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Mobilizing endogenous stem cells for retinal repair

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Abstract

Irreversible vision loss is most often caused by the loss of function and subsequent death of retinal neurons, such as photoreceptor cells—the cells that initiate vision by capturing and transducing signals of light. One reason why retinal degenerative diseases are devastating is that, once retinal neurons are lost, they don't grow back. Stem cell-based cell replacement strategy for retinal degenerative diseases are leading the way in clinical trials of transplantation therapy, and the exciting findings in both human and animal models point to the possibility of restoring vision through a cell replacement regenerative approach. A less invasive method of retinal regeneration by mobilizing endogenous stem cells thus is highly desirable and promising for restoring vision. Although many obstacles remain to be overcome, the field of endogenous retinal repair is progressing at a rapid pace with encouraging results in recent years.

Keywords

retinal regeneration; endogenous stem cells; neuron replacement therapy

Introduction

The retina, as the most accessible part of the central nervous system (CNS), is susceptible to degeneration as a result of genetic mutation or acquired conditions. A variety of diseases can cause retinal neuron degeneration, leading to irreversible blindness. These include conditions that cause photoreceptor death, such as age-related macular degeneration (AMD), retinitis pigmentosa, and cone or rod dystrophy, or damage to the optic nerve and retinal ganglion cells, such as glaucoma and optic neuritis. These diseases share common pathophysiological features: permanent loss of retinal neurons.

Recent advancements in pharmacological therapies, for example the anti-angiogenic treatment for patients with neovascular AMD[1-2], have been successful in slowing down the progression of certain retinal diseases or prevent further deterioration of function. However,

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no treatments are available to completely halt neurodegeneration or enable regeneration and re-establishment of retinal functions in patients once the neurons are lost.

With recent progress, stem cell therapy either by transplanting stem cells or by recruiting endogenous stem cell populations is emerging as a new approach that has the potential to reverse vision loss after retinal degeneration or damage. Attempts have been made in human trials to replace those lost through harvesting and transplanting donor stem cells into the eyes of patients with retinal degenerative diseases, and several clinical trials are in progress[3-4]. The exciting findings in successful restoration of sight in both human and animal models suggest the feasibility of reversing vision loss through a regenerative approach. To this end, new neurons may originate either from an engrafted or endogenous source of stem/progenitor cells. Cell transplantation is still a complex multistep process, even though transplanted stem cells have the capacity to proliferate, differentiate into various cell lineages and repopulate the host retina. Drug-based regenerative therapy that aims at mobilizing the endogenous progenitor cell population to repair the retina may offer many advantages over the transplantation approach. These include less-concerns about immune rejection, neuron integration, tumor formation and disease transmission by implanted cells. The idea of retinal repair through mobilizing endogenous stem cells presents an attractive approach that intends to relieve vision loss in patients by generating and preserving the disease afflicted cells with their own cells. The eye being a relatively small organ presents a special advantage in this approach as it reduces the number of cells required for regenerative therapies – a critical barrier to cell-based approach. To date, the field is rapidly advancing with encouraging results.

Sources of Endogenous Stem Cells/Progenitor Cells

The concept that the adult mammalian CNS contains populations of resident neural stem/progenitor cells was accepted two decades ago[5-6]. Emerging evidence suggests that Müller cells are dormant stem-like cells found throughout the retina and serve as a source of progenitor cells to regenerate retinal neurons after injury[7-8]. In addition, ciliary epithelia-derived cells, retinal pigment epithelium (RPE) and bone marrow–derived cells (BMCs) have also been reported as potential sources of progenitor cells that can be mobilized to the injured retina (Fig. 1, 2).

Lower vertebrates, such as fish and amphibian, are capable of regenerating the retina, and Müller cells are thought to serve as the primary source of retinal progenitors[9]. After injury, quiescent Müller cells re-enter the cell cycle and de-differentiate to form multipotent progenitors that subsequently generate all retinal neuron types that repair the retina and restore visual function[10-16]. Over the past decade, efforts have been placed to investigate whether retinal neuroregeneration can be induced from Müller cells of adult mammals, such as mouse and rat.

