the subretinal space of RCS rats, the cells displayed polarity and integrated well into the host retina. More importantly, these cells showed phagocytic functions. Improvement in visual performance was 100% over untreated controls (spatial acuity was approximately 70% that of normal nondystrophic rats). In the safety evaluation, teratoma formation and other pathological changes were not observed under immunosupression.[9,10] Although the efficiency is relatively low, a significant advantage of this protocol is that no additional reagents (such as Wnt or nodal inhibitors) were supplemented. This protocol mimics the natural generation of RPE cells and avoids the potential contaminations from recombinant proteins or small molecules which were used in other protocols.

However, most of the published protocols used mouse embryonic fibroblast cells as the feeder layer for hESCs and human induced pluripotent stem cells (hiPSCs). Xeno-products used in the differentiation processes pose further challenges, because animal-derived components may carry factors such as sialic acid or Neu5Gc, causing unwanted immunogenicity of the cells,[48,49] or even animal pathogens. Recently, Vaajasaari et al[11] and Zhang et al[12] reported the differentiation of functional RPE-like cells from several hESC lines and one hiPSC line in defined and xeno-free conditions, providing an important step toward a defined and xeno-free culture and differentiation process, enabling easy translation to clinical-quality cell production under Good Manufacturing Practice regulations.

Suspension culture

RPE cells could also be spontaneously differentiated from ESCs by embryonic formation. ESCs were seeded onto a petri dish in the absence of a differentiation antagonist to form embryonic bodies (EBs). Three-dimensional suspension aggregates can mimic embryonic development in vivo. To yield more cells of neuroectodermal lineage, the EBs are replated to a coated dish containing neural differentiation media (coated with extracellular matrix) for adherent culturing.[13,14] Pigmented cells could be found thereafter. In 2011, Advanced Cell Technology (Santa Monica, CA, USA) performed Phase I/II clinical trials by using this protocol to elucidate the efficiencies of hESC-derived RPE transplantation on dry AMD and Stargardt's disease (registration numbers NCT01345006 and NCT01344993).[50] Subsequently, Schwartz et al[24] published the preliminary results of their study, in which two patients (dry AMD and Stargardt's disease, respectively) received subretinal transplantation of 5×10^4 induced RPE cells by vitrectomy. Efficiency evaluations: the cells survived after 4 months of follow-up. The best corrected visual acuities of both patients were slightly improved: 7-letter improvements were achieved in the AMD patient (from 21 to 28 letters) and 5-letter improvements were achieved for the patient with Stargardt's disease (evaluated by the Early Treatment for Diabetic Retinopathy Study visual chart). Safety evaluations: no teratoma formation and immunologic rejection were noticed in both cases. The investigators also found that the phase of cell differentiation was directly associated with cellular attachment and survival: RPE cells with mild depigmentation have better proliferative and adherent abilities. Therefore, choosing donor cells at optimal stages is a crucial step for successful transplantation. Also, hESCs used for differentiation should not contain pathogenic genes, and RPE cell purification is an additional concern.

Subsequently, with the establishment of a three-dimensional culture system, Eiraku et al[51] reported that the optic cup and mature RPE layers could be spontaneously generated by a three-dimensional culture of mouse ESC aggregates. Zhu et al[52] demonstrated the utility of this epithelial culture approach by achieving a quantitative production of RPE cells from hESCs within 30 days. Direct transplantation of this RPE into a rat model of retinal degeneration without any selection or expansion of the cells results in the formation of a donor-derived RPE monolayer that rescues photoreceptor cells. The cyst method for neuroepithelial differentiation of pluripotent stem cells is not only of importance for RPE generation but will also be relevant to the production of other neuronal cell types and for reconstituting complex patterning events from three-dimensional neuroepithelia.

However, three-dimensional culturing is time-consuming and expensive. To enhance the efficiency of RPE generation,

Cho et al[53] conducted a protocol which indicated that RPE cells could be obtained from spherical neural masses. The target cells showed polygonal-shaped epithelial monolayer, and electron microscopy revealed apical microvilli, pigment granules, and tight junctions. These cells also expressed molecular markers of RPE, including ZO-1, RPE65, and bestrophin. On functional evaluation, these cells showed phagocytosis of isolated photoreceptor outer segment (POS) and secretion of soluble factors such as pigment epithelium-derived factor (PEDF) and VEGF. This protocol has remarkable merits: 1) Spherical neural masses have the capability of expansion for long periods without loss of differentiation capability and 2) they are easy to store and thaw, and there is no need for feeder cells. Thus, it could be an efficient strategy for obtaining functional RPE cells for retinal regenerative therapy.

