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Review Article

Stem cell-based therapies for age-related macular degeneration: current status and prospects

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Abstract: Age-related macular degeneration (AMD) is one of the major causes of irreversible blindness both in developed and developing countries. During the past decades, the managements of neovascular AMD (wet AMD) have dramatically progressed. However, still no effective treatment for non-neovascular AMD (dry AMD) which was characterized by geographic macular atrophy. Recent advances in stem cell sciences have demonstrated that retinal pigment epithelium (RPE) cells can be generated from several types of stem cells (including embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells, et al) by cell co-culturing or defined factors. Additionally, studies also showed that visual function could be recovered by transplantation of these cells into subretinal space in vivo. Moreover, the United States Food and Drug Administration already approved several clinical trials to evaluate the efficiencies of stem cell based cell transplantation for dry AMD patients. Till now, a few patients enrolled in these studies achieved promising outcomes. This review will summarize recent advances in stem cell based RPE differentiation, transplantation, and the preliminary results of clinical trials. The obstacles and prospects in this field will also be discussed.

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

Age-related macular degeneration (AMD) is one of the leading causes of irreversible blindness in people over 65 years of age in the world. The incidence rate of AMD is still increasing in the past decades [1-4]. According to the presence or absence of choroidal neovascularization (CNV), AMD can be generally divided into two types: dry AMD and wet AMD. Wet AMD could be controlled by drugs that target vascular endothelial growth factor (VEGF), photodynamic therapy, laser photocoagulation and vitrectomy at different stages of the disease.

Dry AMD is primarily attributed to the accumulation of reactive oxygen free radicals and lipid peroxide which evoke local activation of chronic inflammation and lead to apoptosis of retinal pigment epithelium (RPE) cell, ultimately damage photoreceptors in the outer nuclear layer. Currently, not any drug is available for dry AMD [8]. Therefore, cell replacement and retinal microenvironmental regulation represent potential new approaches for dry AMD.

Stem cells are pluripotent and renewable. They can efficiently differentiate into RPE cells or photoreceptors under defined conditions. Therefore, stem cells have been seemed as unlimited resource of cell transplantation. In addition, stem cells (particularly mesenchymal stem cells, MSCs) perform multiple functions, such as immunoregulation, anti-apoptosis of neurons and neurotrophin secreting. Many studies also suggested that MSCs could maintain and regulate the microenvironment in different models of retinal degeneration. With the progress in basic medical sciences, several phase I/II clinical trials were approved by the FDA and gingerly conducted by some leading ophthalmologists and companies.

This review will focus on the following two aspects: 1, stem cell based RPE replacement;

2. Retinal microenvironmental regulation of MSCs.

Stem cell based RPE replacement

Healthy and vigorous RPE cells are ideal donor cells for patients with dry AMD. According to the source of RPE cells, they can be divided into: 1, stem cell-derived RPE cells; 2, fetal/adult RPE cells; 3, iris pigment epithelial cells; and 4, autologous RPE cells [9-11]. The latter three types of cell are not the only source limited but also lacking in capacity of proliferation. More importantly, isolation and purification of primary RPE cells are time and labour consuming. Therefore, it is very difficult for clinical application.

Embryonic stem cells (ESCs), induced pluripotent stem cells (iPS), and adult stem cells can differentiate into functional RPE cells under certain defined conditions.

ESC-derived RPE cells

Nowadays, ESC-derived RPE cell is a hot spot in regenerative medicine. Seven protocols are now available to generate mature RPE cells from ESCs: 1, spontaneous differentiation; 2, stromal cell-derived inducing activity (SDIA); 3, serum-free floating culture of embryoid body-like aggregates (SFEB); 4, small-molecule induction; 5, retinal determination (RD); 6, spherical neural masses (SNMs) sorting; 7, three-dimensional (3D) culture.

Spontaneous differentiation: Approximately 1% of ESCs can automatically differentiate into RPE-like cells [12] and express the mature markers of RPE cells. After transplantation of these cells into subretinal space of RCS (Royal College of Surgeons) rats (a well-known model of RPE degeneration, which has a mutation in MerTK, is characterized by losing phagocytic function of RPE cells), the donor cells displayed polarity and were demonstrated to integrate well with the photoreceptors of recipient. In functional evaluation, these cells were able to phage the photoreceptor outer segments and recover the visual function of RCS rats [12, 13]. Importantly, teratoma formation and other pathological changes were not observed under immunosuppression.

Although the efficiency is relatively low, a remarkable advantage was noticed in this protocol that it does not depend on recombinant proteins produced in animal or Escherichia coli cells. This protocol provides a solution to the problem of cross-species antigenic contamination in cell-replacement therapy. This protocol has been approved by the FDA as a good manufacturing practice (GMP) standard [14]. In 2011, Advanced Cell Technology (Santa Monica, California, USA) performed phase I/II clinical trials to elucidate the efficiencies of hESC-derived RPE transplantation on dry AMD and Stargardt's disease (registration numbers: NCT01345006 andNCT01344993) [14]. Subsequently, Schwartz et al published the preliminary results: Two patients (dry AMD and Stargardt's disease, respectively) received subretinal transplantation of 5×10^4 induced RPE cells by vitrectomy, the safety and efficiency were analyzed subsequently [15]. Efficiency evaluations: the grafts were continually present within 4 months of follow-up. The best-corrected visual acuity (BCVA) of both patients were slightly improved: 7 letters improvements were achieved in the patient with dry AMD (from 21 letters to 28 letters) and 5 letters improvements were achieved in the patient with Stargardt's disease (from 0 letters to 5 letters) (evaluated by the Early Treatment for Diabetic Retinopathy Study visual chart). Safety evaluations: No signs of abnormal cell proliferation, immune rejection were noticed in both cases. They also found that differentiation stages of RPE cell were associated with cell attachment and survival: RPE cells with mild depigmentation had better proliferative and adhesive abilities.

