Different Applications of Stem Cells Therapy for Degenerative Retinal Diseases

Antonio Florido*, Mauro Budini, Filippo Avogaro and Enzo Maria Vingolo

Department of Biotechnology and Medical-Surgical Sciences, “Sapienza” University of Rome, 04100 Latina, Italy

ABSTRACT
Retinal degenerative diseases, such as Stargardt’s disease (SD), glaucoma, retinitis pigmentosa (RP), age-related macular degeneration (AMD) or diabetic retinopathy (DR), represent the main causes of a decreased quality of vision and blindness worldwide. The progression and management of these conditions have always represented a challenge, but promising new evidences about the efficacy of mesenchymal stem cells (MSCs) as therapy for these diseases has been shown. The therapeutic potential of MSCs lies on its ability to release paracrine factors with neuroprotective, immunomodulatory and anti-angiogenic properties that stimulate the retinal pigment epithelium (RPE) and are even similar to those produced by RPE. In literature we can find many studies conducted animal models, in which MSCs proved their efficacy in stopping the progression of retinal degeneration and for rescuing photoreceptors in the dormant phase. Furthermore, they retain a differentiation potential which allow them to differentiate into various cell types, including the cells of the retina. By all of those properties it is clear how MSCs result an important therapy option in these pathologies. In this review we summarize the various properties of MSCs and their promising applications in various retinal diseases, enhancing a new clinical approach on pathologies which otherwise have a difficult managing and an unfavorable prognosis.

*Corresponding author
Antonio Florido, Department of Biotechnology and Medical-Surgical Sciences, "Sapienza" University of Rome, 04100 Latina, Italy.
E-mail: antonioflorido@hotmail.it

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Introduction
The retina is the innermost layer of the eye and represents its nervous tunic. It consists of ten layers, which are composed of different type of cells which permits the capture and transduction of light stimuli in electrical impulses, then delivered to the visual cortex of the brain. All retinal layers are connected and work together in order to permit the vision, therefore a damage to any of their structure can result in visual impairment. This can derive from a wide range of retinal degenerative disease. While the etiology and pathogenesis of this diseases are different and some still not well known, they share many retinal damage factors, such as ROS, vascular defects, overproduction of pro-inflammatory cytokines, defects of blood-retinal barrier and aging [2-4].

In this review we considered some of the most common retinal degenerative diseases (RDDs), such as age-related macular degeneration (AMD), retinitis pigmentosa (RP), glaucoma and Stargardt’s disease (SD). These diseases have in common the damage of retinal cells, whether they be the ganglion cells, photoreceptors or retinal pigment epithelium (RPE) cells. At the same time, they are responsible of neuro-inflammations, microglial activation, angiogenesis and retinal gliosis. To date, there’s not a proper and effective treatment for RDDs, since retina doesn’t have a regenerative ability. Therefore, new approaches that aim to slow vision loss and/or restore vision are actually object of study, such as gene therapy, neuroprotection, new anti-VEGF molecules (vascular endothelium growth factors), and stem cell therapy [5].

Mesenchymal stem cells (MSCs) have been isolated from different tissue sources like bone marrow (bone marrow-derived MSC, BM-MSC), adipose tissue (adipose-mesenchymal derived stem cells, ASC), dental pulp (dental pulp stem cells, DPSC), umbilical cord blood (umbilical cord-derived MSCs, UC-MSC), Wharton’s jelly MSCs (WJ-MSCs), amniotic membrane. Several studies in literature analyzed the potential regenerative ability of MSCs of retinal cells for therapeutical applications in several retinal degenerative disorders [6]. There are many reasons for considering MSCs as a possible treatment option. Firstly, they can produce paracrine signaling through secretion of neurotrophic factors, which provide a reparative effect on neuro-retinal cells. Secondly, MSCs have shown immunomodulatory properties that can module the pro-inflammatory microenvironment, which is usually present in these diseases. Thirdly, MSCs are capable to secrete anti-angiogenic factors, which counter the the pro-angiogenesis mediators, crucial in the pathogenesis of DR and wet AMD for example [7].
Even though cell-based therapy can be considered as a promising opportunity in these retinal pathological conditions, it is important to report how several patients treated with intraocular injection of autologous MSCs derived from bone marrow/adipose tissue encountered loss of vision after intraocular injection of autologous MSCs derived from bone marrow/adipose tissue. [8, 9]. A probable cause of these outcomes may be the trans-differentiation of injected MSCs into myofibroblast-like cells, which can induce a proliferative vitreoretinopathy (PVR) which can lead retinal detachment.

