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Stem Cell-Based Therapeutic Applications in Retinal Degenerative Diseases

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Abstract Retinal degenerative diseases that target photoreceptors or the adjacent retinal pigment epithelium (RPE) affect millions of people worldwide. Retinal degeneration (RD) is found in many different forms of retinal diseases including retinitis pigmentosa (RP), age-related macular degeneration (AMD), diabetic retinopathy, cataracts, and glaucoma. Effective treatment for retinal degeneration has been widely investigated. Gene-replacement therapy has been shown to improve visual function in inherited retinal disease. However, this treatment was less effective with advanced disease. Stem cell-based therapy is being pursued as a potential alternative approach in the treatment of retinal degenerative diseases. In this review, we will focus on stem cell-based therapies in the pipeline and summarize progress in treatment of retinal degenerative disease.

Keywords Retinal degeneration · Stem cells · Regeneration

Abbreviation

ABCR	ATP-binding cassette retina
AMD	Age-related macular degeneration
BMC	Bone marrow cells
CNV	Choroidal neovascularization
ESC	Embryonic stem cells
FL	flt3 ligand
G-CSF	Granulocyte colony stimulating factor
IPE	Iris pigment epithelium
iPS	Induced pluripotent stem cells
LCA	Leber congenital amaurosis
MSC	Mesenchymal stem cells
RD	Retinal degeneration
RP	Retinitis pigmentosa
RPC	Retinal progenitor cells
RPE	Retinal pigment epithelium
SC	Stem cells
VSEL	Very small embryonic-like stem cells

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Introduction

The field of stem cell-based therapy holds great potential for the treatment of retinal degenerative (RD) disease. Many studies in animal models suggest that stem cells have the capacity to regenerate lost photoreceptors and retinal neurons and improve vision. To date, these cells include retinal progenitor cells (RPC) [1], embryonic stem cells (ESC) [2, 3], induced pluripotent stem cells (iPS) [4, 5], mesenchymal stem cells (MSC) [6], and very small embryonic-like (VSEL) stem cells [7] (Table 1).

Pathophysiology of Retinal Degeneration

Amongst patients that may benefit directly from cellular replacement strategies are those suffering from currently incurable eye diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP). In particular, AMD is the leading cause of irreversible, severe visual loss in the developed world, with 14 million people blind or severely visually impaired because of AMD [8]. RP is the most common retinal degeneration with a prevalence of approximately 1 in 3,000 to

Table 1 Type of stem cells in the treatment of retinal degenerative diseases

Type of stem cells	References
Retinal progenitor cells (RPC)	[1, 20–26]
Embryonic stem cells (ESC)	[2, 3, 41–46]
Induced pluripotent stem cells (iPS)	[4, 101]
Mesenchymal stem cells (MSC)	[6, 71]
Bone marrow-derived VSEL	[7, 78]

1 in 5,000 individuals [9], affecting approximately 1.5 million people worldwide [10]. Retinal degenerations from progressive loss of photoreceptor cells are largely responsible for vision loss. The etiology is attributed primarily to RPE cells (playing an essential role for photoreceptor function and survival) or the photoreceptors themselves [11]. The pathology of retinal degenerations is summarized in Table 2.

Age-Related Macular Degeneration The natural history of dry AMD is progressive, with gradual loss of visual function that may span many years' time. Two types of AMD are known: dry and wet AMD. The dry or non-exudative form accounts for about 90% of all cases. Many patients with dry AMD are asymptomatic and unaware of the disease. In 10–15% of patients with dry AMD, the deterioration is more rapid and extensive and they suffer significant vision loss due to geographic atrophy [8]. The disease is characterized by the presence of lipid-containing deposits called drusen, including hard drusen (associated with localized dysfunction of the RPE), soft drusen (associated with diffuse dysfunction of the RPE), and geographic atrophy. These fundus changes may predispose the eye to develop the neovascular/exudative stages of AMD [12]. The wet or exudative form accounts for only 10% of all AMD cases. However, most patients who develop the exudative form of AMD will develop central visual impairment. Wet AMD is characterized by choroidal neovascularization (CNV). The abnormal blood vessel growth begins in the vascular choroid; eventually the vessels break through the basement membrane (Bruch's membrane) of the RPE and invade the outer retina. The blood vessels are not of the "continuous" type and leak blood and fluid, which secondarily damage the photoreceptor cells. Wet AMD advances rather rapidly to a stage referred to as a disciform scar. This process usually takes place over several months and typically results in a fibrotic scar underlying the macula accompanied by a central scotoma with severe central vision loss. Patients with neovascular AMD in one eye have a 4–12% per year cumulative risk of developing neovascular AMD in the other eye [8]. Therapeutic approaches for AMD focused almost exclusively upon the exudative form and were only of limited benefit to most patients. However, the introduction of pharmacotherapies against vascular endothelial growth factor-A (VEGF-A), a key factor in the pathogenesis of CNV, for treatment of neovascular AMD brings hope for these patients [13].

Retinitis Pigmentosa RP is a heterogeneous family of inherited retinal disorders characterized by progressive degeneration of

Table 2 Pathology of retinal degenerations

Disease	Pathology	Cause
AMD	Dry AMD: most common form, lipid-containing drusen, slow progressing, geographic atrophy Wet AMD: cause of legal blindness, fast progressing, choroidal neovascularization	Unknown
Stargardt's disease	Most common hereditary macular dystrophy. Central vision loss early of age. Rapid and severe central vision impairment	Mutation in: ATP-Binding Cassette Retina (*ABCR*) gene
Leber congenital amaurosis	Moderate to severe visual impairment at or within few months of birth. Nystagmus and sluggish pupillary responses. Absent electroretinographic responses	Mutations in: Retinal guanylate cyclase (*RET-GCL*), *CRX* and *RPE-65* genes
Usher syndrome	Combined hearing and vision impairments: Type I: most common type, born completely deaf and balance problems, in adolescence exhibition of night blindness and loss of peripheral vision. Type II: born with moderate to severe hearing impairment but no problems. Symptoms of RP develop later in adolescence. Type III: progressive hearing and vision loss.	Mutation in: *Myosin Vlla* or *PCDH15* gene (Type 1) *USH2A* gene (Type II) *CLRN1* gene (Type III)
Retinitis pigmentosa	Group of retinal dystrophies with progressive vision loss. Abnormalities of photoreceptors (rods and cones) or retinal pigment epithelium. Night blindness followed by reduction of peripheral vision (tunnel vision), sometimes late loss of central vision. Genetic forms: Autosomal dominant, autosomal recessive, X-linked retinitis pigmentosa	More than 150 different mutations known

the photoreceptors with subsequent degeneration of RPE. It is the most common inherited retinal degeneration worldwide and characterized by pigment deposits predominantly in the peripheral retina and by a relative sparing of the central retina. The typical manifestations are present between adolescence and early adulthood leads with it a high probability to devastating visual loss [14]. In most of the cases of RP, there is primary degeneration of photoreceptor rods, with secondary degeneration of cones. RP is a long-lasting disease that usually evolves over several decades, initially presented as night blindness, and later in life as visual impairment in diurnal conditions. Currently, there is no therapy that stops the evolution of the disease or restores vision. However, there are several therapeutic strategies, including cell or tissue transplantation, aimed at slowing down the degenerative process, treating the complications and helping patients to cope with the social and psychological impact of blindness [15].

Associated Diseases Stargardt's disease is the most common inherited "juvenile" form of macular degeneration. Children typically begin experiencing central vision loss between 6 and 12 years of age. Although peripheral vision remains unaffected, individuals with Stargardt's disease usually experience rapid and severe central vision impairment. The disease exhibits an autosomal recessive pattern with mutations in the ATP-Binding Cassette Retina (ABCR) gene, which is expressed exclusively and at high levels in the retina and rod photoreceptors, and is responsible for the disease. The ABCR gene may also be responsible for some forms of age-related macular degeneration as well [16].