Although preliminary studies have shown promising prospects for RPE cell transplantation, there are still some problems needed to be overcame: 1. the proliferation and viability of donor cell in vivo were depended on the differentiated stages of RPE cells in vitro, thus, the crucial step for transplantation is choosing donor cell with proper differentiated stage. 2. hESCs used for differentiation should not contain pathogenic genes. 3. The protocol for obtaining highly purified RPE cells is another concern.

SDIA (stromal cell-derived inducing activity): In 2000, Kawasaki et al. identified stromal cell-derived inducing activity (SDIA) protocol that promotes neural differentiation of mouse ES

cells. SDIA accumulates on the surface of PA6 stromal cells and induces efficient neuronal differentiation of cocultured ES cells in serum-free conditions without use of either retinoic acid or embryoid bodies. [16]. Two years later, they found about (8%±4%) pigmented cells could be generated from primate ESCs by this protocol [17]. These cells have the same marker (ZO-1, et al) and phagocytosis function similar with the primary RPE cells. More importantly, these cells could protect the photoreceptors and recover the visual functions of RCS rats in vivo [18]. The biggest advantage of this protocol is no exogenous reagent was used, but the target cells could be contaminated by PA6 stromal cells. In addition, photoreceptor-like cells were not observed in their study. As photoreceptors play a key role in cell replacement, this shortage will restrict the application of this protocol.

SFEB culture: In 2005, Ikeda et al. devised a protocol by which retinal precursors could be directly differentiated from mouse ES cells. Under serum-free suspension conditions (SFEB culture) in the presence of Wnt and Nodal antagonists (Dkk1 and LeftyA), 16% of the total cells could be differentiated into retinal precursor cells (Rax positive) [19]. After 4 years of condition optimized, the efficiency of differentiation has been greatly elevated, 23.8%±2.7%, 11.5%±2.0% and 17.2%±1.8% of the total cells could be generated into RPE precursor cells, cones and rods, respectively [20]. Unfortunately, SFEB-induced cells have a poor capacity to be integrated into the host retina [21] primarily due to the low proportion of retinal precursor cells induced by SFEB. Another reason is that the differentiated cells are usually mature, thus, these cells have a poor integrative ability, although the differentiation mimic the process of retinal development [22].

Small molecule inductions: Using casein kinase I inhibitor CKI-7, the ALK4 inhibitor SB-431542 and the Rho-associated kinase inhibitor Y-27632 in serum-free and feeder-free floating aggregate culture, Osakada et al found that ESC and iPS could be efficiently differentiated into RPE cells. These cells displayed the characteristic morphology of mature RPE cells, protein markers and phagocytic capacity. The small molecule induction has the following advantages: Firstly, the inducing reagents are chemicals, which are consistent between different batches and manufacturers. Secondly, this method avoids contaminations and cross-reactions which were observed in biological inductions. Thirdly, the cost is relatively low, making this method easily applicable. However, till now, not any study evaluates the safety and efficiency of this protocol in vivo.

RD (retinal determination): Using Noggin (an inhibitor of the bone morphogenic protein (BMP) pathway), Dickkopf-1 (DKK1, an inhibitor of the Wnt/β-catenin pathway) and insulin-like growth factor-1 (IGF-1), Lamba D et al. [24] obtained up to 82%±23% Pax+ retinal precursor cells, among which 86% of the cells also express Chx10. After transplanted these cells into the subretinal space of Crx deficient mice (a well-established model of photoreceptor degeneration), restoration of some visual function were noticed. The most prominent benefit of this protocol is that: high percentages of target cells were generated from ESC within an especially short period.

SNMs sorting: In 2008, Cho et al. [26] obtained SNMs by selecting neural precursor cells for further amplification after embryoid body formation. Among the SNMs, approximately 5% of the vesicle-like structures eventually differentiated into RPE cells [27]. The SNMs method has the following advantages: 1. No exogenous reagent is required, thereby avoiding contamination and immune responses. 2. SNM-derived RPE cells are produced in a process similar to the natural process that generates RPE cells. 3. SNMs shortens the transition time from ESCs to RPE cells. However, this method has not been examined stringently with animal experiments to test whether the resulting RPE cells are functional.

Three-dimensional culture: In 2011, Eiraku et al. [28] found the optic cup and mature RPE cells could be generated from a three-dimensional culture of mouse embryonic stem cell aggregates. Subsequently, Zhu et al. [29] demonstrate the utility of this epithelial culture approach by achieving a quantitative production of retinal pigment epithelial (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.