Directed differentiation

ESCs also can directly differentiate into RPE-like cells by supplementing with defined factors.[18,19] Early studies using stepwise differentiation protocols were based on models of telencephalic cell derived from ESCs, and combined EB formation with subsequent culture of attached cells in media containing proteins which control specification of neuronal lineage (such as Dkk1, a Wnt antagonist; LeftyA, a nodal antagonist). In 2005, Ikeda et al[18] conducted a protocol by which retinal precursors could be directly differentiated from mouse ESCs by supplementing Dkk1 and LeftyA under serum-free, feeder-free conditions; 16% of the total cells could be differentiated into retinal precursor cells (Rax positive). After optimizing the protocols, the efficiency of differentiation has been greatly elevated.[19]

In addition, insulin-like growth factor signaling pathways and transforming growth factor beta (TGFβ) signaling pathways (such as bone morphogenetic protein antagonists, nicotinamide, and Activin A) were also reported to play important roles in RPE differentiation. Using Noggin (a bone morphogenetic protein antagonist), Dkk1-1, and insulin-like growth factor 1, Lamba et al[54] found that up to 80% of the H1 line can be directed to the retinal progenitor fate, and express a gene expression profile similar to that of progenitors derived from human fetal retina. The most prominent benefit of this protocol is that high percentages of target cells were generated from hESCs within a short period. In another study, Idelson et al[55] revealed that nicotinamide (belonging to TGFβ superfamily), which presumably patterns RPE development during embryogenesis, promotes the differentiation of hESCs to neural and subsequently to RPE fate. The hESC-derived RPE cells exhibited a morphology, marker expression, and function similar to those of authentic RPE and restored retinal structure and function after transplantation in vivo. Activin A, a member of the TGFβ superfamily, is another critical factor in RPE differentiation. It was secreted by the extracellular mesenchyme during optic cup development. With the addition of Activin A, the yield of RPE cells increased.[55] Alternatively, Activin A may serve to maintain the differentiated RPE cell phenotype in culture.[56]

Although protocols mentioned so far have become more efficient than the report in 2004, they are still a bit time-consuming and inefficient. In a recent study, Buchholz et al[57] found that supplementing with defined factors at specific times could yield approximately 80% of the cells to an RPE phenotype within 2 weeks. They also noticed that culturing with more non-RPE cells led to faster RPE pigmentation, suggesting that these cells may secrete factors that activate melanogenesis.

However, the defined factors in these protocols are all derived from animal cells or *Escherichia coli*, raising the possibility of infection or immune rejection due to cross-species contamination. By contrast, using chemical compounds offers several advantages, compared with the recombinant proteins: 1) the small molecules are chemicals, which are consistent between different lot numbers and manufacturers; 2) the cross-species contaminations and cross-reactions are easily avoided; and 3) the cost is relatively low, making this method applicable. In a serum-free and feeder-free floating aggregate culture, Osakada et al[58] found that ESCs and iPSCs could be efficiently differentiated into RPE cells by supplementing CKI-7 (a Wnt antagonist) and SB-431542 (a nodal antagonist). These cells displayed the characteristic morphology of mature RPE cells, protein markers, and phagocytic capacity. This method provides a solution to cross-species antigenic contamination in transplantation, and is also useful for in vitro modeling of development, disease, and drug screening. However, whether these effects are reversible and transient is largely unknown. More research is needed to evaluate the long-term biological effects.

iPSC-derived RPE cells

In recent years, the most breaking advance in cell biology is probably iPSCs, which was first reported by Takahashi and Yamanaka[59] and Yu et al.[60] These cells reprogrammed by using Thomson factors or Yamanaka factors showed morphological characteristics and differentiation abilities (including iPSCs to RPE) similar to those of the ESCs. Studies by several groups have already demonstrated that human RPE cells could be

generated from iPSCs by spontaneous differentiation[15,16] or directed differentiation.[20–22,61] The iPSC-derived RPE cells were morphologically similar to, and expressed numerous markers of, developing and mature RPE cells. Phagocytosis of isolated POS and secretion of soluble factors (PEDF and VEGF) were also mentioned by several groups.[15,16,20–22] Interestingly, Westenskow et al[62] developed a flow cytometry-based assay to compare the phagocytic function between ARPE-19, human fetal RPE, and two types of iPSCs-RPE. They found that highly differentiated iPSCs-RPE phagocytosed POS more efficiently than did native RPE. In vivo studies also suggested that transplantation of these cells could facilitate the maintenance of photoreceptors through phagocytosis of the POS in the model of RPE degeneration.[15,63]

