Mobilizing endogenous stem cells for retinal repair

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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 is, thus, 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. (Translational Research 2014;163:387–398)

Abbreviations: AC = amacrine cell; Ascl1 = Achaete-Scute complexlike 1; BMC = bone marrow-derived cell; Bp = bipolar cell; BrdU = 5′-bromo-2′-deoxyuridine; CE = ciliary epithelium; CNS = central nervous system; Dnmts = DNA methyltransferases; EGF = epidermal growth factor; ESC = embryonic stem cell; FGF = fibroblast growth factor; HC = horizontal cell; MC = Müller cell; NFL = never fiber layer; Pax6 = paired box gene 6; PR = photoreceptor; PRCs = polycomb repressive complexes; RGC = retinal ganglion cell; RPCs = retinal progenitor cell; RPE = retinal pigment epithelium

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 neurodegeneration, leading to irreversible blindness. These include conditions that cause photoreceptor death, such as age-related macular degeneration, retinitis pigmentosa, and cone or rod dystrophy, or damage to the optic nerve and retinal ganglion cells (RGCs), such as glaucoma and optic neuritis. These diseases share common pathophysiological features: permanent loss of retinal neurons.

Recent advancements in pharmacologic therapies—for example, the antiangiogenic treatment for patients with neovascular age-related macular degeneration,[1,2] have been successful in slowing the progression of certain retinal diseases or preventing further deterioration of function. However, no treatments are available to halt neurodegeneration completely or enable regeneration and reestablishment of retinal functions in patients after 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 by harvesting and transplanting donor stem cells into the eyes of patients

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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, although 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 because it reduces the number of cells required for regenerative therapies—a critical barrier to a cell-based approach. To date, the field is advancing rapidly, with encouraging results.

SOURCE OF ENDOGENOUS STEM CELLS/PROGENITOR CELLS

The concept that the adult mammalian CNS contains populations of resident neural stem/progenitor cells was accepted 2 decades ago.[5,6] Emerging evidence suggests that Müller cells are dormant stemlike cells found throughout the retina and they 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 (Figs 1 and 2).

Lower vertebrates, such as fish and amphibians, 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 reenter the cell cycle and dedifferentiate to form multipotent progenitors that subsequently generate all retinal neuron types that repair the retina and restore visual function.[10-16] During the past decade, efforts have been placed to investigate whether retinal neuroregeneration can be induced from Müller cells in adult mammals, such as mouse and rat.

Müller cells in 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 reenter the cell cycle and display properties of retinal progenitors. Although transcription factor Achaete-Scute complexlike 1 (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 in mice.[29] These results suggest that some part of the regenerative program occurring in nonmammalian 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 in 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 is fairly restricted. There has been scarce evidence suggesting that these cells contribute to retinal regeneration after injury in adult mammals or birds.

In amphibians in the order Urodela, which includes 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 dedifferentiation of pigmented cells and then proceeds to depigmentation, reentrance of the cell cycle, and expression of progenitor cell genes.[43] In mammals, RPE in embryonic rats has also been shown to have 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 in 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