Advances in Treatment and Management: Immunologic and Cell-Based Regenerative Therapies

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The glaucomas are a group of eye diseases characterized by progressive ganglion cell and optic nerve damage, leading to constriction of the visual fields and eventual loss of central vision. Although the disease is typically associated with increased intraocular pressure (IOP), there are patients with low- or normal-tension glaucoma and others with elevated IOP who do not experience optic nerve damage. The ocular pathology observed in glaucoma can affect the trabecular meshwork (TM; the main outflow tract for intraocular fluid), the ganglion cell layer of the retina, and the optic nerve head and associated lamina cribrosa. Therapies for these diseases have largely been directed at lowering IOP through pharmacologically decreasing aqueous humor production in the ciliary body or increasing outflow by targeting the TM with drugs or surgical intervention. Although these approaches can lead to significant lowering of the IOP, most patients still experience progressive visual loss, albeit at a slower rate than occurs if the high pressure is left untreated. More recently, efforts have been directed at providing neuroprotection for the cells primarily responsible for vision loss, the retinal ganglion cells (RGCs). Recent advances in stem cell biology, regenerative medicine, and cell-based therapies provide the opportunity to protect or replace cells damaged by increased IOP or other less well-understood abnormalities associated with glaucoma. In this article, I review recent advances in the areas of stem cell biology and cell-based delivery of neuroprotectants for the treatment of retinal diseases and discuss their potential applications for the treatment of glaucoma. I also review recent advances in metabolomics and transcriptomics that will enable analysis of factors that may be present in patients who have increased IOP but no damage from glaucoma.

GENERAL CONSIDERATIONS

Nothing more dramatically captures the imagination of the visually impaired patient or the ophthalmologist treating that patient than the possibility of rebuilding a damaged retina with stem cells. Defined as pluripotent cells capable of differentiating into a variety of cell types, stem cells can be derived from early embryos and, under appropriate conditions, can differentiate into a variety of tissues, including muscle, kidney, brain, blood, liver, skin, and retina. Stem cells have also been identi-

fied and isolated from adult tissues and presumably represent a pool of progenitor cells that may serve to maintain a supply of cells in various tissue types, as well as rescue and repair damaged tissue after injury or stress. More recently, induced pluripotent stem cells (iPSCs) have been derived from adult somatic tissues such as skin fibroblasts or keratinocytes, raising the therapeutic possibility of preparing autologous grafts to replace damaged tissues.

STEM CELLS AND THE EYE

There is an extensive body of literature on the formation of nervous,¹ muscle,² vascular,^{3,4} and hematopoietic tissue from stem cells. Over the past decade, other literature has emerged that strongly supports the potential for exploiting progenitor cells to maintain and perhaps “fix” abnormal ocular tissues. These studies describe four basic populations of cells that contain dormant progenitor cells that, under appropriate circumstances, may have a therapeutic application in the treatment of retinal disease: (1) retinal stem cells that can give rise to photoreceptors and other retinal neurons; (2) Müller/glial stem cells that can differentiate into retinal neurons; (3) retinal pigment epithelial (RPE) stem cells that can serve not only to replace diseased RPE but perhaps also can be stimulated to differentiate into photoreceptors; and (4) endothelial progenitor cells (EPCs) that can contribute to the retinal vasculature and exert a neurotrophic effect.

ADULT BONE MARROW-DERIVED PROGENITOR CELLS

Adult bone marrow-derived progenitors differentiate into EPCs, target activated astrocytes, and provide vasculo- and neurotrophic rescue. Adult bone marrow is a rich source of hematopoietic stem and progenitor cells (HSCs and HPCs).⁵⁻⁷ These cells differentiate into various cell types including myeloid and endothelial cells. One cell population, first identified and purified from mouse bone marrow, is called lineage-negative (Lin⁻), to distinguish it from the lineage-positive Lin⁺ HSCs fraction with regard to the cells' potential to differentiate into formed elements of the blood. Lin⁻ HSCs are described as a heterogeneous population of progenitors that includes cells that differentiate into vascular endothelial cells and form blood vessels (EPCs).⁸ The EPCs are mobilized from the bone marrow in response to a variety of signaling molecules^{9,10} and target sites of angiogenesis in ischemic peripheral vasculature,⁸ myocardium,¹¹ or experimentally injured eyes.¹² This fraction of HSCs can differentiate into a variety of cell types other than hematopoietic cells, including neurons, glial cells, and muscle cells.^{13,14} The observation that HSCs contain a pool of EPCs that can be incorporated into the retinal vasculature has been demonstrated, but there is continuing controversy as to the precise identity of these cells.¹⁵⁻¹⁸