Additionally, iPSCs could be generated by using less transcription factors, which would reduce the incidence of tumorigenesis. Krohne et al[63] found that 1-factor-iPSC-RPE significantly resembled native RPE cells not only on proteomics and untargeted metabolomic analyses but also on in vivo functional evaluations. They showed that 1-factor-iPSC-RPE mediates anatomical and functional rescue of photoreceptors after transplantation in an animal model of RPE degeneration. Moreover, iPSCs could also be derived from other somatic cells than fibroblasts, including RPE cells. Hu et al[64] reprogrammed primary RPE cells by using OCT4, SOX2, LIN28, and Nanog. The RPE-derived iPSCs exhibited morphologies, gene expressions, and teratoma formation similar to hESCs and other iPS cell lines. After spontaneous differentiation by the removal of fibroblast growth factor 2, the resultant RPE cells showed a marked preference for redifferentiation into RPE. They suggested that target cells retain a memory of their previous state of differentiation.

Despite the fact that most protocols for ESC differentiation are suitable for iPSCs, differentiation efficiencies between iPS cell lines vary. Hirami et al[61] suggested that under identical conditions (SFEB/DL), 201B7 and 253G1 cell lines could differentiate into RPE cells, whereas 201B6 cell lines could not. From the perspective of protein expression, 6 days after differentiation toward RPE cells, Rx^+/Pax^+ cells emerged in an mESC-derived pool of cells, whereas this emergence requires 15 days with cells derived from certain iPS cell lines.

iPSC-derived RPEs have several advantages. First, absence of ethical concerns is the biggest benefit for research. Second, patient-specific iPSCs might have minimal immunogenicity than ESCs or other originated RPE cells. Third, iPSC-derived RPEs could be considered as a well-established model for disease mimicking and drug screening.

However, shortcomings of using iPSC-derived RPEs for transplantation cannot be ignored: 1) cells derived from iPSCs have the potential ability of tumorigenesis, which would restrict their clinical applications; 2) generation of patient-specific iPSCs would be a costly and time-consuming course; and 3) patient-specific iPSCs might have genetic defects that contribute to the disease. Combining iPSC technology with gene therapy is a promising solution.[65]

MSC-derived RPE cells

Although RPE cells are derived from the ectoderm, MSCs have the ability of cross-mesodermal differentiation. Huang et al[17] found that RPE-like cells could be obtained from MSCs by RPE conditional medium supplemented with POS. These cells have morphological features and phagocytic capabilities similar to those of the native RPE cells.

Moreover, studies have also indicated that retinal cells could be differentiated from MSCs and replace the damaged retinal cells under certain conditions.[66,67] Gong et al[66] reported that MSC-originated RPE cells could be found in the sodium iodide-damaged retina after subretinal injection of MSCs for 5 days.

Retinal stem cell-derived RPE cells

The retinal stem cells (RSCs) are situated in the ciliary marginal zone (CMZ) in fish and amphibians. The CMZ can continuously generate new neurons after retinal injury. Despite the fact that the mature retina in mammalians lacks regenerative ability, Tropepe et al[68] noticed that CMZ cells are capable of proliferating and differentiating into retinal cells (rods, bipolar cells, and glial cells) in mature mice. By isolating RSCs and supplementing with linoleic acid, selenite, insulin, transferrin, thyroxin, and other factors into the medium, Aruta et al[69] successfully differentiated RSCs into polarized and phagocytotic RPE-like cells. Similar to the MSC-derived RPE cells described by Huang et al[17] no studies were conducted to evaluate the function and safety of induced RPE cells in vivo.

However, the existence of mammalian RSCs is still under debate. Cicero et al[70] speculated that the so-called RSCs are ciliary epithelial cells. Their study showed that no significant differences in molecular, cellular, and morphological characteristics were observed between RSCs and ciliary epithelial cells. They suggested that ciliary epithelial cells can form colony spheres, undergo self-renewal, and express precursor markers.