Cell replacement by iPS-derived RPE cells

In 2006, Yamanaka et al. [30] reported that mouse fibroblasts could be induced into ESC-like cells. These cells which were named as iPSCs, have similar morphological characteristics and differentiation ability compared with the ESCs. Under various conditions, iPS cells can be easily differentiated into cells of all three germ layers. iPS cells also have several advantages in regenerative medicine: 1. These cells can be generated from a variety of cell types, including the RPE cell, thus, it is convenient to be used as a disease model. 2. Theoretically, these cells are non-immunogenic. Because iPS cells are derived from differentiated cells of their host, transplantation using iPS derived cells avoids cross-species and cross-individual rejection. 3. No ethics concern is linked to iPS applications. 4. iPS cells can also be used to establish disease models and drugs screening.

Similar to ESCs, iPS cells could be easily differentiated into RPE cells and photoreceptors by defined protocols. iPS-derived RPE cells express markers of mature RPE and have phagocytic function in vitro. In vivo studies also demonstrated that subretinal injection of these cells could protect the visual functions of the RPE deficient animals [31-36].

Although most protocols for ESCs differentiation are suitable for iPS, differentiation efficiencies between iPS cell lines are various. Hirami et al. suggested [20] that, under identical conditions (SFEB/DL), 201B7 and 253G1 cell lines can differentiate into RPE cells, whereas 201B6 cell lines cannot. Additionally, Rx+/Pax+ cells could be found after 6 days of differentiation in ESCs, whereas, 15 or more days were required in several iPS cell lines. This phenomenon may be due to the genomic characteristics of specific iPS cells or to the culture environment and/or stages of differentiation.

Despite the numerous advantages of iPS cells, their shortages cannot be ignored. Firstly, iPS cells originate from the patient and likely carry disease genes. iPS-derived cells can be safe for transplanting into the recipient only when the causative gene is repaired [37]. Secondly, iPS cells potentially carry tumorigenic risks. Hirami et al. [20] found that 0.60%±0.04% of the cells express Nanog by day 15 of differentiation.

Cell replacement by MSC-derived RPE cells

Although RPE cells and photoreceptors are derived from the ectoderm, MSCs have the ability of cross-mesodermal differentiation. Huang et al. [38] reported that mesenchymal stem cells (MSCs) could be differentiated into RPE like cells with similar morphological and phagocytic capabilities using the photoreceptor outer segments and RPE conditioned medium.

In addition, under certain conditions, MSCs can be further differentiated when transplanted into damaged retina, thereby replacing damaged retinal cells. Gong et al. [39] injected bone marrow (BM) -MSCs into the subretinal space of sodium iodide damaged retina (a model of RPE degeneration). Five weeks later, some BM-MSCs transformed into RPE cells, photoreceptors and glial cells. Tomita et al. [40] found that MSCs could primarily migrate into the inner nuclear layer and transform into retinal cells that express GFAP, Calbindin, Rhodopsin and Vimentin. Castanheira et al. [41] intravitreally injected MSCs into the vitreous cavity of laser-damaged retina. After eight weeks, the majority of MSCs migrated to the ganglion cell layer, the inner nuclear layer, and the outer nuclear layer. These migrated cells expressed markers of photoreceptor cells, bipolar cells, amacrine cells and Müller glial cells.

Cell replacement with retinal stem cell (RSC)-derived RPE cells

The RSCs of fish and amphibians are located in the ciliary marginal zone (CMZ). Following retinal damage, the CMZ can continuously generate new neurons. Although the mature mammalian retina lacks regenerative ability, Tropepe et al. [42] suggested that CMZ cells in mature mice are capable of proliferating and differentiating into retinal neurons (rods and bipolar cells) and glial cells. They believe that these

cells are RSCs. Upon isolating RSCs, Aruta et al. [43] added linoleic acid selenite, insulin, transferrin, thyroxin and other factors and successfully induced RSCs into polarized and phagocytotic RPE-like cells. Similar to the MSCs derived RPE cells described by Huang et al, not any in vivo studies were conducted to evaluate the safety and efficacy.

However, whether mammalian RSCs exist remains controversial. Cicero et al. [44] speculate that the so-called CMZ-derived RSCs are ciliary epithelial cells. They demonstrated that no significant differences in molecular, cellular, and morphological characteristics were observed between these cells and differentiated ciliary epithelial cells. They also suggested that differentiated cells can form colony spheres, undergo self-renewal, and express precursor markers. Gualdoni et al. [45] found that the so-called RSCs could not activate neurelated lipocalin (Nrl, a key gene of photoreceptor differentiation) in photoreceptor differentiation medium.

In addition, Müller cells were regarded as retinal stem cells. Bernardos et al. [46] reported that Müller cells could express low levels of paired box 6 (Pax6, a marker of retinal precursor cells) and cone-rod homeobox (Crx, a marker of photoreceptor) inzebra fish. Song et al. [47] found that atonal homolog 7 (Atoh7, an inhibitor of the Notch pathway) can promote Müller cell transformation into retinal ganglion cells. Müller cells originate from neural retinal precursors and matureat the last stages of retinogenesis, whereas 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, straightforward transformation of Müller cells into RPE is extremely difficult.