Müller cells of adult mammals share many properties of retinal progenitor cells (RPCs). They express the same neurogenic genes, such as Notch and Wnt, as those found in the fish[17,18], and can be reprogrammed in a dish to become retinal neural or photoreceptor progenitors[19,20]. *In vivo*, it has been shown that, by targeting specific signaling pathways through administering fibroblast growth factor (FGF)[21], Notch[22,23], Wnt[24-26], or sonic hedgehog[27], a significant number of Müller cells can be induced to re-enter the cell cycle and display properties of retinal progenitors. While transcription factor Ascl1a was shown to be required for retinal regeneration in the fish[12, 14, 28], recent report indicates that overexpressing a single transcription factor, Ascl1, is also sufficient to induce a neurogenic state of mature Müller cells of mice[29]. These results suggest that some part of the regenerative program occurring in non-mammalian vertebrates remains in the Müller cells of

mammalian retina, which may be induced for retinal repair in patients with retinal degeneration.

The ciliary marginal zone of lower vertebrates, such as teleosts and amphibians, is also known to harbor a pool of RPCs capable of producing new retinal neurons throughout life[30]. A population of multipotent RPCs has been isolated from the ciliary epithelium (CE) of adult rodents and humans that shows the capacity to generate various retinal cell types *in vitro*[31-32]. However, their ability to proliferate and generate new retinal neurons, such as photoreceptors, appears to be limited *in vivo*[33-34]. Mitogens, including basic FGF, insulin, Wnt3a, and pigment epithelium-derived factor, are found to promote the proliferative potential of CE-derived RPCs[35-39]. Transcription factors, such as OTX2, Crx and Chx10, increase the photoreceptor progeny of CE-derived RPCs[40]. Nevertheless, the neurogenic potential of these cells in birds and mammals are fairly restricted. There has been scarce evidence suggesting that these cells contribute to retinal regeneration after injury in adult mammals or birds.

In urodela of amphibians, such as salamanders, RPE cells located between the retina and choroid are capable of transdifferentiating into neurons and regenerating the entire retina[41-42]. The regenerative process usually starts with the de-differentiation of pigmented cells, which then proceed to depigmentation, re-entrance of the cell cycle, and expression of progenitor cell genes[43]. In mammals, RPE of embryonic rats has also been shown to possess the ability to transdifferentiate into retinal neurons and develop into neural retina, but only during the earliest developmental stage[44]. In addition, peripheral RPE cells of adult rats retain the capacity to enter the cell cycle and complete cellular division *in vivo*, although they divide at a low cycling rate[45]. Interestingly, RPE cells from adult humans are reported as being capable of generating stable RPE and differentiating into mesenchymal lineages *in vitro*[46]. Besides, it has been shown that cultured human RPE cells can differentiate into neurons that are positive for beta-III tubulin, MAP2, and neurofilament proteins; whereas, no photoreceptor or glial marker positive cells were observed in these cultures[47]. Mammalian RPEs appear to be deficient in the regulatory elements that are required for induction of transdifferentiation[45]. The neurogenic potential of these cells in birds and mammals are fairly restricted. Evidence suggesting that these cells contribute to retinal regeneration after injury in adult mammals or birds is limited[48].

Some studies have described the ability of BMCs to cross lineage boundaries and express tissue-specific proteins in different organs[49-51]. In mice, it has been shown that endogenous BMCs can migrate to the subretinal space in the damaged retina, presumably to initiate or participate in neural repair[52-54]. However, no evidence has been suggested that they can transdifferentiate into cells with anatomical or functional characteristics of retinal neurons[53-54]. To date, Müller cells are the best characterized mammalian cell type that shows retinal progenitor cell properties and generates new retinal neurons after injury in adult mammals[55-57].

Niche Signals and Stem Cell Potential

Neuroregenerative potential of RPCs depends on both the intrinsic properties of neural stem cells and the environment, or “niche”, in which stem cells reside. The regenerative properties of Müller cells are evolutionarily conserved. In contrast to lower vertebrates, mammals have lost the ability to regenerate retinal neurons, likely due to the constraints of the non-neurogenic environment of the adult retina[58]. In rodent retinas, for example, the Müller cells become reactive and hypertrophic in response to injury, but few re-enter the cell cycle—a first step toward Müller cell transdifferentiation into RPCs[58]. Treating the retina with exogenous activating factors after damage has been shown to induce proliferation of

endogenous RPCs. For example, Wnt3a[59], epidermal growth factor (EGF)[60], FGF[61], insulin-like growth factor (IGF)[62], retinoic acid[59], Notch[63], Nmethyl-N-nitrosourea[64] and α-aminoadipic acid (α-AA)[55] have all been reported to stimulate the proliferation from at least a subpopulation of Müller cells.