In addition, Müller cells were once considered as retinal stem cells. Bernardos et al[71] reported that Müller cells could

express Pax6 and Crx at a low level in zebra fish. Song et al[72] found that Atoh7 could promote the transformation of Müller cells into retinal ganglion cells. However, Müller cells originate from neural retinal precursors and mature at the last stages of retinogenesis, and RPE precursors, and neural retinal precursors divided during early embryonic development (neural retinal cells develop in the following order: retinal ganglion cells, cone cells, amacrine cells, horizontal cells, rod cells, bipolar cells, and Müller cells). Therefore, direct transformation of Müller cells into RPE will be extremely difficult.

Reprogrammed RPE cells (somatic cell-derived RPE cells)

With the development of cell biology, direct cell reprogramming shows a promising prospect in the generation of target cells from other types of somatic cells. The most important and interesting advantage of this technique is that direct lineage conversion could bypass the pluripotent state, and therefore might reduce the risk of tumor formation. In addition, the process of direct lineage conversion requires less time than does the conventional differentiation by iPSCs or ESCs.

Currently, using defined transcription factors, direct lineage conversion has been applied to generate various cell types, including neurons,[73–75] kidney cells,[76] endocrine beta cells,[77] hepatocytes,[78] oligodendroglial cells,[79] as well as RPE cells.[23] Zhang et al[23] reported that defined transcription factors (cMyc, Mitf, Otx2, Rax, and Crx) could reprogram human fibroblasts into RPE cells by supplementation with retinoic acid and sonic hedgehog in a matrigel-based culture condition. These cells exhibit specific morphological and molecular features of RPE lineage and are capable of pigmentation. The most significant weakness in this study was that the suspected cells were not further evaluated by a functional test. However, this study still provided a novel direction to learn the nature of cellular identity and plasticity of RPE lineage, and also conducted a new approach to obtain functional RPE cells for regenerative medicine.

MSC-based microenvironmental regulation

Oxidative stress, overexpression of inflammatory cytokines, and retinal nutritional deficiency are some common mechanisms of AMD.[5] MSCs have various biological effects,[28] including secreting neurotrophins, promoting angiogenesis, regulating immune responses, inhibiting apoptosis, promoting extracellular matrix remodeling, and activating adjacent host stem cells. Furthermore, due to the low immunogenicity, MSCs are also ideal vehicles for introducing exogenous neurotrophic genes which could be expressed in the host retina. Therefore, MSCs are excellent candidates for dry AMD treatment.

On the basis of different origins, MSCs can be classified into bone marrow-derived MSCs (BM-MSCs), umbilical cord blood MSCs, placenta-derived MSCs, adipose-derived MSCs, and so on. BM-MSCs are the most well-studied groups of MSCs. This section will focus on the recent applications of BM-MSCs in AMD therapy.

Roles of BM-MSCs on retinal microenvironmental regulation

BM-MSCs can secrete neurotrophins

Inoue et al[80] reported that conditioned medium of BM-MSCs could inhibit photoreceptor apoptosis in vitro. After intravitreal injection of BM-MSCs, photoreceptor apoptosis was also delayed, and retinal function was slightly restored in RCS rats. These results indicated that soluble factors secreted by BM-MSCs may inhibit photoreceptor apoptosis. In another study, Zhang and Wang[81] found that intravitreally injected BM-MSCs could express brain-derived neurotrophic factor (BDNF) and protect the outer nuclear layer in light-damaged retina. Xu et al[29,30] also reported that MSCs could secret basic fibroblast growth factor and exhibit neuroprotective effects in light-damaged retina. Importantly, not only intravitreal injection but also intravenous injection of MSC could achieve retinal protective effects. Wang et al[82] reported that intravenous injection of 1×10^6 MSCs increased the survival of photoreceptors and restored the visual functions in RCS rats. Reverse transcriptase polymerase chain reaction and immunohistochemistry suggested that the protective effects were attributed to the retinal neurotrophins secreted by MSCs.

BM-MSCs can alleviate retinal inflammation

Xu et al[29,30] found that intravitreal injection of BM-MSCs could suppress microglia activation, thereby reducing the retinal injury.

BM-MSCs can inhibit neuronal apoptosis

Otani et al[83] showed that retinal antiapoptotic genes were significantly upregulated after intravitreal injection of BM-MSCs. These genes included low-molecular-weight heat shock proteins and transcription factors.

BM-MSCs integrate into the host retina

Arnhold et al[84] found that intravitreal injection of BM-MSCs could significantly protect photoreceptors in rhodopsin

knockout retinitis pigmentosa mice. They also showed that the transplanted BM-MSCs were well integrated into the RPE layer and the neurosensory layer of the host retina.