Leber congenital amaurosis (LCA) is the designation for a group of autosomal recessive retinal dystrophies that represent the most common genetic causes of congenital visual impairment of retinal origin in infants and children. LCA is characterized by moderate to severe visual impairment identified at or within a few months of birth, infantile nystagmus, sluggish pupillary responses, and absent or poorly recordable electroretinographic responses early in life. LCA has been shown to be caused by mutations in three different genes: guanylate cyclase, CRX and RPE-65 [17].

Usher syndrome is an autosomal recessive disease that is characterized by moderate to profound hearing impairment and progressive vision loss due to RP. It is the major cause of a combined hearing and visual impairment. Three types of the syndrome are known. Type 1 is diagnosed in individuals who are born profoundly (completely) deaf and who experience problems with balance. The R245X mutation of the *PCDH15* gene may be the most common cause of this type of the disease. In adolescence, they usually begin to exhibit the first signs of RP which are night blindness and loss of peripheral vision. Type II is diagnosed in individuals with moderate to severe hearing impairment at birth, but who do not have balance problems. Symptoms of RP develop later in adolescence. Type III is characterized by hearing loss and vision loss due to RP, and both symptoms are progressive [18].

Type of Stem Cells

Retinal Progenitor Cells RPC are derived from fetal or neonatal retinas, and comprise an immature cell population that is responsible for generation of all retinal cells during embryonic development [19]. Previous studies reported that RPC can proliferate and generate new neurons and specialized retinal support cells in vitro [20–22]. RPC can migrate into all retinal layers and develop morphological characteristics of various retinal cell types in vivo [1, 23–26]. These results support the hypothesis that RPC transplants are a potential treatment for retinal degenerative diseases.

Embryonic Stem Cells (ESC) ESC are derived from the inner cell mass of blastocyst-stage embryos, with self-renewal capabilities as well as the ability to differentiate into all adult cell types derived from the three embryonic germ layers [27–29]. Therefore, ESC hold great therapeutic promise in the generation of functional cell types relevant for neurons [30–34], cardiomyocytes [35, 36], hepatocytes [37], lung epithelium [38] and pancreatic beta cells [39, 40]. Studies have shown that ESC can differentiate into photoreceptor progenitors, photoreceptor, or retinal pigment epithelium (RPE) in mice and humans [3, 41–46]. A recent study showed that transplantation of retinal cells derived from human ESC into the subretinal space of adult Crx($^{-/-}$) mice promoted the differentiation of hESC-derived retinal cells into functional photoreceptors, and the procedure improved light responses in these animals [2]. Although ESC are promising in retinal replacement therapies, there remain ethical and immune rejection issues to be considered. ESC have also been associated with teratoma formation [27–29].

Induced Pluripotent Stem Cells (iPS) iPS derived from adult tissues were first described by Takahashi et al. in 2006 [47]. iPS are pluripotent ESC-like cells reprogrammed in vitro from terminally differentiated somatic cell by retroviral transduction of four transcription factors: Oct3/4, Sox2, Klf4 and c-Myc [5, 47–50]. iPS have similar developmental potential as ESC such as morphology, proliferation, and teratoma formation, and they contribute to the development of all cell types in chimeric animals, including the germ line [47, 48]. A study showed that irradiated genetically identical adult recipient mice can be rescued after transplantation with hematopoietic progenitor cells derived from iPS in vitro, with a multilineage reconstitution [51]. The therapeutic benefit of iPS-derived hematopoietic cells has recently been

evaluated in a humanized mouse model of sickle cell anemia where it was shown that autologous genetically corrected iPS-derived hematopoietic progenitor cells phenotypically and functionally corrected the sickle cell defect [52]. Recent studies have shown that patient-specific iPS taken from skin biopsies of an amyotrophic lateral sclerosis possess properties similar to human ESC and were successfully directed to differentiate into motor neurons [53]. Another study also showed that iPS can differentiate into neural precursor cells and develop into neuronal and glial cell types both in vitro and in vivo [54]. It has been reported that human iPS have a similar potential of ESC to mimic normal retinogenesis [4]. The use of defined reprogramming factors for the generation of specific iPS offers: 1) a treatment regimen that does not require the use of immunosuppressive drugs to prevent rejection; 2) the opportunity to repair genetic defects; 3) the ability to expand a desired cell type in vitro; and 4) the absence of ethical problems faced when using ESC transplants. However, major issues include reducing the risk of viral integrations and oncogene expression for generation of iPS [55]. Therefore, specific-iPS could be an excellent choice for cell-replacement therapy if the major problems involving the use of this therapy could be solved, as they could provide unlimited number of cells needed for such procedures.

Mesenchymal Stem Cells (MSC) MSC are a bone marrow-derived cell population, independent of the hematopoietic system, which have the ability to self-renew as well as give rise to multiple tissue types [56]. Other sources of MSC have been described including adipose tissue, placenta, cord blood and liver [57–59]. MSC are capable of differentiating into mesoderm-type cells such as osteoblasts, adipocytes and chondrocytes [60–62]. Recent evidence also shows that MSC can differentiate into non-mesoderm cells such as cardiomyocytes [63–66], neural cells [67–69], and insulin-producing cells [70]. It was reported that MSC can be induced into cells expressing photoreceptor lineage-specific markers in vitro using activin A, taurine, and epidermal growth factor [6]. In addition, an in vivo animal model demonstrated that MSC injected into the subretinal space can slow down retinal cell degeneration and integrate into the retina and differentiate into photoreceptors in Royal College of Surgeons (RCS) rats [6, 71].

Bone Marrow (BM)-derived $CD45^-/Sca\text{-}1^+/Lin^-$ VSEL VSEL are a rare homogenous population in mouse bone marrow that exhibit similar morphology to and express several markers of undifferentiated ESC [72]. VSEL are capable of differentiating into cells from all three germ-layers in vitro [72]. VSEL are a mobile pool of stem cells that are mobilized into peripheral blood following: 1) acute myocardial infarction in mice and humans [73, 74]; 2) stroke in mouse and humans [72, 75]; 3) after toxic liver or skeletal muscle injury [76]; and 4) STZ-induced diabetes [77]. Previous studies have shown that when bone marrow cells (BMC) are cultured directly with RPE in a cell to cell contact manor, BMC can adopt a flat, epithelial cell-like RPE morphology and promote the expression of specific-cell markers of RPE lineage including cytokeratin, RPE65, and MITF [78]. Mice treated with growth factors (FL+granulocyte colony stimulating factor [G-CSF]) significantly mobilized BMC into peripheral blood, and these cells home to $NaIO_3$ damaged tissue in the subretinal space [78]. More recent studies indicate that VSEL are present in the murine retina and comprise 1.5% of neonatal retina cells [7]. VSEL can also differentiate into cells expressing markers of retinal neurons and cells resembling RPE [7].

Biology of Regenerative Medicine

The ultimate goal of the recent field of regenerative medicine is to present solutions for chronic diseases which cannot be sufficiently repaired by the body's own mechanisms. A wide array of major diseases with therapeutic needs, including congestive heart failure, osteoporosis, Alzheimer's disease, diabetes mellitus, etc., underscores the importance of this goal. Today, a growing body of convergent data from bioengineering, genetic, cell-based, or growth factor-based approaches for different diseases gives hope for problem-based solutions. All of these approaches could be pursued, at least experimentally, in ophthalmology for the treatment of retinal degenerations.

In retinal diseases, degeneration can be slowed by intraocular injection of soluble growth/survival factors including acidic and basic fibroblast growth factor, brain-derived neurotrophic factor, ciliary neurotrophic factor, leukemia inhibitory factor, interleukin-1β, and pigment epithelium-derived factor [79, 80]. There is evidence that at least some of these protective effects may be mediated indirectly via other retinal cells [81]. However, these proteins have relatively short half-lives and their effects are usually transient, even after repeated applications. More sustained release and thus more prolonged protection may be obtained by grafting cells that express, perhaps by ex vivo genetic modification, particular growth or trophic factors.

The use of physiologically intact retinal cells to replace damaged tissue is crucial when developing new therapies for retinal degenerations. Therefore, two main sources are considered: 1) the use of RPE cells to rescue photoreceptors, and 2) the use of photoreceptor precursors to repair the degenerating neural retina.