Stem cell based microenvironmental regulation

Oxidative stress, inflammatory cytokines, and retinal nutritional deficiency are some of the pathogenic mechanisms of dry AMD [8]. Stem cells, particularly MSCs, have numerous biological effects, including secreting neurotrophins, promoting angiogenesis, regulating immune responses, antagonizing apoptosis, promoting extracellular matrix remodeling and activating adjacent host stem cells [48]. Furthermore, due to their low immunogenicity, MSCs are also an ideal carrier for introducing exogenous neurotrophic factors. These factors may also be expressed in the host retina and play biological effects. Therefore, MSCs are excellent candidates for treating dry AMD.

Based on different sources, MSCs can be classified into BM-MSCs, umbilical cord blood (UCB)-MSCs, umbilical cord (UC)-MSCs, placenta-derived (PD)-MSCs, adipose-derived mesenchymal stem cells (ASCs) etc. BM-MSCs are the most extensively studied groups of MSCs. This article will focus on reviewing BM-MSC researches and applications in treating dry AMD.

Roles of MSC on retinal microenvironmental regulation

MSCs can secrete neurotrophins: Inoue et al. [49] found that BM-MSC conditioned medium could delay photoreceptor apoptosis. After intravitreal injection of BM-MSCs, photoreceptor degeneration was decelerated, and retinal function was slightly protected in RCS rats. These results suggested that BM-MSCs may secrete soluble factors that inhibit photoreceptor apoptosis. In light-damaged retina, Zhang et al. [50] found that intravitreally injected BM-MSCs can express brain-derived neurotrophic factor (BDNF) and protect the outer nuclear layer. Xu et al. [51, 52] also reported that MSCs could release basic fibroblast growth factor (bFGF) and protect neurons in light-damaged retina. Wang et al. [53] injected 1×10^6 BM-MSCs into the tail veins of RCS rats, and found that the survival of the outer nuclear layer cells in the injected group was significantly greater than that of the control group. Rats in the injected group achieved significant visual improvements and electrophysiological recordings, as well as alleviated vascular leakage. Additionally, RT-PCR and immunohistochemistry also confirmed that the injected group had increased levels of growth factors and retinal neurotrophins.

MSCs can inhibit local inflammation: Xu et al. [51, 52] found that intravitreal injection of BM-MSCs could suppress microglia activation, thereby reducing retinal injury.

MSCs can inhibit neuronal apoptosis

Otani et al. [54] showed that retinal anti-apoptotic gene expression was significantly up-regulated after intravitreal injection of BM-MSCs. These genes included low molecular weight heat shock proteins and transcription factors.

MSCs integrate into the host retina

Arnhold et al. [55] found that intravitreal injection of BM-MSCs could significantly protect photoreceptors in rhodopsin knockout retinitis pigmentosa (RP) mice. They also showed the transplanted BM-MSCs were well integrated into the RPE layer and the neurosensory layer of the host retina.

Notably, 1, MSCs with diverse origins differ in their abilities to survive and to integrate into the host retina. Intravitreally injected UCB-MSCs rarely migrated to the retina and only survived for three weeks [56], whereas BM-MSCs survived for up to 20 weeks and had a good integrative ability [57]. 2, different species and types of MSCs have different protective effects on retinal cells. Levkovitch-Verbin et al. [58] found that human BM-MSCs could protect the retinal ganglion cells, whereas rat BM-MSCs had no protective effect. A study by Huang et al. [59] also suggested that fractalkine (CX3CL1) which were secreted by MSCs have the strongest effects on restoration of light-damaged retina. 3, retinal protective effects of MSCs are not identical in different types of transplantation. Tzameret et al. [57] compared the effects between intravitreal injection and subretinal injection. They discovered the therapeutic effects of these two types of transplantation lasted 12 weeks and 20 weeks, respectively. The b-wave amplitudes of electroretinogram (ERG) were 56.4 μV in the intravitreal injection group and 66.2 μV in the subretinal injection group ($P<0.01$). 4, different retinal microenvironments in host eyes also affect MSC functions.

Based on the successful experimental studies in vivo, several phase I/II clinical trials of MSCs were prudently conductedby some leading ophthalmologists. In 2005, Kumar et al. [60] conducted intravitreal injections of autologous BM-MSCs for 25 patients with dry AMD and RP. One month and 3 months post-injection, the best-corrected visual acuity of such patients has been mild improved. In 2010, Jonas et al. [61] (registration number: NCT01068561) reported the primary outcomes of three cases received BM-MSCs intravitreal injection (including 1 case of dry AMD). The initial visual acuities of patients were poor in terms of light perception (poor light positioning). Twelve months after BM-MSC injection, no significant improvement in visual acuity and no serious complication were observed. The only effect was fluctuations of intraocular pressure (15 mmHg-30 mmHg) at four weeks after treatment. Siqueira et al. [62] intravitreally injected 1×10^7 BM-MSCs per eye for three RP patients and two cone-rod dystrophy patients. The results indicated that the visual acuities improved more than one row in four patients after one week and that these improvements were maintained at the end of the follow-up. Electrophysiological recordings of two patients have been mild improved. However, no significant changes in angiography, optical coherence tomography and visual field were observed. No complication was noted throughout the study. Although the current clinical trials have not shown promising results, we must consider the following factors: 1. The patients enrolled were relatively old, and their BM-MSCs have limited proliferative capacity and viability. 2. The patients had advanced stage of diseases and poor eyesight. Therefore, vision recovery in these patients is difficult.