In 2004, we demonstrated that bone marrow-derived EPCs, injected directly into the vitreous of neonatal mice, are stably

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incorporated into forming vessels as a result of targeting activated astrocytes (Fig. 1). This astrocytic template is closely associated with the retinal vasculature as a functional template for both developmental and injury-associated retinal angiogenesis. If the bone marrow-derived progenitor cells are injected into the vitreous of mice with inherited retinal degeneration (e.g., rd1 and rd10 mice), they completely prevent the retinal vascular degeneration observed in these models and rescue the neuronal retinal component.¹⁹ It is also possible to use this population of cells to express a potent angiostatic peptide and profoundly inhibit retinal angiogenesis.²⁰ The use of stem cells in cell-based delivery systems has the advantage over more traditional systemic drug administration of selectively and potentially delivering drugs to the back of the eye in physiological doses.

ADULT BONE MARROW-DERIVED MYELOID PROGENITOR CELLS

Recent data support the concept that myeloid cells are involved in the regulation of angiogenesis and neovascularization independent of the observed effects of EPCs.²¹ Myeloid progenitor cells have been reported to rescue and maintain the function of ischemia-damaged endothelial cells of the hind limb²² and to prevent vascular abnormalities in a mouse model of ischemic injury: oxygen-induced retinopathy.²¹ In this model, a subpopulation of Lin⁻ cells that express high levels of the hyaluronic acid receptor CD44 (CD44^{hi}) enhances vascular repair after oxygen-induced vascular obliteration and stabilizes hypoxia-driven neovascularization. The high level of expression of myeloid-specific markers, cellular morphology, and localization of cells external to the lumen of the blood vessels define these cells as microglia and suggest that they may be useful in treating ischemic retinopathies.²¹

CORD BLOOD AS A SOURCE OF PROGENITOR CELLS

Human umbilical cord blood (UCB) is a well-described source of HSCs that are used for transplantation in the treatment of hematologic and genetic diseases.^{23,24} Since the first human transplantation of UCB-derived cells in 1988, many studies have contributed to the extensive characterization of these progenitor cells. Compared with those obtained from adult bone marrow, UCB cells are present at higher density and with a higher capacity to form hematopoietic colonies and differentiate into multiple blood cell lineages. Clinical observations suggest that patients who undergo UCB-derived stem cell trans-

plantation have a relatively low incidence of graft-versus-host disease, probably because of the lower immunogenicity of these cells when compared with those derived from bone marrow.

EPCs derived from UCB are a heterogeneous population of cells expressing CD34 and CD11b antigens. When UCB-derived EPCs are grown in culture, they upregulate the expression of endothelial markers such as Tie-2 and Ang-1.^{25,26} This differentiation potential suggests that the stem cells circulating in the UCB not only give rise to hematopoietic cells but also play a role in repairing vascular endothelium after injury and surgery. Thus, UCB-derived cells that can regulate inflammation cascades and hypoxia-driven neovascularization may be useful in treating diseases including retinopathy of prematurity and diabetic retinopathy, in which an early phase of vaso-obliteration is followed by inflammation-associated neovascularization.²⁷ That UCB is much easier to obtain than bone marrow from a newborn child and is a less immunocompetent source than peripheral blood and adult bone marrow makes the use of this stem cell source in treating retinopathies such as ROP very appealing.

In recent years, the importance of circulating monocytes/macrophages in neovascularization has been demonstrated in ischemic diseases.^{28,29} Monocytes, which are derived from monoblasts, the HSC precursors in the bone marrow, circulate in the bloodstream before extravasating into tissues of the body and promoting angiogenesis related to inflammatory reactions.³⁰ The monoblasts and their monocytic progeny are attracted to hypoxic areas and begin to differentiate into tissue macrophages, dendritic cells, and microglia.

Macrophages in tissue have been known to be polarized populations: M1 and M2 subsets.^{31,32} Whereas M1 macrophages are proinflammatory and phagocytose pathogens, M2 macrophages modulate the inflammatory response and help with angiogenesis and tissue repair. Recent reports confirm that most monocytes from UCB become M2 polarized cells, which are less inflammatory and more angiogenic.^{33,34} This reduced inflammation could be explained by the immaturity of the immune- and inflammation-stimulatory functions of UCB. For these reasons, the progeny of UCB-derived myeloid progenitor cells/monocytes and macrophages may provide a promising alternative to stem cell transplantation for treatment of ocular diseases, in that the cells can promote arteriogenesis and angiogenesis and can reduce the inflammation processes in various ischemic retinopathies.