Approaches to use RPE as a therapeutic tool in retinal degeneration began in earnest in the mid 1980s when del Cerro and colleagues transplanted strips of retinal tissue with the RPE still attached [82] and Gouras et al.

accomplished the first successful transplantation of RPE into the subretinal space in monkeys [83]. RPE cell replacement in AMD has been pursued since then and its feasibility has been demonstrated as reports of human trials for wet as well as dry AMD indicate. Results by Binder et al. (2004) showed that patients undergoing CNV removal plus autologous RPE transplantation reached significant better reading acuity and higher multifocal electroretinogram response density than patients who underwent membrane excision alone [84]. However, visual results in patients receiving RPE transplantation additional to CNV removal have been rather disappointing. Weisz and colleagues transplanted a suspension of fetal retinal RPE in a patient with non-vascular AMD (geographic atrophy) and found that the patient's vision remained unchanged for 5 months after the surgery [85]. Although it is surgically feasible to transplant RPE to the subretinal space of AMD patients, allogeneic transplants without immunosuppression lead to fibrosis and rejection. Therefore, autologous transplantations of full thickness RPE/choroid from periphery to macular have been implemented recently [86, 87]. Autologous RPE transplantation can in principle restore vision in neovascular AMD, but surgical complications remain high.

In the 1990s, the idea of iris pigment epithelial (IPE) transplantation to use easily available autologous tissue was introduced [88]. IPE cells have many properties that make them suitable for clinical transplantation. They can be easily obtained, and they originate from the inner and outer layers of the optic vesicle, as do the neural retina and RPE, respectively. Autologous IPE cells transplanted into the subretinal space also display no signs of rejection. Thereby, it was shown that autologous IPE transplants were able to delay photoreceptor degeneration for a prolonged period of time but did not improve visual acuity or only on a very low level.

Retinal cell transplantation describes the introduction of healthy photoreceptor cells into the host in order to decrease the rate of disease progression and/or to replace degenerated cells. The therapeutic benefit in retinal degenerative disease would come through either the reestablishment of a complete neural retinal network or the rescue of residual cone photoreceptors [89]. Transplantation of either embryonic dissociated cells or retinal sheets into the subretinal space of rodent models, as well as in RP patients, has demonstrated that transplants survive and differentiate [90, 91]. Neuronal fibers originating from the transplant develop synapses with the remaining host retina which are at least sufficient to mediate a simple improvements in some photoreceptor function, but restoration of vision has not been well established [90, 91]. This shortcoming has been attributed to the failure of transplanted tissue to form functional connections with the host's neurons, the loss of synaptic receptivity of the host's retinal neurons, and neural remodeling and formation of glial seal in the degenerated retina [92].

The advantages of transplantation of already differentiated retinal cells over stem cell transplantation is that the former integrate well into the host retinal layers and express specific retinal cell markers. However, due to immunological issues, surgical complexity and genetic defects in autologously derived cells, the use of stem cells as a source of material for retinal repair is a valuable alternative [93].

The ability of pluripotent stem cells to differentiate into various functional cell types is the major value for use in regenerative medicine. Approaches to employ stem cells (SC) for retinal degeneration include ESC, as well as adult stem/progenitor cells. In contrast to ESC, adult SC differentiate into a more narrow range of cell types. Phenotypes are mostly restricted to the germ layer of their origin. However, transdifferentiation has been reported for several adult stem cell types (see below).

The field of ESC research holds significant potential for regenerative medicine, as these cells are able to differentiate into all three germ layers. Different culture techniques exist to maximize induction of neural progenitor cells from ESC (see review [94]). In the case of retinal degeneration, derivation of retinal cell types (especially of photoreceptor and RPE), is the focus of many research groups. Commitment of mouse, non-human primate, and human ESC to a retinal lineage was shown with variable success [2, 95]. However, tumor formation after transplantation of naïve ESC is the greatest obstacle for the use of these cells in regenerative medicine [96]. To circumvent this phenomenon, predifferentiation before transplanting the cells showed promising results. Haruta's report (2004) has indicated survival of pigmented cells derived from non-human primate ESC in vivo with an absence of tumor formation [97]. Lund et al. (2006) found similar results including rescue of visual function with human ESC [98]. However, even after solving all methodological and scientific issues, ethical concerns about the use of ESC still remain high.

An alternative source of cells showing embryonic properties could be VSEL. These cells were first described by Ratajczak and colleagues in 2006 [72], and their properties have been intensively investigated. By employing multiparameter sorting, a homogenous population of rare (~0.02%) $Lin^-/Sca\text{-}1^+/CD45^-$ cells that express ESC markers such as SSEA-1, Oct-4, Nanog and Rex-1 was identified in murine BM. Microscopic analysis revealed that these cells are small (~2–4 mm), possess large nuclei, are surrounded by a narrow rim of cytoplasm, and contain open-type chromatin (euchromatin) that is also typical for embryonic stem cells. In vitro these cells were able to differentiate into all three germ-layer lineages. Recently, the same group isolated a similar VSEL cell population from human cord blood [99]. The cells could also be found in different organs including neurosensory retina and RPE [7, 73]. The overall concept that embryonic cells could reside in adult organs/tissues was presented almost 150 years ago by Rudolf

Virchow and Julius Connheim, who hypothesized that adult tissues may contain embryonic remnants that were 'lost' during developmental organogenesis and lie dormant [100]. The population of VSEL might be deposited early in development in BM and/or other organs and could possibly be employed for tissue/organ regeneration.

Recently, the successful reprogramming of differentiated somatic cells into a pluripotent state has opened new possibilities in regenerative medicine. The so-called iPS cells were reprogrammed from fibroblasts, human as well as mouse, and similar to ESC in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Furthermore, these cells could differentiate into cell types of the three germ layers in vitro and form teratomas [47, 48]. So far, several publications have shown the induction of retinal cell types. Induced pluripotent stem cells were able to acquire features of advanced retinal differentiation in a sequence and time course that mimics retinal development in situ [4]. Carr and colleagues found also protective effects of iPS-derived RPE after transplantation into RCS dystrophic rats [101].

Pluripotency of adult SC has been under investigation for the last decade. Their presumed differentiation potential across germ layers has led to renewed interest in adult SC for tissue repair [102]. These findings may imply that organ-specific stem cells can overcome their intrinsic restrictions upon exposure to a novel environment (transdifferentiated), perhaps via genomic reprogramming. However, in the absence functional criteria it remains possible that organ-specific adult stem cells cannot differentiate into functioning cells of another organ, but rather can only take on the shape and express some of the proteins characteristic of transdifferentiated cells. Therefore, it is of critical importance, especially in regard to any use in regenerative medicine, to apply high standards in defining transdifferentiation. Possible landmarks were proposed by Anderson and colleagues in 2001: 1) The donor population should be prospectively isolated and transplanted without intervening culture manipulations. It will be critical to compare the behavior of cultured and uncultured cells. 2) The transplanted stem cells should give rise to robust and sustained regeneration of the target tissues. 3) The phenotype of the converted cells needs to be demonstrated not only anatomically and molecularly, but also functionally [103].

In retinal regeneration the following candidate SC might be used to replace damaged cells: Neural stem cells have been found in different areas of embryonic and adult mouse and human brains [104, 105]. These cells are highly plastic cells capable of differentiating into oligodendrocytic, astrocytic and neuronal phenotypes under appropriate culture conditions [106] as well as of fate conversion/transdifferentiation events [107, 108]. In regard to retina regeneration, it has been shown that NSC are able to differentiate in vitro and in vivo into retinal cells like opsin-positive cells [109] and RPE [110]. However, it still must be confirmed that the converted cells execute physiological function of photoreceptors or RPE.