Given these possible complications, it is crucial to understand better the interaction between administered MSCs and the degenerative retinal environment of the patient. There’s still the need of evidence about the safety and efficacy of stem/progenitor cell-based transplantation therapy, but there are several promising results to date [10].

**Table 1: Summary of ongoing and concluded clinical trials of stem-cell-derived RPE transplantation [11].**

<table>
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<tr>
<th>References</th>
<th>Source cells</th>
<th>HLA matching</th>
<th>Immunosuppression</th>
<th>Form</th>
<th>Dose/size</th>
<th>Scaffold</th>
<th>Disease</th>
<th>Participants</th>
<th>NCT or UMIN number</th>
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Possible Roles of Mscs in Treatment of Retinal Diseases

Until date, these retinal diseases have no curative treatment. The main goal of stem cell therapy is to slow down the tissue degeneration caused by the underlying pathology. By delivering ESCs, MSCs and iPSCs into the eye, it is possible to exert a renewal effect on the damaged retinal tissue. Moreover, they dampen the pro-inflammatory factors which contribute to retinal cells death [12]. In particular, MSCs have been gathered from several tissue sources such as bone marrow, adipose tissue, dental pulp, umbilical cord blood, amniotic membrane and they are considered in many studies among the best candidates for a therapy regimen in RDDs. The reason for this is that they secrete paracrine factors, exosomes and mitochondria into host cells. As follow we resume some of the key points of these features:

Paracrine neuroprotective factors

Bone marrow derived mesenchymal stem cells (BMSCs) are capable to produce and secrete a wide range of neurotrophic factors (NTFs) such as ciliary neurotrophic factor (CNTF), BDNF, glial cell derived neurotrophic factor (GDNF), platelet derived growth factor (PDGF), nerve growth factor (NGF), neurotrophin-3, 4/5 (NT-3, 4/5), insulin-like growth factor 1 (IGF1), basic Fibroblast growth factor (FGF2), PEDF and erythropoietin (EPO). In particular, these neurotrophic factors find their receptors on retinas cells and enhance their differentiation, neural cell survival, axonal outgrowth, neural cell attachment, while stopping neural cell apoptosis. The signaling pathways activated by the NTFs, such as PI3K/AKT, P13K/JAP, PLC/IP3/PKC, MAPK/ERK and JAK/STAT3 have neuroprotective effect on the neuro-retinal cells. In many studies it has been shown how the beneficial effects of these mediators. The neuroprotective role was demonstrated in a study by Cui et al conducted in in-vivo, where co-culturing BMSCs with retinal ganglion cells (RGCs) reduced hydrogen peroxide (H2O2) induced injury in RGCs through the expression of neurotrophins, BDNF, CNTF and reduced the expression of pro-inflammatory factors interleukin 1β (IL1β) and tumor necrosis factor α (TNFα) by RGCs[13]. In another work, Osborne et al and Johnson et al showed how PDGF secreted by BMSCs can exert a protection on RGCs in both an ex vivo and preclinical models [14]. Mead et al stated that NGF, BDNF and NT-3 secreted by BMSCs have protective effects on RGCs; moreover, they showed that this effect is lost when tropomyosin related kinase (TrK) and PDGF receptor α (PDGFRα) get inhibited in RGCs [15]. Intravitreal transplantation of BMSCs, which were able to secrete GDNF and BDNF, was associated with a higher number of RGCs compared to the control group in an experimental optic nerve crush model. In the same way, it was possible to observe long-term neuroprotection and axon regeneration of RGCs after transplantation of BMSCs, which was attributed to an increased expression of FGFR2 and ILβ in the RGC layer that activated the PI3/AKT signaling cascade and rescued RGCs. In a study of Martin et al it was observed a significant increase in neuroprotective (DI14, Crim-1, Glupican-3, Ctnn1), anti-inflammatory (Transforming Growth Factor β and IL10, 13, 11, 4) molecules as well as proteins associated with anti-oxidant (haptoglobin), anti-apoptotic (Apex1) activity and protein homeostasis (Hsp10, Hsp60, Hsp70, Hsp20, Hsp27, Kctd10, Pyk2, clusterin) in the secretome of human BMSCs co-cultured with neuroretinal explants [16].