Müller cell proliferation has been studied in a variety of species, both *in vitro* and *in vivo*, and some of these mitogenic factors are better characterized. In the post hatch chick, a combination of insulin and FGF causes a large percentage of the Müller cells to proliferate. P2Y-receptor activation stimulates Müller cells proliferation in guinea pig, as does the activation of the platelet-derived growth factor receptor[65]. Perhaps the best-studied mitogenic factor for Müller cells is EGF, which stimulates Müller cells proliferation of mice[60], rats[66], rabbits[67], guinea pigs[65], and humans[68]. Intraocular injection of EGF significantly increases the number of BrdU-positive Müller cells in adult rats after light damage[66]. Wnt3a also induces BrdU-positive Müller cells in retinal explant cultures, where retinal damage inevitably occurs during culture preparation[59].

Several groups have studied retinal damage-induced Müller cell proliferation in mice[25,55,56] and reported that mouse Müller cells could be induced to proliferate when neurotoxic damage was coupled with growth factor stimulation. Interestingly, mouse Müller cells can be stimulated to proliferate in the absence of neural death by a subtoxic dose of glutamate or alpha-aminoadipate (α-AA)[55]. Together, these observations implicate that the non-neurogenic environment of adult mammals may present an inhibitory niche that suppresses the regenerative potential of Müller cells. Similar to Müller cells, CE-derived RSCs have also been found to be capable of re-entering the cell cycle in the presence of certain mitogens, including bFGF, insulin, Wnt3a, and pigment epithelium-derived factor[35-39]. Likely, there is a large overlap in the molecular pathways that regulate the proliferative and regenerative potentials of RPCs of different sources.

Recent studies have also begun to unveil the components of the negative niche factors that inhibit the regenerative potential of the adult retina and brain. Among them, ephrin-A and EphA receptors are thought to act as important players[69-71]. For example, adult neurogenesis is detected in two restricted areas, the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ) of the CNS; however, neural stem cells are found widely distributed throughout the adult CNS, including those that are considered the non-neurogenetic areas[72]. The astrocytes in areas outside of the neurogenic SGZ and SVZ of adult mice express high levels of ephrin-A2 and -A3, which present an inhibitory niche negatively regulating neural stem cell growth[73]. Adult mice lacking ephrin-A2 and -A3 display active ongoing neurogenesis throughout the CNS.[73] Interactions of ephrin-A/EphA family with neurogenic signals, such as Wnt and FGF, have been documented[74-76], further supporting a role of ephrin-As in the regulation of neural stem cell behavior. Recent reports indicate that ephrin-As also play a key inhibitory role in the developing retina and adult ciliary epithelium to suppress stem cell proliferation and retinogenesis via suppressing Wnt signaling[77]. Together, these data indicate a novel mechanism associated with ephrinA/EphAs as endogenous modulators in the control of neurogenesis and regeneration in the adult CNS.

Studies in the brain and retina have suggested that the limitation of retinal neuroregeneration can be attributed to an assortment of factors, including the presence of inhibitory extracellular matrix or cell adhesion molecules. Among these factors, injury-induced inhibitory molecules, such as chondroitin sulfate proteoglycans, neurocan, hyaluronan-binding glycoprotein CD44, semaphorins and ephrins, are particularly abundant in the retina[77-80]. These molecules have previously been shown to function as inhibitory cues for neurite and axonal extension and block the regeneration of nerve fibers as well as neural

stem cell growth[78, 81]. The retina, as part of the CNS, upregulates these growth inhibitory molecules after injury. Both neurocan and CD44 are expressed in the normal retina, and injury stimulates the increased production of these proteins in reactive retinal astrocytes and Müller cells[82]. Upregulation of these inhibitory molecules leads to an inhospitable environment for the proliferation of RPCs and neuron regeneration[77], and hinders successful RPC transplantation by blocking donor-host integration[83]. In addition, the most recent study showed that BMP2, BMP4 and sFRP2 secreted by the adult lens and cornea inhibit CE-derived RPCs and contribute to adult RPCs quiescence in the adult mammalian eye[84]. Thus, to induce regeneration and restore visual function through transplantation, neutralization of these inhibitory proteins may be necessary.

Together, both stimulating the growth pathways and blocking the effects of extracellular inhibitory molecules of neurons have been proved to be beneficial for promoting retinal regeneration after diseases or injury. Although the detailed mechanisms involved remain to be determined, a two-pronged approach might be the most efficient way of promoting retinal regeneration and repair after injury.