Retinal stem cells (RSC) have been successfully isolated from the pigmented ciliary bodies of several mammalian species, including humans [22, 24, 111]. By modulating time and environment in vitro these cells can yield significant numbers of lineage-specific cells like photoreceptors [112]. Transplantation of these cells in normal and degenerative rodent retina was only minimally successful due to the limited ability of the cells to invade and integrate into the host retina [23, 113]] In contrast, transplantation of immature RSC from the developing retina (postnatal day 1) improves retinal integration. But capability of subretinally or intravitreously injected RSC to invade and integrate into the neural retina remains restricted to sites of retinal injury [1].

Bone marrow-derived stem cells (BMSC) may also provide an alternative source for regeneration of diseased ocular tissue. However, the bone marrow contains several types of pluripotent/multipotent cells: hematopoietic stem cells, the source of all blood cells, and mesenchymal stem cells (MSC), a heterogeneous population of non-hematopoietic cells that differentiate into mesenchymal tissues but possibly also into other tissue types. Additionally, it has been reported that bone marrow contains tissue-committed stem cells that hide out in the marrow and are already committed to lineage-specific cells types like heart or neurons [114]. Two approaches can be pursued to guide the cells to the injured tissue: First, an endogenous mobilization of the BMSC into the periphery using growth factors such as G-CSF. There they would follow an SDF-1 gradient towards the eye. We have shown in the murine sodium iodate model of RPE degeneration mobilization, migration, mobilized BMSC home to the site of RPE damage and express cell markers of RPE lineage [78]. However, to apply this to the human system, a pivotal precondition would be secretion of SDF-1 from the degenerative area, and this is not yet known for AMD and RP. Second, a subretinal or systemic transplantation of BMSC has been harvested exogenously. In the latter case, the cells would also need to migrate to the area of damage in the eye. We reported a proof of principle in the murine sodium iodate model in 2006 [115]. This approach could also allow pre-committing the BMSC in vitro towards the desired lineages.

Collaborative Cells: i.e., Facilitating Cells

We were the first to discover $CD8^+/TCR^-$ graft facilitating cells (FC), a novel cell population in bone marrow that potently enhances engraftment of HSC in both allogeneic and syngeneic recipients [78, 116–122]. FC are a heterogeneous

cell population, with the predominant subpopulation resembling $B220^+/CD11c^+/CD11b^-$ plasmacytoid precursor dendritic cells (p-preDC) [119]. FC suppress graft-versus-host disease in vivo by producing $CD4^+/CD25^+/FoxP3^+$ regulatory T cells (T_{reg}) [123] and induce antigen-specific T_{reg} in vitro in the presence of CpG (Fig. 1) [124].

Combined administration of both Flt3-ligand (FL) and granulocyte colony-stimulating factor (G-CSF) has a synergistic effect in mobilization of HSC and FC into the periphery [125]. The engraftment potential of FL+G-CSF-mobilized PBMC was superior to G-CSF-treatment alone [125]. Mobilization of bone marrow-derived cells by using growth factors (FL + G-SCF) has been suggested to repair cardiac tissue and improve heart function after acute myocardial infarction [126, 127]. Studies have investigated whether bone marrow-derived cells can be induced to adopt a retinal pigment epithelium (RPE) cell phenotype in vitro and can home to the site of RPE damage after FL + G-CSF mobilization in vivo [78, 128]. Their data show that bone marrow-derived cells can differentiate into RPE-like cells and express RPE-specific markers of cytokeratin, RPE65, and MITF in vitro. In $EGFP^+$ chimeras treated with FL+G-CSF, $EGFP^+$ donor bone marrow derived cells were mobilized to the site of $NaIO_3$ induced RPE damage and expressed RPE lineage markers [78]. To test whether $CD8^+/TCR^-$ FC enhance bone marrow-derived cell migration to the subretinal space, sorted $EGFP^+/Sca\text{-}1^+/c\text{-}Kit^+$ cells with or without FC or whole bone marrow were transplanted into mice treated with $NaIO_3$ by intravenous injection. Data show that the combined transplantation of $Sca\text{-}1^+/cKit^+$ cells plus FC improved survival of the $Sca\text{-}1^+/c\text{-}Kit^+$ cells in the damaged subretinal space [129].

Animal Models for Retinal Degenerations/Retinitis Pigmentosa

Mutations in five genes (rhodopsin, peripherin/RDS, Neural Retina Leucine/NRL, RP1 protein and CRX) have been shown to result in the dominant form of RP. Many other autosomal dominant RP loci have been identified but the gene causing mutations have not been identified and characterized. Identification and development of suitable animal models for retinitis pigmentosa is therefore focused on the known defects. The rodent models described below are most widely used in retinitis pigmentosa research and can be applied to test efficiency and efficacy of stem cell-based regenerative approaches.

Mutations in the rhodopsin gene account for more than 10% of all cases and represent the most common cause of autosomal dominant retinitis pigmentosa. Mice carrying a targeted disruption of the rhodopsin ($Rho^{-/-}$) gene resulting in impaired development of rod outer segments and photoreceptor loss over a 3 month period have been established by Humphries et al. [130].

The retinal degeneration slow (rds) mouse is a non-transgenic, natural mutant with a mutation in the rds/peripherin gene similar to that found in human RP families. In the homozygous rds mice, the receptor layer remains rudimentary and the outer segment does not develop but the other retinal layers show a normal trend of growth during the first 2 weeks after birth. Thereafter, the morphological layers containing visual cell structures (photoreceptors, outer nuclear and outer plexiform layers) begin to thin over time. The inner parts of the retina, including inner nuclear, inner plexiform, and ganglion cell layers, remain morphologically unaffected until irregular vascularization follows total loss of visual cells [131].

Natural mutant mice homozygous for the *rd* mutation display hereditary retinal degeneration and the classic rd lines serve as a model for human RP. In affected animals the retinal rod photoreceptor cells begin degenerating at about postnatal day 8, and by 4 weeks no photoreceptors are left. In all regions of the eye, rapid rod degeneration precedes much slower cone degeneration [132]. Scientists first identified the biochemical problem in the retina of the rd mouse as a deficit in the ROS enzyme phosphodiesterase (PDE) that leads to an abnormal accumulation of cyclic GMP and subsequent photoreceptor cell degeneration and death. The lesion is manifest early in the postnatal period of

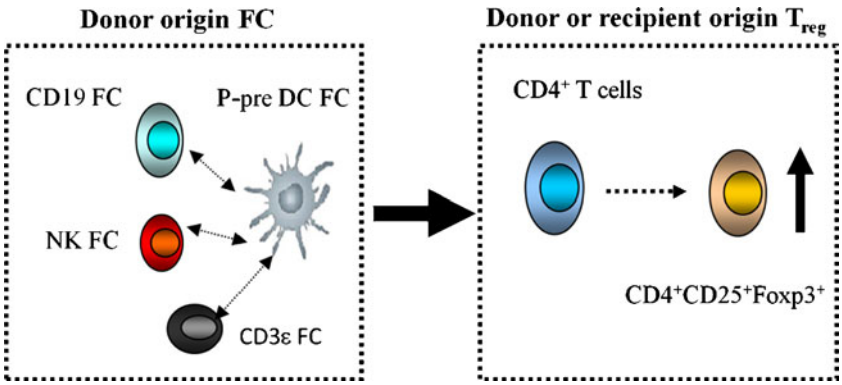

Fig. 1 $CD8^+/TCR^-$ FC are a heterogeneous cell population, with the predominant subpopulation resembling $B220^+/CD11c^+/CD11b^-$ p-pre DC. Other subpopulations include $CD19^+$, $NK1.1^+/DX5^+$ and $CD3\varepsilon^+$. FC has been shown to induce T_{reg} generation in vivo and in vitro

development. Further studies localized the molecular defect to a mutation in the gene coding for the beta subunit of the PDE enzyme [133].

Mutations in the *RPE65* gene can cause severe blindness from birth or early childhood. Although its exact function is unknown, the RPE65 protein is associated with vitamin A metabolism. RPE65-deficient mice ($RPE^{-/-}$) exhibit changes in retinal physiology and biochemistry. Outer segment discs of rod photoreceptors are slightly disorganized and rod function, as measured by electroretinography, is abolished. Rhodopsin is lacking, but not the opsin apoprotein. Disruption of the RPE-based metabolism of all-trans-retinyl esters to 11-cis-retinal thus appears to underlie this RP-like phenotype. The RPE-65 knock out mouse is used as a model for both retinitis pigmentosa and Leber's congenital amaurosis [134, 135].