Mscs derived extracellular vesicles (MSC-EVs)

MSC-EVs or exosomes are secreted, bilipid layered, nano dimensional micro vesicles. These structures can encapsulate functional molecules, like proteins, lipids, miRNAs which are able to exert important therapeutic effects. These vesicles were observed to be endocytosed by retinal neurons, microglia and RGCs via caveolar mediated endocytic pathway, facilitated by heparin sulfate proteoglycans (HSPGs). Furthermore, their interaction with proteins of the vitreous humor resulted in a prolonged retention of EVs in the eye. Yu and co-workers observed how the administration of MSC-EVs in vitreous was equally efficient when compared with transplanted MSCs, not only in reducing apoptosis and damage, but even in augmenting vision in an experimental model of retinal laser injury. Additionally, MSC-EVs dampened retinal damage processes through the downregulation of pro-inflammatory mediators, intercellular adhesion molecule 1 (ICAM1), monocyte chemoattractant protein 1 (MCP1), VEGF-A and TNFα [17].

Mscs weaken inflammatory responses

The so called ocular immune privilege is the capability of the eye to restrain intraocular inflammation, so that there’s a protective effect on the visual components from damage and therefore preserving visual acuity. This is an articulated phenomenon made possible by the blood-retinal barrier (BRB) which efficiently separates the eye from the immune system along with local inhibition of both adaptive and innate immune responses by the ocular microenvironment, and ocular-specific mechanisms cause systemic activation of immunosuppressive regulatory T cells. Nevertheless, in every retinal degenerative diseases such as RP, AMD, glaucoma and DR, there’s an abundance of proinflammatory cytokines associated with an infiltration of immune cells leading to breakdown of the BRB.

Many studies in literature highlighted how intravitreal and peri-orbital administration of BMSCs granted a significant reduction of inflammatory cytokines in the retinal microenvironment, infiltration of macrophages and CD4+ T cells [18].

Mscs regulate angiogenesis

Pathological retinal angiogenesis, leads to derangement and produce aberrant blood vessels that compromise the retinal tissue organization. These neovascularizations break through the outer retina and the macular pit, which is physiologically an avascular zone. The disruption of macular structure compromises the normal vision. Many retinal pathological conditions lead to aberrant angiogenesis with progressive loss of vision. Basically, every time there are low levels of oxygen, tissues may respond...
multifactorial etiology, but they share the fact that they lead to visual impairment and lately to blindness. Moreover, they have in common the damage and death of retinal cells and the degeneration of photoreceptors [28, 29]. In AMD the degeneration of RPE and Bruch’s membrane leads to the loss of photoreceptors. RP is characterized by the disruption of the rod photoreceptors at first, then of the cones, due to a genetic defect. In DR, since early stages, a damage to pericytes, endothelial cells and retina’s neuronal cells is observed. SD is an hereditary disease where the loss of RPE and photoreceptors brings to loss of vision in young patients. In glaucoma, an high intraocular pressure (IOP) causes a progressive damage to retinal ganglion cells (RGCs) [30, 31]. The treatment currently available for these diseases is limited, in fact it is mainly directed to the slowing of the disease progression. Late stages of RD can only benefit from laser photoacoagulation and intraocular injection of inhibitors of vascular endothelial growth factors (VEGF), the latter used also in wet AMD. In some cases, the treatment is surgery, such as vitrectomy or microsurgery strategies, which are much more invasive and can lead to risky complications. On the other hand, stem cells could represent a novel approach to these pathologies, due to their potential to regenerate the damaged retinal tissue, as happens in these age-related retinal disorders. There are already many studies on animals demonstrating the therapeutical potential of MSCs on degenerative retinal diseases [32 - 34]. In fact, evidences proved how these cells can provide a regeneration of many retinal cells, such as RPE cells, photoreceptors and axons, and also augment the survival of RGCs [35, 36].