Effects of gene-modified MSCs

With the development of cell engineering, MSCs have gradually become a promising source of cell vehicle. Guan et al. [63] injected gene-modified MSCs into the subretinal spaces of sodium iodate damaged eyes. An increased level of erythropoietin (EPO) in gene-modified group was noticed and these cells conducted stronger protective effects on retinal neurons than conventional MSCs. Machalinska et al. [64] 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 expressing neurotrophin-4 (NT-4) can upregulate signals and transcription factors associated with cell survival, such as crystallin β-γ superfamily members. In addition, gene-modified MSCs expressing NT-4 also increase the expression of proteins that are associated with visual perception, visual signal reception, eye

development and other related functions. Park et al. [65] observed the effects of subretinal and intravitreal transplantations of gene-modified BM-MSCs over expressing BNDF. They found that approximately 15.7% of the MSCs integrated into the retina after 4 weeks. Additionally, the protein and mRNA level of BDNF was greatly increased in the retina.

In addition, to regulate the retinal microenvironment, gene-modified MSCs also have distinct functions corresponding to that of the introduced genes and, therefore, have promising prospects of application. However, gene types and gene introduction methods for dry AMD remain to be elucidated. Additionally, the safety and efficiency of this approach require further evaluation.

Prospects

In-depth studies on biological characteristics of stem cell derived RPE, differentiation protocols, and transplantation methods are gradually changing the current stem cell based therapy from a dream to reality. However, there continue to be many difficulties in using stem cell based sciences into clinical practice. Firstly, the sizes of the current trials are extremely small. The safety of stem cell based therapy is still to be subjected to multi-center studies. Secondly, although the subretinal space is considered to be immune privilege, studies have shown that [66] the transplanted cells in the host eye still require long-term immune suppression for survival. Thus, the duration of immunosuppression and the recommended dose remains to be discussed in detail. Thirdly, distinct mechanisms of disease and pathological processes could affect the visual recovery from RPE transplantation. The optimal cell types, differentiation stages, cell numbers and transplantation strategies are needed to be further explored.

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References

[1] Friedman DS, O'Colmain BJ, Muñoz B, Tomany SC, McCarty C, de Jong PT, Nemesure B, Mitchell P, Kempen J; Eye Diseases Prevalence Research Group. Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol 2004; 122: 564-572.

[2] Vingerling JR, Dielemans I, Hofman A, Grobbee DE, Hijmering M, Kramer CF, de Jong PT. The prevalence of age-related maculopathy in the Rotterdam Study. Ophthalmology 1995; 102: 205-210.

[3] Klein R, Knudtson MD, Lee KE, Gangnon RE, Klein BE. Age-period-cohort effect on the incidence of age-related macular degeneration: the Beaver Dam Eye Study. Ophthalmology 2008; 115: 1460-1467.

[4] Klein R, Klein BE, Lee KE, Cruickshanks KJ, Gangnon RE. Changes in visual acuity in a population over a 15-year period: the Beaver Dam Eye Study. Am J Ophthalmol 2006; 142: 539-549.

[5] Gonzales CR; VEGF Inhibition Study in Ocular Neovascularization (V.I.S.I.O.N.) Clinical Trial Group. Enhanced efficacy associated with early treatment of neovascular age-related macular degeneration with pegaptanib sodium: an exploratory analysis. Retina 2005; 25: 815-827.

[6] Colquitt JL, Jones J, Tan SC, Takeda A, Clegg AJ, Price A. Ranibizumab and pegaptanib for the treatment of age-related macular degeneration: a systematic review and economic evaluation. Health Technol Assess 2008; 12: iii-iv, ix-201.

[7] Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T; ANCHOR Study Group. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. Ophthalmology 2009; 116: 57-65.

[8] Parmeggiani F, Romano MR, Costagliola C, Semeraro F, Incorvaia C, D'Angelo S, Perri P, De Palma P, De Nadai K, Sebastiani A. Mechanism of inflammation in age-related macular degeneration. Mediators Inflamm 2012; 2012: 546786.

[9] Zhang T, Hu Y, Li Y, Wu J, Zhao L, Wang C, Liu Y, Yin Z, Ma Z. Photoreceptors repair by autologous transplantation of retinal pigment epithelium and partial-thickness choroid graft in rabbits. Invest Ophthalmol Vis Sci 2009; 50: 2982-2988.

[10] Ma Z, Han L, Wang C, Dou H, Hu Y, Feng X, Xu Y, Wang Z, Yin Z, Liu Y. Autologous transplantation of retinal pigment epithelium-Bruch's membrane complex for hemorrhagic age-related macular degeneration. Invest Ophthalmol Vis Sci 2009; 50: 2975-2981.