Intracellular Signals and Transcriptional Regulation

Niche factors must signal through intracellular pathways to regulate stem cell behavior. The self-renewal of stem cells is tightly controlled through concerted actions of intrinsic transcription factors and networks. Moreover, it has been shown that aging generally has a negative impact on the proliferation and regenerative potential of neural stem cells. Adult neurogenesis in the brain decreases with aging, both as a result of reduced proliferation and differentiation of newborn cells; in parallel with it, an age-associated decline in cognitive performance is often observed. The age of the animal at the time of testing also seems to be a crucial determinant of the ability of Müller cells to re-enter the cell cycle. There is a large decline in the response of Müller cells to mitogens during the second postnatal week in mice[56]. This loss in responsiveness to EGF correlates with the down-regulation of EGF receptor (EGFR) that occurs over the same period. These studies support the notion that the failure of retinal neurons to regenerate in the adult is not a mere outcome of changes in the extraneuronal milieus; rather, the intrinsic nature of RPCs also contributes critically to their regenerative capacity.

Recent years, some key intracellular signals and transcription factors that control the intrinsic growth programs of retinal progenitors have been identified. Ascl1 is emerging as a key regulator determining the neuronal fate of glial lineage neural stem cells. Forced expression of Ascl1 induced a neurogenic state of mature Müller cells of mice,[29] suggesting it as a potential target for stimulating retinal neuroregenerative therapy after disease or injury. Moreover, it is evident that expression of Ascl1 along with two other transcription factors, Brn2 and Myt1l, using viral gene delivery directly converted fibroblasts into neurons[85], suggesting a crucial involvement of Ascl1 in neuronal fate determination.

Pax6 gene encodes a transcription factor controlling retinal neurogenesis and regeneration[86-88]. In the vertebrate retina, Pax6 is highly expressed during retinal development to maintain the multipotency and proliferation capacity of retinal progenitors[89]. Pax6 is also detected in a subpopulation of Müller cells of adult mice where it may be involved in the molecular response to retinal injury[11, 18]. It has been showed that photoreceptor injury induces migration of Pax6-positive Müller cell nuclei toward the outer retinal layers. These cells express markers of cell cycle, implicating their potential to re-enter the cell cycle similarly to that is seen in lower vertebrates[90]. Moreover, Pax6 is upregulated in the Müller cells of mice after N-methyl-D-aspartate glutamate induced neural damage or Wnt3a treatment, as are components of the Notch pathway, Dll1, Notch1 and

Nestin[56, 59, 91]. Studies with the human retina have indicated that many progenitor cell genes (e.g. Pax6 and Sox2) are reactivated in the mammalian Müller cells after damage and mitotic stimulation[68].

Crx, NeuroD, Nrl, and Nr2e3 are the major transcription factors known to be involved in photoreceptor genesis during development thus far. Crx and NeuroD are expressed in photoreceptors of the developing and mature retina, and are essential for precise differentiation and maturation[92]. Nrl is exclusively expressed in rod photoreceptors and is essential for their development and maintenance[93]. *Ex vivo* studies show that Nr2e3 acts synergistically with Nrl and inhibits the activation of cone genes by Crx[94-95].

Even though with the increased understanding of the roles of the molecular signals in the regulation of retinal regeneration, to date, successful repair of the damaged or diseased retina remains a challenge. The critical issue hampering our understanding of the mechanisms controlling retinal regeneration lies in the complexity of the problem and its potential involvement of multiple factors. In order to develop clinically feasible and applicable therapies, studies are needed to further elucidate the interactive effects of these factors as well as the mechanisms underlying the regulation of the proliferation and regenerative behavior of RPCs.

Epigenetic Regulation of Stem Cell Potential

Epigenetics is one of the most promising and expanding fields in the current biomedical research landscape. The term generally refers to chromatin modifications that persist from one stage of cell division to the next stage. It involves heritable alterations of gene expression without changes in DNA sequence, and contributes to the diversity of gene expression and memory of cell lineage. Epigenetics is believed to play a major role in retinal development and cell specification, partly through stabilizing transcriptional programs in embryonic progenitors and differentiated descendants, and establishing and maintaining gene expression in RPCs in the postnatal life. Thus, epigenetic mechanism is a likely avenue which should be explored to change the plasticity of RPCs and enhance the endogenous regenerative potential of the retina.

Epigenetic regulation includes histone modifications, DNA methylation, and other mechanisms, which work together to establish and maintain the global and local condensed or decondensed chromatin states to determine gene expression[96-98]. Disruption of epigenetic machineries is known to provoke aberrant gene expression patterns that give rise to developmental defect. Histone modifications, including histone methylation and acetylation, are areas of intensive interest. In part, this is because chemical compounds that manipulate these processes have been recently identified and some have been shown to affect retinal neurons survival[99-100].