The RCS rat model of retinal degeneration has been known for many years. In this model, the RPE fail to phagocytose photoreceptor rod outer segments which leads to the accumulation of a layer of membranous debris between the retinal photoreceptors and the RPE and induces retinal degeneration in the developing retina [136]. The gene mutation in the RCS rat has recently been found to be in the receptor tyrosine kinase *Mertk* gene [137].

Pharmacological models of retinal degeneration exist additionally to the various genetic RP models. N-Methyl-N-Nitrosourea, for example, has been used in research as a cancer-inducing agent for a long time [138]. But the alkylating compound is also known to induce degeneration in the retina in a concentration-dependent manner by causing apoptotic death of photoreceptor cells [139]. Thus, the model provides a unique system in which onset and severity of photoreceptor degeneration can be actively manipulated. In combination with functional measurements it might be useful to test the effectiveness of cell replacement therapy.

Animal Models for AMD As the macula is a highly specialized area of the retina and not present in many laboratory mammals, the use of animal models such as mouse strains is very limited. Today, no "ideal" AMD animal model is known to the scientific community. Therefore, models that are currently available show some, but not all aspects of the pathology of AMD. However, models which mimic features of human AMD, panretinal and cone degenerations will give knowledge about physiology and visual processing relevant to the disease. Key pathologic events seen in human AMD, such as drusen formation, thickened Bruch's membrane, various types of retinal degeneration, choroidal neovascularization, and RPE regional dropout have all been seen in various mouse strains [140]. Ambati and colleagues published a murine AMD model, which developed cardinal features of the disease, including accumulation of lipofuscin, development of drusen, photoreceptor atrophy and CNV [141]. These observations in $Ccl2^{-/-}$ or $Ccr2^{-/-}$ mice implicated also an involvement of macrophage dysfunction in AMD. Choroidal neovascularization has also been induced by laser treatment in several species, such as rats [142], rabbits [143], and monkeys [144]. However, only a few of the known models are more widely used. Attempts have also been made pharmacologically to induce changes in the retina resembling AMD. One example is the sodium iodate ($NaIO_3$) model of RPE degeneration. $NaIO_3$ is known to specifically target RPE, and as secondary events, photoreceptors and choriocapillaris will be damaged [145]. The degeneration is time and concentration dependent and results in functional and morphological changes, which can be used to investigate experimental approaches to refurbish areas of RPE atrophy [128, 146].

A better understanding of the disease at a basic level will be useful in pursuing experimental and treatment approaches in humans. However, it is important to keep in mind that the available animal models involve artificial elicitation of disease in relatively "young" animal eyes, whereas authentic human AMD occurs only after decades have passed.

In conclusion, stem cell based regenerative repair for disorders of the eye hold great promise. However, further elucidation of the biology and immunology of such approaches are needed before the full potential can be realized.

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References

1. MacLaren, R. E., Pearson, R. A., MacNeil, A., et al. (2006). Retinal repair by transplantation of photoreceptor precursors. *Nature, 444*(7116), 203–207.
2. Lamba, D. A., Gust, J., & Reh, T. A. (2009). Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx-deficient mice. *Cell Stem Cell, 4*(1), 73–79.
3. Lamba, D. A., Karl, M. O., Ware, C. B., et al. (2006). Efficient generation of retinal progenitor cells from human embryonic stem cells. *Proceedings of the National Academy of Science USA, 103*(34), 12769–12774.
4. Meyer, J. S., Shearer, R. L., Capowski, E. E., et al. (2009). Modeling early retinal development with human embryonic and

induced pluripotent stem cells. *Proceedings of the National Academy of Science USA, 106*(39), 16698–16703.
5. Yu, J., Vodyanik, M. A., Smuga-Otto, K., et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science, 318*(5858), 1917–1920.
6. Kicic, A., Shen, W. Y., Wilson, A. S., et al. (2003). Differentiation of marrow stromal cells into photoreceptors in the rat eye. *Journal of Neuroscience, 23*(21), 7742–7749.
7. Liu, Y., Gao, L., Zuba-Surma, E. K., et al. (2009). Identification of small Sca-1(+), Lin(-), CD45(-) multipotential cells in the neonatal murine retina. *Experimental Hematology, 37*(9), 1096–107–1107.
8. Gehrs, K. M., Anderson, D. H., Johnson, L. V., et al. (2006). Age-related macular degeneration–emerging pathogenetic and therapeutic concepts. *Annals of Medicine, 38*(7), 450–471.
9. Boughman, J. A., Conneally, P. M., & Nance, W. E. (1980). Population genetic studies of retinitis pigmentosa. *American Journal of Human Genetics, 32*(2), 223–235.
10. Berson, E. L. (1993). Retinitis pigmentosa. The Friedenwald Lecture. *Investigative Ophthalmology and Visual Science, 34*(5), 1659–1676.
11. Gaillard, F., & Sauve, Y. (2007). Cell-based therapy for retina degeneration: the promise of a cure. *Vision Research, 47*(22), 2815–2824.
12. Bressler, N. M., Bressler, S. B., & Fine, S. L. (1988). Age-related macular degeneration. *Survey of Ophthalmology, 32*(6), 375–413.
13. Mitchell, P., Korobelnik, J. F., Lanzetta, P., et al. (2009). Ranibizumab (Lucentis) in neovascular age-related macular degeneration: evidence from clinical trials. *British Journal of Ophthalmology, 94*(1), 2–13.
14. Shintani, K., Shechtman, D. L., & Gurwood, A. S. (2009). Review and update: current treatment trends for patients with retinitis pigmentosa. *Optometry, 80*(7), 384–401.
15. Hamel, C. (2006). Retinitis pigmentosa. *Orphanet Journal of Rare Diseases, 1*, 40.
16. Westerfeld, C., & Mukai, S. (2008). Stargardt's disease and the ABCR gene. *Seminars in Ophthalmology, 23*(1), 59–65.
17. Dharmaraj, S. R., Silva, E. R., Pina, A. L., et al. (2000). Mutational analysis and clinical correlation in Leber congenital amaurosis. *Ophthalmic Genetics, 21*(3), 135–150.
18. Brownstein, Z., Ben-Yosef, T., Dagan, O., et al. (2004). The R245X mutation of PCDH15 in Ashkenazi Jewish children diagnosed with nonsyndromic hearing loss foreshadows retinitis pigmentosa. *Pediatric Research, 55*(6), 995–1000.
19. Reh, T. A. (2006). Neurobiology: right timing for retina repair. *Nature, 444*(7116), 156–157.
20. Anchan, R. M., Reh, T. A., Angello, J., et al. (1991). EGF and TGF-alpha stimulate retinal neuroepithelial cell proliferation in vitro. *Neuron, 6*(6), 923–936.
21. Reh, T. A., & Levine, E. M. (1998). Multipotential stem cells and progenitors in the vertebrate retina. *Journal of Neurobiology, 36*(2), 206–220.
22. Tropepe, V., Coles, B. L., Chiasson, B. J., et al. (2000). Retinal stem cells in the adult mammalian eye. *Science, 287*(5460), 2032–2036.
23. Qiu, G., Seiler, M. J., Mui, C., et al. (2005). Photoreceptor differentiation and integration of retinal progenitor cells transplanted into transgenic rats. *Experimental Eye Research, 80*(4), 515–525.
24. Coles, B. L., Angenieux, B., Inoue, T., et al. (2004). Facile isolation and the characterization of human retinal stem cells. *Proceedings of the National Academy of Science USA, 101*(44), 15772–15777.
25. Klassen, H. J., Ng, T. F., Kurimoto, Y., et al. (2004). Multipotent retinal progenitors express developmental markers, differentiate into retinal neurons, and preserve light-mediated behavior. *Investigative Ophthalmology and Visual Science, 45*(11), 4167–4173.
26. Chacko, D. M., Rogers, J. A., Turner, J. E., et al. (2000). Survival and differentiation of cultured retinal progenitors transplanted in the subretinal space of the rat. *Biochemical and Biophysical Research Communications, 268*(3), 842–846.
27. Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., et al. (1998). Embryonic stem cell lines derived from human blastocysts. *Science, 282*(5391), 1145–1147.
28. Reubinoff, B. E., Pera, M. F., Fong, C. Y., et al. (2000). Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nature Biotechnology, 18*(4), 399–404.
29. Cowan, C. A., Klimanskaya, I., McMahon, J., et al. (2004). Derivation of embryonic stem-cell lines from human blastocysts. *New England Journal of Medicine, 350*(13), 1353–1356.
30. Reubinoff, B. E., Itsykson, P., Turetsky, T., et al. (2001). Neural progenitors from human embryonic stem cells. *Nature Biotechnology, 19*(12), 1134–1140.
31. Schuldiner, M., Eiges, R., Eden, A., et al. (2001). Induced neuronal differentiation of human embryonic stem cells. *Brain Research, 913*(2), 201–205.
32. Zhang, S. C., Wernig, M., Duncan, I. D., et al. (2001). In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nature Biotechnology, 19*(12), 1129–1133.
33. Martinat, C., Bacci, J. J., Leete, T., et al. (2006). Cooperative transcription activation by Nurr1 and Pitx3 induces embryonic stem cell maturation to the midbrain dopamine neuron phenotype. *Proceedings of the National Academy of Science USA, 103*(8), 2874–2879.
34. Yan, Y., Yang, D., Zarnowska, E. D., et al. (2005). Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. *Stem Cells, 23*(6), 781–790.
35. Laflamme, M. A., Chen, K. Y., Naumova, A. V., et al. (2007). Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nature Biotechnology, 25*(9), 1015–1024.
36. Yang, L., Soonpaa, M. H., Adler, E. D., et al. (2008). Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. *Nature, 453*(7194), 524–528.
37. Shirahashi, H., Wu, J., Yamamoto, N., et al. (2004). Differentiation of human and mouse embryonic stem cells along a hepatocyte lineage. *Cell Transplantation, 13*(3), 197–211.
38. Samadikuchaksaraei, A., Cohen, S., Isaac, K., et al. (2006). Derivation of distal airway epithelium from human embryonic stem cells. *Tissue Engineering, 12*(4), 867–875.
39. Soria, B., Roche, E., Berna, G., et al. (2000). Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes, 49*(2), 157–162.
40. Assady, S., Maor, G., Amit, M., et al. (2001). Insulin production by human embryonic stem cells. *Diabetes, 50*(8), 1691–1697.
41. Zhao, X., Liu, J., & Ahmad, I. (2002). Differentiation of embryonic stem cells into retinal neurons. *Biochemical and Biophysical Research Comunications, 297*(2), 177–184.
42. Zhao, X., Liu, J., & Ahmad, I. (2006). Differentiation of embryonic stem cells to retinal cells in vitro. *Methods in Molecular Biology, 330*, 401–416.
43. Ikeda, H., Osakada, F., Watanabe, K., et al. (2005). Generation of Rx+/Pax6+ neural retinal precursors from embryonic stem cells. *Proceedings of the National Academy of Science USA, 102*(32), 11331–11336.
44. Vugler, A., Carr, A. J., Lawrence, J., et al. (2008). Elucidating the phenomenon of HESC-derived RPE: anatomy of cell genesis, expansion and retinal transplantation. *Experimental Neurology, 214*(2), 347–361.
45. Klimanskaya, I., Hipp, J., Rezai, K. A., et al. (2004). Derivation and comparative assessment of retinal pigment epithelium from human embryonic stem cells using transcriptomics. *Cloning and Stem Cells, 6*(3), 217–245.