[11] Hu Y, Zhang T, Wu J, Li Y, Lu X, Qian F, Yin Z, Ma Z. Autologous transplantation of RPE with partial-thickness choroid after mechanical de-

bridement of Bruch membrane in the rabbit. Invest Ophthalmol Vis Sci 2008; 49: 3185-3192.
[12] Lund RD, Wang S, Klimanskaya I, Holmes T, Ramos-Kelsey R, Lu B, Girman S, Bischoff N, Sauvé Y, Lanza R. Human embryonic stem cell-derived cells rescue visual function in dystrophic RCS rats. Cloning Stem Cells 2006; 8: 189-199.
[13] Klimanskaya I, Hipp J, Rezai KA, West M, Atala A, Lanza R. Derivation and comparative assessment of retinal pigment epithelium from human embryonic stem cells using transcriptomics. Cloning Stem Cells 2004; 6: 217-245.
[14] Lu B, Malcuit C, Wang S, Girman S, Francis P, Lemieux L, Lanza R, Lund R. Long-term safety and function of RPE from human embryonic stem cells in preclinical models of macular degeneration. Stem Cells 2009; 27: 2126-2135.
[15] Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, Mickunas E, Gay R, Klimanskaya I, Lanza R. Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet 2012; 379: 713-720.
[16] Kawasaki H, Mizuseki K, Nishikawa S, Kaneko S, Kuwana Y, Nakanishi S, Nishikawa SI, Sasai Y. Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. Neuron 2000; 28: 31-40.
[17] Kawasaki H, Suemori H, Mizuseki K, Watanabe K, Urano F, Ichinose H, Haruta M, Takahashi M, Yoshikawa K, Nishikawa S, Nakatsuji N, Sasai Y. Generation of dopaminergic neurons and pigmented epithelia from primate ES cells by stromal cell-derived inducing activity. Proc Natl Acad Sci U S A 2002; 99: 1580-1585.
[18] Haruta M, Sasai Y, Kawasaki H, Amemiya K, Ooto S, Kitada M, Suemori H, Nakatsuji N, Ide C, Honda Y, Takahashi M. In vitro and in vivo characterization of pigment epithelial cells differentiated from primate embryonic stem cells. Invest Ophthalmol Vis Sci 2004; 45: 1020-1025.
[19] Ikeda H, Osakada F, Watanabe K, Mizuseki K, Haraguchi T, Miyoshi H, Kamiya D, Honda Y, Sasai N, Yoshimura N, Takahashi M, Sasai Y. Generation of Rx+/Pax6+ neural retinal precursors from embryonic stem cells. Proc Natl Acad Sci U S A 2005; 102: 11331-11336.
[20] Hirami Y, Osakada F, Takahashi K, Okita K, Yamanaka S, Ikeda H, Yoshimura N, Takahashi M. Generation of retinal cells from mouse and human induced pluripotent stem cells. Neurosci Lett 2009; 458: 126-131.
[21] West EL, Gonzalez-Cordero A, Hippert C, Osakada F, Martinez-Barbera JP, Pearson RA, Sowden JC, Takahashi M, Ali RR. Defining the integration capacity of embryonic stem cell-derived photoreceptor precursors. Stem Cells 2012; 30: 1424-1435.
[22] Lakowski J, Baron M, Bainbridge J, Barber AC, Pearson RA, Ali RR, Sowden JC. Cone and rod photoreceptor transplantation in models of the childhood retinopathy Leber congenital amaurosis using flow-sorted Crx-positive donor cells. Hum Mol Genet 2010; 19: 4545-4559.
[23] Osakada F, Jin ZB, Hirami Y, Ikeda H, Danjyo T, Watanabe K, Sasai Y, Takahashi M. In vitro differentiation of retinal cells from human pluripotent stem cells by small-molecule induction. J Cell Sci 2009; 122: 3169-3179.
[24] Lamba DA, Karl MO, Ware CB, Reh TA. Efficient generation of retinal progenitor cells from human embryonic stem cells. Proc Natl Acad Sci U S A 2006; 103: 12769-12774.
[25] Lamba DA, Gust J, Reh TA. Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx deficient mice. Cell Stem Cell 2009; 4: 73-79.
[26] Cho MS, Lee YE, Kim JY, Chung S, Cho YH, Kim DS, Kang SM, Lee H, Kim MH, Kim JH, Leem JW, Oh SK, Choi YM, Hwang DY, Chang JW, Kim DW. Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. Proc Natl Acad Sci U S A 2008; 105: 3392-3397.
[27] Cho MS, Kim SJ, Ku SY, Park JH, Lee H, Yoo DH, Park UC, Song SA, Choi YM, Yu HG. Generation of retinal pigment epithelial cells from human embryonic stem cell-derived spherical neural masses. Stem Cell Res 2012; 9: 101-109.
[28] Eiraku M, Takata N, Ishibashi H, Kawada M, Sakakura E, Okuda S, Sekiguchi K, Adachi T, Sasai Y. Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature 2011; 472: 51-56.
[29] Zhu Y, Carido M, Meinhardt A, Kurth T, Karl MO, Ader M, Tanaka EM. Three-dimensional neuroepithelial culture from human embryonic stem cells and its use for quantitative conversion to retinal pigment epithelium. PLoS One 2013; 8: e54552.
[30] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126: 663-676.
[31] Comyn O, Lee E, MacLaren RE. Induced pluripotent stem cell therapies for retinal disease. Curr Opin Neurol 2010; 23: 4-9.
[32] Jin ZB, Okamoto S, Mandai M, Takahashi M. Induced pluripotent stem cells for retinal degenerative diseases: a new perspective on the challenges. J Genet 2009; 88: 417-424.
[33] Parameswaran S, Balasubramanian S, Babai N, Qiu F, Eudy JD, Thoreson WB, Ahmad I. Induced pluripotent stem cells generate both retinal ganglion cells and photoreceptors: ther-