The histone methyltransferase complex, termed polycomb repressive complexes (PRCs), controls key steps in developmental transitions and cell fate choices[99, 101]. PRC2 methyltransferase activity, for example, catalyzes the addition of histone H3 lysine 27 trimethylation (H3K27me3) to specific genomic loci, which act as docking sites for recruiting additional repressive complexes. PRC2 regulates the progression of retinal progenitors from proliferation to differentiation. In *Xenopus*, the PRC2 core components are enriched in retinal progenitors and downregulated in differentiated cells. Knockdown of the PRC2 core component Ezh2 leads to reduced retinal progenitor proliferation, in part due to upregulation of the Cdk inhibitor p15Ink4b. In addition, although PRC2 knockdown does not alter eye patterning or the expression of retinal progenitor genes, such as Sox2, it does cause suppression of proneural bHLH gene expression. These studies indicate that PRC2 is

crucial for the initiation of neural differentiation in the retina. Consistent with these observations, knocking down or blocking PRC2 function constrains the generation of most retinal neural cell types and promotes a Müller glial cell fate decision[99].

It is thought that histone acetylation promotes a more open chromatin structure that allows for gene transcription, while histone methylation in general stabilizes transcriptional programs in progenitor cells and their differentiated descendants. Thus, histone modification is crucial for establishing and maintaining gene expression in cell's postnatal life[102-103]. Accordingly, alterations in histone acetylation may cause behavioral changes of RPCs, such as death and aberrant differentiation. In contrast, mutations associated with histone methylation are likely to result in long-term consequences on cell survival and function. Histone deacetylation generally represses gene promoters and is used to silence genes during differentiation. Loss of histone deacetylatase 1 (HDAC1) in zebrafish leads to retinal overproliferation and inhibition of differentiation through activation of the Wnt and Notch pathways[104]. In mouse retinal explants, HDAC inhibition resulted in defects in rod differentiation but also, unlike in zebrafish, in a reduction in proliferation[105].

Another major form of epigenetic regulation is DNA methylation. Although the role of cell-specific DNA methylation in the retina is still unclear, one potential mechanism may be that it helps direct proper lineage decisions and differentiation of retinal precursor cells. Recent study demonstrates that DNA methyltransferases (Dnmts) are involved in development of the vertebrate eyes. High levels of mouse Dnmts expression are observed during early stages of retinal differentiation[106]. In the zebra fish embryo, knockdown of the maintenance Dnmts by a translation-blocking antisense morpholino results in a profound disorganization of all retinal layers[107].

Epimutation, defined as abnormal transcriptional repression or activation of genes caused by mutations in epigenetic modulators[108], is generally considered reversible in comparison to genetic mutations. The potential to pharmacologically modify gene expression through the manipulation of epigenetic regulation is currently an area of intense interest. The research in this field may unveil novel pathways underlying stem cell regulation and lead to new epigenetic drug targets for boosting the regenerative potential of endogenous stem cells for treating and reversing vision loss.

Functional Restoration of Retinal Neurons

Translation of stem cell biology into clinical application for retinal degenerative disorders via endogenous stem cells must overcome three major obstacles: The first obstacle concerns the development of methods to mobilize endogenous stem cells to sufficiently proliferate and restore the lost cell numbers; the second obstacle regards directing the targeted differentiation of endogenous stem cells into retinal progenitors capable of regenerating desired retinal cell types *in vivo*; finally, to enable restoration of sight newly-generated neurons must integrate into the neural circuitry, form synaptic contacts with the existing neurons, and establish functional connectivity. By repopulating an injured retina with newly-generated neurons in fish, these cells have been shown to develop functional connections with the existing circuitry and restore sight[109]. Emerging studies on stem cell-based therapy for targeted neuron replacements, such as photoreceptors and retinal ganglion cells, indicate that directed neurodifferentiation by endogenous stem cells could also be achieved in adult mammals.

Photoreceptors are photosensitive cells, and their degeneration is a major cause of blindness worldwide, partly due to their incapability of regeneration or self-repair. Stem-cell therapy for photoreceptor replacement provide an exciting prospect for restoring sight in those

whose vision is significantly impaired by retinal disease affecting primarily the photoreceptors. Currently, no treatments are available that can effectively reverse vision loss due to photoreceptor degeneration. Various sources of cells, including neural precursors[110], embryonic and postnatal RPCs[111], neural stem cell lines[112] and bone marrow stem cells[113], have been tested for their ability to differentiate and replace photoreceptors. An appropriate source of precursor cells is a key for photoreceptor cell replacement therapy. Post-mitotic photoreceptor precursor cells could easily be derived from RPCs isolated from neonatal mouse retinas (P1 – P5)[114]. However, equivalent human retinal progenitor cells would have to be derived from second-trimester fetus[114]. Aside from ethical considerations, such tissues are in limited provision and might not provide a consistent source of cells for retinal cell transplantation[114].