**Use of MSCs in age-related macular degeneration**

Age-related macular degeneration is listed as a leading cause of blindness in the elderly population; in particular, people over 70 years of age are at highest risk [37]. The vision loss is due to the disruption of RPE, without which the photoreceptors loose its nutrition and homeostasis’ maintenance function, causing their progressive death. The pathogenesis of AMD derives from many genetic and environmental factors [38], which leads in early stages to an accumulation of amorphous deposits under the RPE, visible as drusen during fundoscopy exam or optical coherence tomography (OCT). These deposits can trigger inflammation, which pushes towards degeneration of retinal cells [39]. Late stages of the disease include two different forms, dry AMD, called also geographic atrophy, and wet AMD, which consist in a choroidal neovascularization. Dry AMD is a result of the degradation of RPE and Bruch’s membrane, with consequential loss of photoreceptors. On the other hand, the accumulation of materials between RPE and Bruch’s membrane can results in its detachment leading to wet AMD. The consequent neovascularization is characterized by abnormal and leaky capillaries, which causes the accumulation of subretinal fluid or macular hemorrhages and thus the central vision loss [40]. Currently available therapies are effective only on wet AMD, while geographic atrophy, which affects most of the AMD population, remains incurable. Wet AMD patients can benefit from anti-VEGF or steroids intraocular injections, retinal photodynamic and laser photoacoagulation therapy in order to alleviate neovascularization [41, 42]. In studies on animals, where AMD disease was artificially provoked by using sodium iodate (NaI03), which normally cause degeneration of RPE and photoreceptors, MSCs implantation granted protection of these retinal cells from NaI03 [43]. Recently, some studies have also been conducted on humans. In 2012, Schwartz et al. provided preliminary results about human ESCs subretinal transplantation in patients suffering from AMD and SD, showing the safety of the procedure on the patient’s eyes in the next 4 months. After a 22 month follow up, which recruited 9 dry AMD and 9 SD
patient’s eyes, a benefit in term of BCVA was observed in 10 patients, while it remained stable in 7 eyes and decreased in 1 eye. Untreated eyes showed no improvement in BCVA [44, 45]. In a work of Kumar and colleagues bone marrow MSCs were administrated through intravitreal injections in 60 advanced dry AMD patients. After a 6-month follow up, authors observed an improvement BCVA, as well as electrophysiological and anatomical amelioration [46]. In a study of Limoli et. al, authors used the Limoli Retinal Restoration Technique on 36 eyes of 25 patients suffering from dry AMD. The technique consisted in surgical implantation in the suprachoroidal space of autologous cells, platelets derived from platelet-rich plasma and adipose derived MSCs. After 6 months, treated patients showed better BCVA compared to baseline values, especially in patients with higher retinal thickness [47, 48].

Use of MSCs in retinitis pigmentosa

Retinitis Pigmentosa is a hereditary degenerative retinal disease which represent one of the main causes of vision loss below middle age population, with a world prevalence of 1:4000 [49]. It may occur with autosomal recessive (AR), autosomal dominant (AD) or X-linked inheritance patterns. Although it involves only the retina, RP can present also as part of a syndrome: among these, Usher’s syndrome is the most common one [50]. At first, patients suffer from loss of night vision (nyctalopia) and limitation of peripheral vision, due to the disruption of the rod photoreceptors caused by gene mutations, then in later stages it hits cones, causing the loss of central vision and colours discrimination [51]. To date, RP cannot be caused by almost 3000 mutations of almost 200 genes [52]. RP pathogenesis takes place from its characteristic gene’s mutations, affecting rods in peripheral retina. As a result, the disrupted rods have a lower demand in term of metabolic processes. At the same time, the availability of a rich blood supply and high metabolic rate could lead to oxidative stress affecting cone’s function. Moreover, in RP there’s an increased expression of proinflammatory cytokines secondary to extrabulbar inflammatory processes, which contributes to the cone’s dysfunction [53, 54]. From 20 to 30% of the AD cases is caused by rhodopsine gene (RHO), while Usherin (USH2A) is the most common gene involved in AR form (15% of cases), and the X-linked one is due to the retinitis pigmentosa GTPase regulator (RPGR, 70 to 90% of cases) [55]. Treatment of RP is currently very limited, basing mostly on support therapy. According to some evidence, antioxidant vitamins could slow disease’s progression, such as the augmented production of reactive oxygen species, advanced glycation end-products, inflammatory factors and VEGF [67, 68]. DR can be classified in two stages: non-proliferative DR (NPDR) and proliferative DR (PDR). In early non-proliferative stages, a progressive damage of microvasculature takes place, with the loss of pericytes and endothelial cells, and there’s also inflammation and degradation of retinal neuron cells [69]. There’s a progression of the disease in proliferative DR, a macular edema can develop, resulting in vision loss, and local ischemia can lead to proliferation of abnormal new vessels in the retina and in the eye, causing hemorrhages [70]. In DR treatment, since the early stages, a precise control of glucose levels and blood pressure is needed [71]. In late stages, in order to slow the progression of the disease, treatment can include retinal laser therapy, intravitreal corticosteroids and anti-VEGF. Steam cells therapies have been hypothesized as a valid solution for RD, considering the retinal cell loss during the progression of the disease. Among MSCs, ASC shown an efficacy when implanted in rat eyes with early-stage of DR, as they improve retinal function [72], and they act as pericytes, so that they could exert a repair of retinal blood vessels [73]. Moreover, they demonstrated the ability of reducing oxidative damage and promote the secretion of neurotrophic factors [74]. In a study of Gu et. al, 10 patients with severe NPDR and 7 with PDR received intravenous administration of autologous BM MSCs. After the 6 months from the therapy, NPDR patients showed a significant improve in BCVA and reduction of macular thickness, while PDR patients had only benefit of slight effects [75].