apeutic implications in degenerative changes in glaucoma and age-related macular degeneration. Stem Cells 2010; 28: 695-703.
[34] Carr AJ, Vugler AA, Hikita ST, Lawrence JM, Gias C, Chen LL, Buchholz DE, Ahmado A, Semo M, Smart MJ, Hasan S, da Cruz L, Johnson LV, Clegg DO, Coffey PJ. Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. PLoS One 2009; 4: e8152.
[35] Buchholz DE, Hikita ST, Rowland TJ, Friedrich AM, Hinman CR, Johnson LV, Clegg DO. Derivation of functional retinal pigmented epithelium from induced pluripotent stem cells. Stem Cells 2009; 27: 2427-2434.
[36] Kokkinaki M, Sahibzada N, Golestaneh N. Human induced pluripotent stem-derived retinal pigment epithelium (RPE) cells exhibit ion transport, membrane potential, polarized vascular endothelial growth factor secretion, and gene expression pattern similar to native RPE. Stem Cells 2011; 29: 825-835.
[37] Meyer JS, Howden SE, Wallace KA, Verhoeven AD, Wright LS, Capowski EE, Pinilla I, Martin JM, Tian S, Stewart R, Pattnaik B, Thomson JA, Gamm DM. Optic vesicle-like structures derived from human pluripotent stem cells facilitate a customized approach to retinal disease treatment. Stem Cells 2011; 29: 1206-1218.
[38] Huang C, Zhang J, Ao M, Li Y, Zhang C, Xu Y, Li X, Wang W. Combination of retinal pigment epithelium cell-conditioned medium and photoreceptor outer segments stimulate mesenchymal stem cell differentiation toward a functional retinal pigment epithelium cell phenotype. J Cell Biochem 2012; 113: 590-598.
[39] Gong L, Wu Q, Song B, Lu B, Zhang Y. Differentiation of rat mesenchymal stem cells transplanted into the subretinal space of sodium iodate-injected rats. Clin Experiment Ophthalmol 2008; 36: 666-671.
[40] Tomita M, Adachi Y, Yamada H, Takahashi K, Kiuchi K, Oyaizu H, Ikebukuro K, Kaneda H, Matsumura M, Ikehara S. Bone marrow-derived stem cells can differentiate into retinal cells in injured rat retina. Stem Cells 2002; 20: 279-283.
[41] Castanheira P, Torquetti L, Nehemy MB, Goes AM. Retinal incorporation and differentiation of mesenchymal stem cells intravitreally injected in the injured retina of rats. Arq Bras Oftalmol 2008; 71: 644-650.
[42] Tropepe V, Coles BL, Chiasson BJ, Horsford DJ, Elia AJ, McInnes RR, van der Kooy D. Retinal stem cells in the adult mammalian eye. Science 2000; 287: 2032-2036.
[43] Aruta C, Giordano F, De Marzo A, Comitato A, Raposo G, Nandrot EF, Marigo V. In vitro differentiation of retinal pigment epithelium from adult retinal stem cells. Pigment Cell Melanoma Res 2011; 24: 233-240.
[44] Cicero SA, Johnson D, Reyntjens S, Frase S, Connell S, Chow LM, Baker SJ, Sorrentino BP, Dyer MA. Cells previously identified as retinal stem cells are pigmented ciliary epithelial cells. Proc Natl Acad Sci U S A 2009; 106: 6685-6690.
[45] Gualdoni S, Baron M, Lakowski J, Decembrini S, Smith AJ, Pearson RA, Ali RR, Sowden JC. Adult ciliary epithelial cells, previously identified as retinal stem cells with potential for retinal repair, fail to differentiate into new rod photoreceptors. Stem Cells 2010; 28: 1048-1059.
[46] Bernardos RL, Barthel LK, Meyers JR, Raymond PA. Late-stage neuronal progenitors in the retina are radial Müller glia that function as retinal stem cells. J Neurosci 2007; 27: 7028-7040.
[47] Song WT, Zhang XY, Xia XB. Atoh7 promotes the differentiation of retinal stem cells derived from Müller cells into retinal ganglion cells by inhibiting Notch signaling. Stem Cell Res Ther 2013; 4: 94.
[48] Siqueira RC, Voltarelli JC, Messias AM, Jorge R. Possible mechanisms of retinal function recovery with the use of cell therapy with bone marrow-derived stem cells. Arq Bras Oftalmol 2010; 73: 474-479.
[49] Inoue Y, Iriyama A, Ueno S, Takahashi H, Kondo M, Tamaki Y, Araie M, Yanagi Y. Subretinal transplantation of bone marrow mesenchymal stem cells delays retinal degeneration in the RCS rat model of retinal degeneration. Exp Eye Res 2007; 85: 234-241.
[50] Zhang Y, Wang W. Effects of bone marrow mesenchymal stem cell transplantation on light-damaged retina. Invest Ophthalmol Vis Sci 2010; 51: 3742-3748.
[51] Xu W, Wang X, Xu G, Guo J. Basic fibroblast growth factor expression is implicated in mesenchymal stem cells response to light-induced retinal injury. Cell Mol Neurobiol 2013; 33: 1171-1179.
[52] Xu W, Wang X, Xu G, Guo J. Light-induced retinal injury enhanced neurotrophins secretion and neurotrophic effect of mesenchymal stem cells in vitro. Arq Bras Oftalmol 2013; 76: 105-110.
[53] Wang S, Lu B, Girman S, Duan J, McFarland T, Zhang QS, Grompe M, Adamus G, Appukuttan B, Lund R. Non-invasive stem cell therapy in a rat model for retinal degeneration and vascular pathology. PLoS One 2010; 5: e9200.
[54] Otani A, Dorrell MI, Kinder K, Moreno SK, Nusinowitz S, Banin E, Heckenlively J, Friedlander M. Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells. J Clin Invest 2004; 114: 765-774.
[55] Arnhold S, Absenger Y, Klein H, Addicks K, Schraermeyer U. Transplantation of bone marrow-derived mesenchymal stem cells rescue