Considerable progress has been made in differentiating embryonic stem cells (ESCs) *in vitro* toward a neural retinal precursor phenotype that is competent to generate photoreceptor-like cells[111, 115]. Opsin- and rhodopsin-positive cells are obtained after subretinal grafting of human ESCs, indicating the potential of human ESCs to differentiate into retinal cells, while the subretinal microenvironment supports their differentiation toward a photoreceptor cell fate[116]. New rod and cone photoreceptors have also been successfully generated from ESCs from mouse, monkey and human[117-122]. Most recent study has demonstrated that retinal stem cells isolated from the adult retina have the potential of producing functional photoreceptor cells that can integrate into the retina, morphologically resembling endogenous photoreceptors, and forming synapses with resident retinal neurons[123]. Both structural integration of grafted cells and improvement of pupillary reflex have been reported after transplantation of photoreceptor precursors into a mouse model of retinal degeneration[124].

Currently, many labs have reported an increase in proliferation of mammalian Müller cells —an endogenous source of RPCs—and their migration into the injured areas of the retina[25, 55, 91, 125]. However, it remains unclear if the newly-developed neurons can integrate and allow restoration or improvement of visual function. A number of studies using a transplantation approach further support the extraordinary potential of cell-replacement therapy in functionally refurbishing damaged retinal tissues. These include the studies directed towards the creation of new photoreceptors[118, 126] or RPE by grafted stem cells[127-129]. A variety of different cell types have been tested in their ability to restore retinal function. ESCs, RPCs and photoreceptor precursor cells have all been shown to form new functioning photoreceptors and improve retinal function following transplantation into the degenerative retinal hosts[118, 124]. Moreover, there has been evidence that transplanted cells are capable of forming synaptic contacts with local retinal neurons, suggesting that functional communication between newly generated cells with native retinal neurons can be developed for improvement of visual function.

Compared with photoreceptors, replacing lost RGCs is a more challenging task. This is because successful replacement of RGCs requires not only the survival, migration and integration of donor cells into the ganglion cell layer and differentiation into RGC-like cells. These cells must also extend long axons which navigate through the optic disc, entering the optic nerve through the lamina cribosa. Newly generated RGC axons must be properly myelinated in the optic nerve and continuously extend and make the right way (cross or not cross) into the chiasm, and finally establish functional and topographical connections to the central visual targets. Due to these challenges, efforts at transplantation-based replacement of RGCs are still lagging behind.

ESCs[133-134], RPCs[135] and Müller cell–derived stem cells[131] have been investigated for their potential of replacing RGCs in treating retinal degenerative diseases caused by RGC

dysfunction. Interestingly, these results suggest that the stem cells are capable of migrating and integrating into the retina depleted of RGCs or populated by apoptotic RGCs, expressing RGC markers and extending neurites[136]. iPSCs-derived RGC-like cells with electrophysiological properties similar to RGCs have also been generated from mouse fibroblasts through adenoviral gene delivery[137]. However, these cells showed limited ability to integrate into the normal retina after transplantation. Some evidence suggests that a glaucomatous retina presents even a less permissive environment to the integration of transplanted cells[138]. Mesenchymal stem cells[139], on the other hand, failed completely to migrate into the injured eye after intravenous engraftment; nevertheless, some neuroprotective effect was observed following transplantation into a rat model of glaucoma[140-141]. To date, evidence for synaptic integration and functional improvement by stem cell-derived RGCs remains elusive.

Conclusions

One reason why retinal damage or degeneration is so devastating is that, once neurons are lost, they do not grow back. Intense efforts and substantial progress have been made in this field in the last decade. Emerging evidence suggests that the mammalian retina contains a subpopulation of stem-like cells, primarily Müller cells, as well as CE derived RPCs, in addition to RPE and BMC populations, which may retain the capacity for neuronal regeneration under a certain condition. Limitation of retinal regeneration in adult mammals reflects both intrinsic inability of retinal neurons to reinitiate robust regeneration and lack of a permissive environment for such growth.