46. Osakada, F., Ikeda, H., Mandai, M., et al. (2008). Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. *Nature Biotechnology, 26*(2), 215–224.
47. Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell, 126*(4), 663–676.
48. Takahashi, K., Tanabe, K., Ohnuki, M., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell, 131*(5), 861–872.
49. Nakagawa, M., Koyanagi, M., Tanabe, K., et al. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nature Biotechnology, 26*(1), 101–106.
50. Wernig, M., Meissner, A., Foreman, R., et al. (2007). In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature, 448*(7151), 318–324.
51. Kyba, M., Perlingeiro, R. C., & Daley, G. Q. (2002). HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. *Cell, 109*(1), 29–37.
52. Hanna, J., Wernig, M., Markoulaki, S., et al. (2007). Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science, 318*(5858), 1920–1923.
53. Dimos, J. T., Rodolfa, K. T., Niakan, K. K., et al. (2008). Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science, 321*(5893), 1218–1221.
54. Wernig, M., Zhao, J. P., Pruszak, J., et al. (2008). Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proceedings of the National Academy of Science USA, 105*(15), 5856–5861.
55. Puzio-Kuter, A. M., & Levine, A. J. (2009). Stem cell biology meets p53. *Nature Biotechnology, 27*(10), 914–915.
56. Friedenstein, A. J., Gorskaja, J. F., & Kulagina, N. N. (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Experimental Hematology, 4*(5), 267–274.
57. Campagnoli, C., Roberts, I. A., Kumar, S., et al. (2001). Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood, 98*(8), 2396–2402.
58. In 't Anker, P. S., Scherjon, S. A., Kleijburg-van der Keur, C., et al. (2004). Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells, 22*(7), 1338–1345.
59. Lee, O. K., Kuo, T. K., Chen, W. M., et al. (2004). Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood, 103*(5), 1669–1675.
60. Bianco, P., & Robey, P. G. (2001). Stem cells in tissue engineering. *Nature, 414*(6859), 118–121.
61. Bianco, P., Riminucci, M., Gronthos, S., et al. (2001). Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells, 19*(3), 180–192.
62. Abdallah, B. M., & Kassem, M. (2008). Human mesenchymal stem cells: from basic biology to clinical applications. *Gene Therapy, 15*(2), 109–116.
63. Makino, S., Fukuda, K., Miyoshi, S., et al. (1999). Cardiomyocytes can be generated from marrow stromal cells in vitro. *Journal of Clinical Investigation, 103*(5), 697–705.
64. Xu, W., Zhang, X., Qian, H., et al. (2004). Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype in vitro. *Experimental Biology and Medicine (Maywood), 229*(7), 623–631.
65. Nagaya, N., Kangawa, K., Itoh, T., et al. (2005). Transplantation of mesenchymal stem cells improves cardiac function in a rat model of dilated cardiomyopathy. *Circulation, 112*(8), 1128–1135.
66. Chen, S. L., Fang, W. W., Ye, F., et al. (2004). Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *American Journal of Cardiology, 94*(1), 92–95.
67. Jiang, Y., Jahagirdar, B. N., Reinhardt, R. L., et al. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature, 418*(6893), 41–49.
68. Chopp, M., Zhang, X. H., Li, Y., et al. (2000). Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation. *NeuroReport, 11*(13), 3001–3005.
69. Chen, J., Li, Y., Wang, L., et al. (2001). Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke, 32*(4), 1005–1011.
70. Karnieli, O., Izhar-Prato, Y., Bulvik, S., et al. (2007). Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem Cells, 25*(11), 2837–2844.
71. Inoue, Y., Iriyama, A., Ueno, S., et al. (2007). Subretinal transplantation of bone marrow mesenchymal stem cells delays retinal degeneration in the RCS rat model of retinal degeneration. *Experimental Eye Research, 85*(2), 234–241.
72. Kucia, M., Reca, R., Campbell, F. R., et al. (2006). A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4(+) stem cells identified in adult bone marrow. *Leukemia, 20*, 857–869.
73. Zuba-Surma, E. K., Kucia, M., Dawn, B., et al. (2008). Bone marrow-derived pluripotent very small embryonic-like stem cells (VSELs) are mobilized after acute myocardial infarction. *Journal of Molecular and Cellular Cardiology, 44*(5), 865–873.
74. Wojakowski, W., Tendera, M., Kucia, M., et al. (2009). Mobilization of bone marrow-derived Oct-4^{+} SSEA-4^{+} very small embryonic-like stem cells in patients with actue myocardial infarction. *Journal of the American College of Cardiology, 53*, 1–9.
75. Paczkowska, E., Kucia, M., Koziarska, D., et al. (2009). Clinical evidence that very small embryonic-like stem cells are mobilized into peripheral blood in patients after stroke. *Stroke, 40*(4), 1237–1244.
76. Kucia, M. J., Wysoczynski, M., Wu, W., et al. (2008). Evidence that very small embryonic-like stem cells are mobilized into peripheral blood. *Stem Cells, 26*(8), 2083–2092.
77. Huang, Y., Kucia, M., Hussain, L. R., et al. (2010). Bone marrow transplantation temporarily improves pancreatic function in streptozotocin-induced diabetes: Potential involvement of very small embryonic-like cells. *Transplantation, 89*, 677–685.
78. Li, Y., Atmaca-Sonmez, P., Schanie, C. L., et al. (2007). Endogenous Bone marrow derived cells express retinal pigment epithelium cell markers and migrate to focal areas of RPE damage. *Investigative Ophthalmology and Visual Science, 48*(9), 4321–4327.
79. Lavail, M. M., Li, L., Turner, J. E., et al. (1992). Retinal pigment epithelial cell transplantation in RCS rats: normal metabolism in rescued photoreceptors. *Experimental Eye Research, 55*(4), 555–562.
80. Chaum, E. (2003). Retinal neuroprotection by growth factors: a mechanistic perspective. *Journal of Cellular Biochemistry, 88*(1), 57–75.
81. Wahlin, K. J., Campochiaro, P. A., Zack, D. J., et al. (2000). Neurotrophic factors cause activation of intracellular signaling pathways in Muller cells and other cells of the inner retina, but not photoreceptors. *Investigative Ophthalmology and Visual Science, 41*(3), 927–936.
82. Parysek, L. M., del Cerro, M., & Olmsted, J. B. (1985). Microtubule-associated protein 4 antibody: a new marker for astroglia and oligodendroglia. *Neuroscience, 15*(3), 869–875.