Use of MSCs in stargardt disease

Stargardt’s disease is an hereditary blinding disease characterized by macular degeneration, due to deposition of lipofuscin-like substance in RPE, leading to photoreceptors cell death. It occurs below the first two decades of life [76, 77]. The most common gene involved in this disease is ABCR gene, which disfunction causes the accumulation of lipofuscin molecule components in RPE [78, 79]. To date, there is no valid therapeutical approach available to cure or slow the progression of SD. Therefore, the use of stem cell therapy in order to restore damaged retinal photoreceptors could represent a valid strategy for the disease. Song et al. showed how subretinal transplantation of human ESC-derived cells of RPE were well tolerated in 2 asian patients with SD, showing no adverse effect. Moreover, there was a significative visual acuity improvement during the follow-up period [80]. We already mentioned the work of Schwartz et al., where authors demonstrated the safety and efficacy in terms of BCVA recovery through human ESCs subretinal transplantation in patient’s eyes [44, 45].
Use of MSCs in glaucoma

In glaucoma, as like as other retinal degenerative disease, there’s a progressive death of retinal cells, in particular RGCs, due to high values of IOP. This result in the disruption of optic nerve head and consequential vision loss, starting as a reduction in peripheral vision [81, 82]. Moreover, there are several other actors in retinal ganglion cell’s degeneration, such as hypoxia, ischemic insults, neuroinflammation, rise in levels of ROS in RGC layer and reduction of neurotrophic factors, occurring as a consequence of high IOP and neuronal damage [81]. Current therapy is based on IOP reduction, through medical treatment involving pharmacological agents, or by using different surgical techniques, ranging from trabeculectomy and drainage implants to MIGS and non-penetrating glaucoma treatments [82]. More recent approaches involved gene therapy, studied to both reduce IOP and increasing of secretion of neurotrophic factors [83]. In recent years the attention has also moved towards mesenchymal stem cells transplantation. In a study on rats with glaucoma, Hu et al. showed how BM MSCs can provide a neuroprotective effect, through which they can prevent RGCs damage and degeneration [84]. Limoli et. al conducted a study on 35 eyes of 25 patients suffering from glaucomatous optic neuropathy, where a control group included 21 eyes, while the LRRT group included 14 eyes, which were treated with suprachoroidal autograft of mesenchymal stem cells using Limoli Retinal Restoration Technique. Results after 6 months follow-up showed a significant increase in visual function, regarding BCVA, sensitivity and also close-up visus [85].

Conclusion

Therapeutical application of stem cells, firstly observed and confirmed on animal models, has opened new perspective of treatment of degenerative retinal disease. In fact, while most of the therapeutic options since now available has the power of slowing the progression of the disease, stem cells could provide a regeneration of the degenerated cells in the eye. In particular, MSCs appear to exert their therapeutic mechanism through a paracrine effect, so that they could be used to sustain retinal neuron’s function and to stimulate glia to provide a neural repair effect. Thanks to their non-invasive, non-tumorigenic, immunosuppressive and cells supporting effects, MSCs could represent a valid innovative therapy for eye diseases, especially the ones which currently doesn’t have a proper cure.

References


Different Applications of Stem Cells Therapy for Degenerative Retinal Diseases.