The regenerative strategy by mobilizing endogenous stem cells to participate in retinal repair has several advantages over cell transplantation therapy as it does not need to face the shortage of donor cells nor diseases or disorders that may be transmitted via implant; it avoids potential immune rejections. Moreover, endogenously derived RPCs are generally thought to be better programmed to differentiate into retinal neurons and integrate into the existing neural circuitry than exogenously transplanted stem cells. Recent research has shown that adult human and mouse Müller cells can be induced to re-enter the cell cycle and regenerate new neurons *in vitro* and *in vivo* following stimulation by a single compound, although the number and diversity of regenerative neurons is still limited. Practically, a drug therapy for stimulating residential RPCs derived from the patient's own retina would be clinically more viable than transplanting exogenous cells. This is because the (drug therapy) method reduces the concerns over the ethical issues associated with the use of embryonic stem cells, while injection of a drug solution into the eye is a clinically established procedure and considered less invasive than cell transplantation. Patient's native Müller (stem-like) cells are likely to be competent to generate retinal specific cells. Currently, the primary challenge of inducing retinal regeneration from Müller progenitors falls onto the limited number of Müller cells that can be activated to re-enter the cell cycle and participate in regeneration and repairing process. The tumorogenic potential of Müller progenitors is much less of an issue as compared to transplanted hESCs and iPSCs. To our knowledge, there hasn't been any report of tumor formation by Müller progenitors *in vitro* or *in vivo* upon mitogen stimulation. Although barriers to regenerative cell survival, migration, and integration as well as long-term efficacy and safety concerns remain to be overcome, endogenous retinal repair is progressing at a rapid pace and may soon turn the endogenous stem cell approach into a viable therapy.

Acknowledgments

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Abbreviations

CNS	central nervous system
AMD	age-related macular degeneration
RGCs	retinal ganglion cells
PCs	Progenitor cells
RPCs	retinal progenitor cells
CE	ciliary epithelium
BMCs	bone marrow–derived cells
FGF	fibroblast growth factor
CMZ	ciliary marginal zone
EGF	epidermal growth factor
IGF	insulin-like growth factor
α-AA	α-aminoadipic acid
PRCs	polycomb repressive complexes
H3K27me3	histone H3 lysine 27 trimethylation
HDAC1	histone deacetylatase 1
Dnmts	DNA methyltransferases
ESCs	Embryonic stem cells
hESCs	human embryonic stem cells
iPSCs	induced pluripotent stem cells
MSCs	mesenchymal stem cells
RPE	retinal pigment epithelium
PR	photoreceptor
HC	horizontal cell
BP	bipolar cell
MC	Müller cell
AC	amacrine cell
NFL	never fiber layer

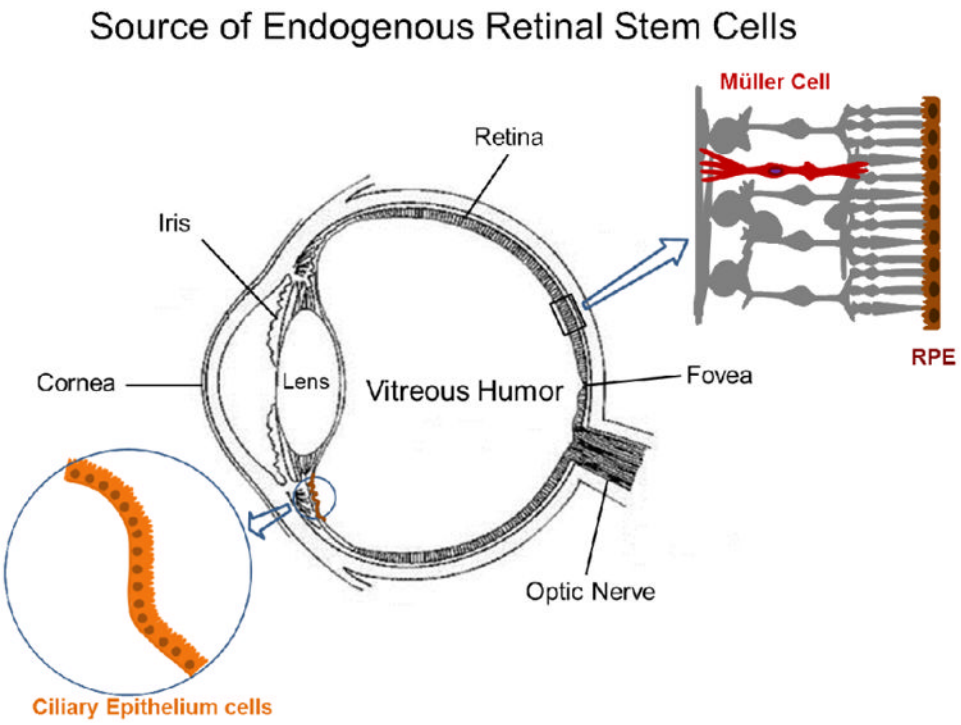

Figure 1.
Sources of endogenous retinal stem cells. The retina has been shown to contain a population of endogenous stem cells, including Müller cells in neural retina, retinal pigment epithelium and ciliary epithelium cells in the ciliary margin zone.