83. Gouras, P., Flood, M. T., Kjedbye, H., et al. (1985). Transplantation of cultured human retinal epithelium to Bruch's membrane of the owl monkey's eye. *Current Eye Research, 4*(3), 253–265.
84. Binder, S., Krebs, I., Hilgers, R. D., et al. (2004). Outcome of transplantation of autologous retinal pigment epithelium in age-related macular degeneration: a prospective trial. *Investigative Ophthalmology and Visual Science, 45*(11), 4151–4160.
85. Weisz, J. M., Humayun, M. S., de Juan, E. J., et al. (1999). Allogenic fetal retinal pigment epithelial cell transplant in a patient with geographic atrophy. *Retina, 19*(6), 540–545.
86. MacLaren, R. E., Uppal, G. S., Balaggan, K. S., et al. (2007). Autologous transplantation of the retinal pigment epithelium and choroid in the treatment of neovascular age-related macular degeneration. *Ophthalmology, 114*(3), 561–570.
87. van Meurs, J. C., ter Averst, E., Hofland, L. J., et al. (2004). Autologous peripheral retinal pigment epithelium translocation in patients with subfoveal neovascular membranes. *British Journal of Ophthalmology, 88*(1), 110–113.
88. Rezai, K. A., Lappas, A., Farrokh-Siar, L., et al. (1997). Iris pigment epithelial cells of long evans rats demonstrate phagocytic activity. *Experimental Eye Research, 65*(1), 23–29.
89. Perrault, I., Rozet, J. M., Gerber, S., et al. (1999). Leber congenital amaurosis. *Molecular Genetics and Metabolism, 68* (2), 200–208.
90. Aramant, R. B., & Seiler, M. J. (1994). Human embryonic retinal cell transplants in athymic immunodeficient rat hosts. *Cell Transplantation, 3*(6), 461–474.
91. Radtke, N. D., Aramant, R. B., Petry, H. M., et al. (2008). Vision improvement in retinal degeneration patients by implantation of retina together with retinal pigment epithelium. *American Journal of Ophthalmology, 146*(2), 172–182.
92. Marc, R. E., Jones, B. W., Watt, C. B., et al. (2003). Neural remodeling in retinal degeneration. *Progress in Retinal and Eye Research, 22*(5), 607–655.
93. Lund, R. D., Kwan, A. S., Keegan, D. J., et al. (2001). Cell transplantation as a treatment for retinal disease. *Progress in Retinal and Eye Research, 20*(4), 415–449.
94. Hoffman, L. M., & Carpenter, M. K. (2005). Characterization and culture of human embryonic stem cells. *Nature Biotechnology, 23* (6), 699–708.
95. Idelson, M., Alper, R., Obolensky, A., et al. (2009). Directed differentiation of human embryonic stem cells into functional retinal pigment epithelium cells. *Cell Stem Cell, 5*(4), 396–408.
96. Arnhold, S., Klein, H., Semkova, I., et al. (2004). Neurally selected embryonic stem cells induce tumor formation after long-term survival following engraftment into the subretinal space. *Investigative Ophthalmology and Visual Science, 45*(12), 4251–4255.
97. Haruta, M., Sasai, Y., Kawasaki, H., et al. (2004). In vitro and in vivo characterization of pigment epithelial cells differentiated from primate embryonic stem cells. *I Investigative Ophthalmology and Visual Science, 45*(3), 1020–1025.
98. Lund, R. D., Wang, S., Klimanskaya, I., et al. (2006). Human embryonic stem cell-derived cells rescue visual function in dystrophic RCS rats. *Cloning and Stem Cells, 8*(3), 189–199.
99. Kucia, M., Halasa, M., Wysoczynski, M., et al. (2007). Morphological and molecular characterization of novel population of CXCR4+ SSEA-4+ Oct-4+ very small embryonic-like cells purified from human cord blood: preliminary report. *Leukemia, 21*(2), 297–303.
100. Virchow, R. (1855). Leukemia. *Archiv für pathologische Anatomie und Physiologie, und für klinische Medizin, 8*, 23–54.
101. Carr, A. J., Vugler, A. A., Hikita, S. T., et al. (2009). Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. *PLoS One, 4*(12), e8152.
102. Lagasse, E., Connors, H., Al Dhalimy, M., et al. (2000). Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nature Medicine, 6*(11), 1229–1234.
103. Anderson, D. J., Gage, F. H., & Weissman, I. L. (2001). Can stem cells cross lineage boundaries? *Nature Medicine, 7*(4), 393–395.
104. Svendsen, C. N., & Smith, A. G. (1999). New prospects for human stem-cell therapy in the nervous system. *Trends in Neurosciences, 22*(8), 357–364.
105. Shimazaki, T. (2003). Biology and clinical application of neural stem cells. *Hormone Research, 60*(Suppl 3), 1–9.
106. Bez, A., Corsini, E., Curti, D., et al. (2003). Neurosphere and neurosphere-forming cells: morphological and ultrastructural characterization. *Brain Research, 993*(1–2), 18–29.
107. Clarke, D. L., Johansson, C. B., Wilbertz, J., et al. (2000). Generalized potential of adult neural stem cells. *Science, 288* (5471), 1660–1663.
108. Bjornson, C. R., Rietze, R. L., Reynolds, B. A., et al. (1999). Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. *Science, 283*(5401), 534–537.
109. Dong, X., Pulido, J. S., Qu, T., et al. (2003). Differentiation of human neural stem cells into retinal cells. *NeuroReport, 14*(1), 143–146.
110. Enzmann, V., Howard, R. M., Yamauchi, Y., et al. (2003). Enhanced induction of RPE lineage markers in pluripotent neural stem cells engrafted into the adult rat subretinal space. *Investigative Ophthalmology and Visual Science, 44*(12), 5417–5422.
111. Ahmad, I., Tang, L., & Pham, H. (2000). Identification of neural progenitors in the adult mammalian eye. *Biochemical and Biophysical Research Comunications, 270*(2), 517–521.
112. Merhi-Soussi, F., Angenieux, B., Canola, K., et al. (2006). High yield of cells committed to the photoreceptor fate from expanded mouse retinal stem cells. *Stem Cells, 24*(9), 2060–2070.
113. Ahmad, I. (2001). Stem cells: new opportunities to treat eye diseases. *Investigative Ophthalmology and Visual Science, 42* (12), 2743–2748.
114. Ratajczak, M. Z., Kucia, M., Reca, R., et al. (2004). Stem cell plasticity revisited: CXCR4-positive cells expressing mRNA for early muscle, liver and neural cells 'hide out' in the bone marrow. *Leukemia, 18*(1), 29–40.
115. Friedlander, M., Dorrell, M. I., Ritter, M. R., et al. (2007). Progenitor cells and retinal angiogenesis. *Angiogenesis, 10*(2), 89–101.
116. Gandy, K. L., Domen, J., Aguila, H. L., et al. (1999). $CD8^{+}TCR^{+}$ and $CD8^{+}TCR^{-}$ cells in whole bone marrow facilitate the engraftment of hematopoietic stem cells across allogeneic barriers. *Immunity, 11*(5), 579–590.
117. Schuchert, M. J., Wright, R. D., & Colson, Y. L. (2000). Characterization of a newly discovered T-cell receptor beta-chain heterodimer expressed on a CD8+ bone marrow subpopulation that promotes allogeneic stem cell engraftment. *Nature Medicine, 6*(8), 904–909.
118. Huang, Y., Rezzoug, F., Chilton, P. M., et al. (2004). Matching at the MHC Class I K locus is essential for long-term engraftment of purified hematopoietic stem cells: a role for host NK cells in regulating HSC engraftment. *Blood, 104*, 873–880.
119. Fugier-Vivier, I., Rezzoug, F., Huang, Y., et al. (2005). Plasmacytoid precursor dendritic cells facilitate allogeneic hematopoietic stem cell engraftment. *Journal of Experimental Medicine, 201*(3), 373–383. PMCID: PMC2213023.
120. Rezzoug, F., Huang, Y., Tanner, M. K., et al. (2008). TNFa is critical to facilitation of hematopoietic stem cell engraftment and function. *Journal of Immunology, 180*(1), 49–57.
121. Grimes, H. L., Schanie, C. L., Huang, Y., et al. (2004). Graft facilitating cells are derived from hematopoietic stem cells and functionally require CD3, but are distinct from T lymphocytes. *Experimental Hematology, 32*(10), 946–954.
122. Huang, Y., Fugier-Vivier, I., Miller, T., et al. (2008). Plasmacytoid precursor dendritic cells from NOD mice exhibit impaired function:

are they a component of diabetes pathogenesis? *Diabetes, 57*, 2360–2370. PMCID: PMC2518487.
123. Colson, Y. L., Christopher, K., Glickman, J., et al. (2004). Absence of Clinical GVHD and the In Vivo Induction of Regulatory T cells following Facilitating Cell Transplantation. *Blood, 104*, 3829–3835.
124. Taylor, K. N., Shinde-Patil, V. R., Cohick, E., et al. (2007). Induction of FoxP3+CD4+25+ regulatory T cells following hemopoietic stem cell transplantation: role of bone marrow-derived facilitating cells. *Journal of Immunology, 179*(4), 2153–2162.
125. Neipp, M., Zorina, T., Domenick, M. A., et al. (1998). Effect of FLT3 ligand and granulocyte colony-stimulating factor on expansion and mobilization of facilitating cells and hematopoietic stem cells in mice: kinetics and repopulating potential. *Blood, 92*(9), 3177–3188.
126. Dawn, B., Guo, Y., Rezazadeh, A., et al. (2006). Postinfarct cytokine therapy regenerates cardiac tissue and improves left ventricular function. *Circulation Research, 98*(8), 1098–1105.
127. Sanganalmath, S. K., Stein, A. B., Guo, Y., et al. (2009). The beneficial effects of postinfarct cytokine combination therapy are sustained during long-term follow-up. *Journal of Molecular and Cellular Cardiology, 47*(4), 528–535. PMCID: PMC2760590.
128. Li, Y., Reca, R., Sonmez, P., et al. (2006). Retinal pigment epithelium damage enhances expression of chemoattracts and migration of bone marrow-derived stem cells. *Investigative Ophthalmology and Visual Science, 47*(4), 1646–1652.
129. Atmaca-Sonmez, P., Li, Y., Yamauchi, Y., et al. (2006). Systemically transferred hematopoietic stem cells home to the subretinal space and express RPE-65 in a mouse model of retinal pigment epithelium damage. *Experimental Eye Research, 83*, 1295–1302.
130. Humphries, M. M., Rancourt, D., Farrar, G. J., et al. (1997). Retinopathy induced in mice by targeted disruption of the rhodopsin gene. *Nature Genetics, 15*(2), 216–219.
131. Sanyal, S., De Ruiter, A., & Hawkins, R. K. (1980). Development and degeneration of retina in rds mutant mice: light microscopy. *Journal of Comparative Neurology, 194*(1), 193–207.
132. Carter-Dawson, L. D., Lavail, M. M., & Sidman, R. L. (1978). Differential effect of the rd mutation on rods and cones in the mouse retina. *Investigative Ophthalmology and Visual Science, 17*(6), 489–498.
133. Bowes, C., Li, T., Danciger, M., et al. (1990). Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. *Nature, 347*(6294), 677–680.
134. Redmond, T. M., Yu, S., Lee, E., et al. (1998). Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nature Genetics, 20*(4), 344–351.
135. Liu, S. Y., & Redmond, T. M. (1998). Role of the 3'-untranslated region of RPE65 mRNA in the translational regulation of the RPE65 gene: identification of a specific translation inhibitory element. *Archives of Biochemistry and Biophysics, 357*(1), 37–44.
136. Lavail, M. M., Unoki, K., Yasumura, D., et al. (1992). Multiple growth factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light. *Proceedings of the National Academy of Science USA, 89*(23), 11249–11253.
137. D'Cruz, P. M., Yasumura, D., Weir, J., et al. (2009). Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. *Human Molecular Genetics, 9*(4), 645–651.
138. Jobst, K. (1967). Teratogenous changes and tumors in rats following treatment with methylnitroso-urea (MNU). *Neoplasma, 14*(4), 435–436.
139. Nagar, S., Krishnamoorthy, V., Cherukuri, P., et al. (2009). Early remodeling in an inducible animal model of retinal degeneration. *Neuroscience, 160*(2), 517–529.
140. Marano, R. J., & Rakoczy, P. E. (2006). An improved method using densitometry for evaluating severity of laser photocoagulation induced CNV. *Biotechnic & Histochemistry, 81*(2–3), 59–62.
141. Ambati, J., Anand, A., Fernandez, S., et al. (2003). An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nature Medicine, 9*(11), 1390–1397.
142. Frank, R. N., Das, A., & Weber, M. L. (1989). A model of subretinal neovascularization in the pigmented rat. *Current Eye Research, 8*(3), 239–247.
143. elDirini, A. A., Ogden, T. E., & Ryan, S. J. (1991). Subretinal endophotocoagulation. A new model of subretinal neovascularization in the rabbit. *Retina, 11*(2), 244–249.
144. Ohkuma, H., & Ryan, S. J. (1982). Vascular casts of experimental subretinal neovascularization in monkeys: a preliminary report. *Japan's Journal of Ophthalmology, 26*(2), 150–158.
145. Noell, W. K. (1953). Experimentally induced toxic effects on structure and function of visual cells and pigment epithelium. *American Journal of Ophthalmology, 36*(6:2), 103–116.
146. Enzmann, V., Row, B. W., Yamauchi, Y., et al. (2006). Behavioral and anatomical abnormalities in a sodium iodate-induced model of retinal pigment epithelium degeneration. *Experimental Eye Research, 82*(3), 441–448.