photoreceptor cells in the dystrophic retina of the rhodopsin knockout mouse. Graefes Arch Clin Exp Ophthalmol 2007; 245: 414-422.
[56] Hill AJ, Zwart I, Tam HH, Chan J, Navarrete C, Jen LS, Navarrete R. Human umbilical cord blood-derived mesenchymal stem cells do not differentiate into neural cell types or integrate into the retina after intravitreal grafting in neonatal rats. Stem Cells Dev 2009; 18: 399-409.
[57] Tzameret A, Sher I, Belkin M, Treves AJ, Meir A, Nagler A, Levkovitch-Verbin H, Barshack I, Rosner M, Rotenstreich Y. Transplantation of human bone marrow mesenchymal stem cells as a thin subretinal layer ameliorates retinal degeneration in a rat model of retinal dystrophy. Exp Eye Res 2014; 118:135-44.
[58] Levkovitch-Verbin H, Sadan O, Vander S, Rosner M, Barhum Y, Melamed E, Offen D, Melamed S. Intravitreal injections of neurotrophic factors secreting mesenchymal stem cells are neuroprotective in rat eyes following optic nerve transection. Invest Ophthalmol Vis Sci 2010; 51: 6394-6400.
[59] Huang L, Xu W, Xu G. Transplantation of CX3CL1-expressing mesenchymal stem cells provides neuroprotective and immunomodulatory effects in a rat model of retinal degeneration. Ocul Immunol Inflamm 2013; 21: 276-285.
[60] Kumar A, Pahwa VK, Tandon R, Kumar L, Mohanty S. Use of autologous bone marrow derived stem cells for rehabilitation of patients with dry age related macular degeneration and retinitis pigmentosa: phase-1 clinical trial. Indian J Med Paediatr Oncol 2005; 26 Suppl 3: 12-14.
[61] Jonas JB, Witzens-Harig M, Arseniev L, Ho AD. Intravitreal autologous bone-marrow-derived mononuclear cell transplantation. Acta Ophthalmol 2010; 88: e131-2.
[62] Siqueira RC, Messias A, Voltarelli JC, Scott IU, Jorge R. Intravitreal injection of autologous bone marrow-derived mononuclear cells for hereditary retinal dystrophy: a phase I trial. Retina 2011; 31: 1207-1214.
[63] Guan Y, Cui L, Qu Z, Lu L, Wang F, Wu Y, Zhang J, Gao F, Tian H, Xu L, Xu G, Li W, Jin Y, Xu GT. Subretinal transplantation of rat MSCs and erythropoietin gene modified rat MSCs for protecting and rescuing degenerative retina in rats. Curr Mol Med 2013; 13: 1419-1431.
[64] Machalińska A, Kawa M, Pius-Sadowska E, Stępniewski J, Nowak W, Rogińska D, Kaczyńska K, Baumert B, Wiszniewska B, Józkowicz A, Dulak J, Machaliński B. Long-term neuroprotective effects of NT-4-engineered mesenchymal stem cells injected intravitreally in a mouse model of acute retinal injury. Invest Ophthalmol Vis Sci 2013; 54: 8292-305.
[65] Park HY, Kim JH, Sun Kim H, Park CK. Stem cell-based delivery of brain-derived neurotrophic factor gene in the rat retina. Brain Res 2012; 1469: 10-23.
[66] West EL, Pearson RA, Barker SE, Luhmann UF, Maclaren RE, Barber AC, Duran Y, Smith AJ, Sowden JC, Ali RR. Long-term survival of photoreceptors transplanted into the adult murine neural retina requires immune modulation. Stem Cells 2010; 28: 1997-2007.