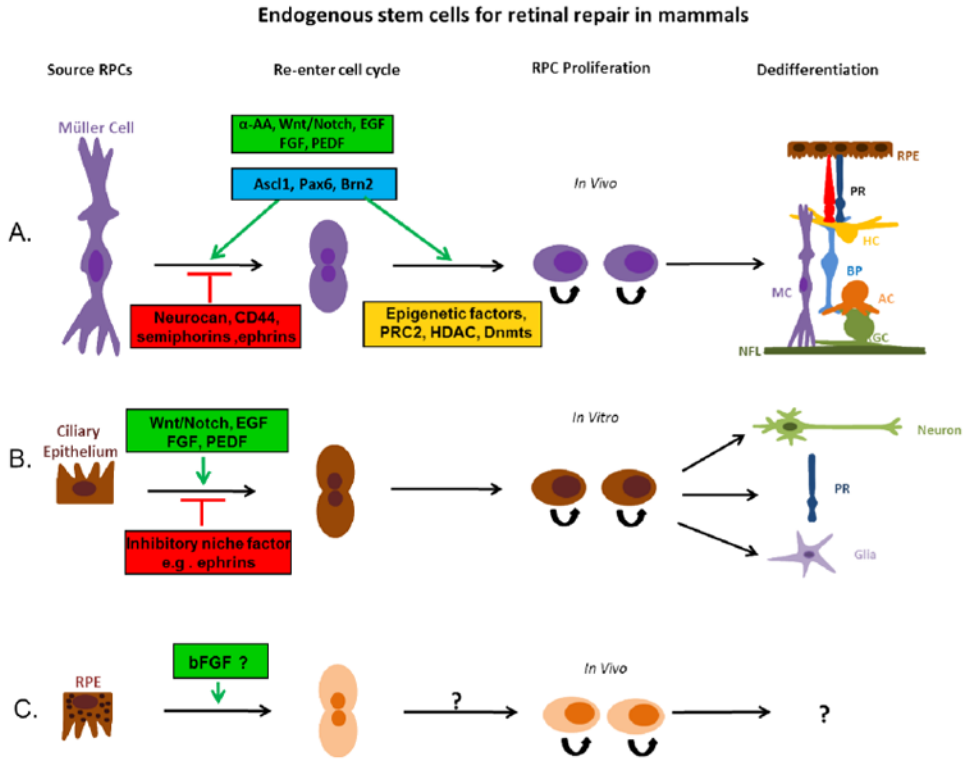

Figure 2.

Müller cells, retinal pigment epithelium and Ciliary epithelium cells are reported as retinal stem cells for retinal repair in mammals. (A) Latent Müller cells are stimulated or suppressed to re-enter the cell cycle by different factors (green or red boxes). Müller cells can be induced to proliferate by growth factors and transcription factors listed in the green boxes and differentiate into various retinal cell types. Modification of epigenetic factors may also contribute to the regulation of the stem cell potency of Müller cells. (B) It has been shown that *in vitro*, ciliary epithelium cells isolated from mature retina can be stimulated (green box) or suppressed (red box) to re-enter the cell cycle, differentiating into new neurons, including photoreceptors (PR), and glia cells. (C) Retinal pigment epithelium has also been shown to possess the ability to proliferate and transdifferentiate into retinal neurons, but the neurogenic potential of these cells in mammals are fairly restricted and appear to be deficient in the regulatory elements that are required for induction of transdifferentiation. EGF: Epidermal Growth Factor; FGF: Fibroblast Growth Factor; PEDF: Pigment Epithelium-Derived Factor; α-AA: α-Aminoadipic Acid; Ascl1: Achaete-Scute Complex-like 1; Pax6: Paired Box Gene 6; Brn2: Brain-2; PRC2: polycomb repressive complex-2; HDAC: Dnmts: DNA methyltransferases; RPC: Retinal Progenitor cell; PR: Photoreceptor; RPE: Retinal Pigment Epithelium; HC: Horizontal cell; BP: Bipolar cell; MC: Müller cell; AC: Amacrine cell; RGC: Retinal Ganglion cell; NFL: Nerve Fiber layer