Notably, 1) the survival or integration of MSCs originated from different tissues might be very diverse. Intravitreally injected UCB-MSCs rarely migrated to the retina and only survived for 3 weeks,[85] whereas BM-MSCs survived for up to 20 weeks and had a good integration ability.[86] 2) The neuroprotective effects of MSCs might be different between species. A study conducted by Levkovitch-Verbin et al[87] revealed that protection of retinal ganglion cells was merely noticed in human BM-MSCs, but not in rat BM-MSCs. 3) Methods for transplantation always relate to the experimental outcomes. Tzameret et al[86] compared the effects of intravitreal injection and subretinal injection in RCS rats. They found that the therapeutic effects lasted 12 and 20 weeks, respectively. The b-wave amplitudes in the electroretinogram were 56.4 μV in the intravitreal injection group and 66.2 μV in the subretinal injection group. 4) Retinal microenvironments in the host eyes also affect the functions of MSCs.

On the basis of the successful works in vivo, several Phase I/II clinical trials of MSCs were prudently conducted by some leading ophthalmologists. In 2005, Kumar et al[88] reported the outcomes of intravitreal injections of autologous BM-MSCs in 25 patients with dry AMD and retinitis pigmentosa. The mild improvement in BCVA was noticed after 1 or 3 months of injection. In 2010, Jonas et al[89] (registration number NCT01068561) reported the primary outcomes of three cases that received BM-MSC intravitreal injection (including one case of dry AMD). The initial BCVA of patients was poor in terms of light perception (poor light positioning). Twelve months after BM-MSC injection, no significant improvement in visual acuity and no serious complications were observed. The only effect was fluctuations of intraocular pressure (15–30 mmHg) at 4 weeks after treatment. Siqueira et al[90] intravitreally injected 1×10^7 BM-MSCs per eye in three retinitis pigmentosa patients and two cone–rod dystrophy patients. The results indicated that the visual acuities improved more than one row in four patients after 1 week and that these improvements were maintained at the end of the follow-up. Electrophysiological recordings of two patients were mildly improved. However, no significant changes in angiography, optical coherence tomography, and visual field were observed. Although the current clinical trials have not shown promising results, we must bear in mind the following: 1) Patients enrolled were relatively old, and their BM-MSCs have limited proliferative capacity and viability and 2) the patients were in advanced stages of disease. Therefore, vision recovery in these patients is sometimes difficult.

Effects of gene-modified MSCs

As an alternative to a viral vector, the application of stem cells to transfer specific genes is under investigation in various organs, including the eye.[31] Guan et al[32] found that after transplanting gene-modified MSCs into the subretinal spaces of sodium iodate-damaged eyes, a significant increase in erythropoietin was noticed and gene-modified MSCs showed stronger protective effects on retinal neurons than did conventional MSCs. Machalinska et al[33] also found that gene-modified MSCs stably expressing the NT-4 gene could migrate to the retinal damage area and protect the damaged cells. More importantly, gene-modified MSCs could upregulate the signals and transcription factors related to cell survival, such as crystallin β–γ superfamily members. In addition, gene-modified MSCs also increased the expression of proteins related to visual perception, visual signal reception, and eye development. In another study, Park et al[34] evaluated the integration ability of gene-modified BM-MSCs and their BDNF secretion in vivo. They found that approximately 15.7% of the MSCs integrated into the retina after 4 weeks. The protein and mRNA levels of BDNF were greatly increased in the host retina. The function of gene-modified MSCs is largely dependent on the genes they deliver. Choosing suitable genes and delivery protocols will enable us to establish a new direction for ADM treatment.

Prospects

In-depth studies on the biological characteristics of stem cell-derived RPEs, differentiation protocols, and transplantation methods are gradually changing the current stem cell-based therapy from a dream to reality. However, there are still several obstacles before their clinical application. Transplanted RPE cells showed limited adhesion and survival in human eyes, and aged Bruch's membrane did not likely support adhesion, survival, differentiation, and function of grafted RPE cells.[91–94] Therefore, the use of genetic engineering to overexpress integrins or integrin activators in the RPE cells[95–97] or the use of RPE cells growing on scaffolds might show promising prospects. Second, although subretinal space was once considered to have immune privilege, studies also have indicated that the long-term survival of the transplanted cells in the host eyes still required immune suppression.[25–27] Thus, the course of immunosuppression and the drugs used for immunosuppression have to be further discussed.

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Disclosure

The authors report no conflicts of interest in this work.

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