APPLICATION OF STEM CELL TECHNOLOGIES TO THE TREATMENT OF GLAUCOMA

At least two populations of cells in the TM and ganglion cell layer appear to undergo degeneration in glaucoma, leading to the observed pathologies. Several groups have begun to explore potential therapeutic applications of TM-derived stem cells. It has long been known that a decrease in TM cellularity accompanies the onset of primary open-angle glaucoma (POAG).^{35,36} POAG is typically characterized by an increase in IOP, which can lead to visual field loss and blindness as a result of ganglion cell death and optic nerve atrophy. Although the precise etiology of POAG is still unknown, a possible mechanism is the obstruction of the outflow of aqueous humor resulting from the malfunction of the TM outflow tract, including Schlemm's canal.³⁷ The discovery of a putative stem cell-like population of TM cell progenitors (termed insert cells because of their proximity to the area where the TM inserts into the cornea beneath Schwalbe's line) by Acott et al.³⁸ and others^{39,40} raised the prospect of expanding and ultimately implanting these cells into the TM of glaucomatous eyes. Al-

FIGURE 1. Lin⁻ HSCs from green fluorescent protein (GFP) transgenic mice (yellow cells, arrow) selectively targeted activated astrocytes (green cells) when injected intravitreally into 3-day-old mice.

though several groups have successfully isolated putative TM progenitor cells from free-floating neurospheres derived from human TM cells, there are phenotypic differences between these cells and mature TM cells.^{41,42} Furthermore, little progress has been made with regard to applying these cells clinically.

The RGCs undergo progressive damage leading to visual field deficits and the eventual blindness associated with glaucoma. Although the idea of replacing dead ganglion cells with new, embryonic, or induced pluripotent stem cell-derived ganglion cells is appealing, the reality may be very different. Once the ganglion cells die and their axons degenerate, it is unlikely that appropriate retinal-tectal projections will be re-established. Instead, it seems more reasonable to provide neuroprotection for RGCs stressed by increased IOP, as seen in glaucoma. Targeting of autologous bone marrow-derived progenitor cell populations toward activated glia in the nerve fiber layer, as described earlier, may provide trophic rescue of associated diseased RGCs. This hypothesis can be readily tested in animal models of ganglion cell degeneration.⁴³ Alternatively, it may be possible to generate autologous grafts of TM cells, microglia, or ganglion cells from iPSCs in a fashion similar to that used to prepare RPE.⁴⁴ If this approach were to be used, it seems logical to intervene early, before loss of ganglion cell projections into the visual cortex. Actual cell replacement, augmentation, or trophic support could serve to provide therapeutic benefit.

OCULAR HYPERTENSION AND LOW-TENSION GLAUCOMA: THE SECRETS TO CURING GLAUCOMA?

It is well recognized that there are patients with increased IOP who do not develop glaucomatous damage (ocular hypertension). Similarly, there are patients with severe glaucomatous damage and vision loss who have normal or low IOP (low-tension glaucoma). The challenge, perhaps key to understanding glaucoma, is to analyze these patients to determine whether there are identifiable factors that place those with low-tension glaucoma at higher risk than the general population or whether there are protective factors present in ocular hypertensives that protect them from glaucomatous damage. Clues to understanding susceptibility to glaucomatous damage of various groups may be found in analyzing the ocular tissues directly affected by the disease (e.g., ganglion cells, optic nerve head/lamina cribrosa, and TM). Alternatively, if there is an immunologic component to the disease, analysis of circulating immune cells (e.g., monocytes, dendritic cells) may reveal factors that are protective or damaging. There is an increasing awareness of the importance of interactions between circulating immune cells and alterations in vasculature and neurons observed in several retinal vascular and degenerative diseases. It is not unreasonable to think that various circulating immune cells would modulate the neuronal response to alterations in IOP. For example, these cells could provide neurotrophic protection under conditions of stress, such as those that occur in glaucoma, not dissimilar to that observed in outer retinal vascular and neuronal tissues in response to ischemia.⁴⁵

The recently emerging fields of transcriptomic and metabolomic analysis may help identify factors that can exacerbate or moderate response to glaucomatous conditions. High-precision measurements of gene expression can be accomplished with the use of quantitative, real-time polymerase chain reaction assay systems optimized for precision, as measured by tight replicate coefficients of variation and for matched amplification efficiencies of the primer/probe sets. RNA transcript biomarkers can be designed and used to analyze tissues from patients at various stages of glaucoma and individuals with

ocular hypertension who do not have glaucomatous damage. Analysis of tissues from animal models of glaucoma with this technology would also prove informative. Metabolomic analysis can be used to measure the levels of various metabolites in human aqueous, vitreous, and retina (when available) from patients with glaucoma, ocular hypertension, or low-tension glaucoma. We have used such an approach to help in understanding the mechanisms of hypoxic stress-induced vasculopathy as well as to identify potential mediators of progenitor cell-based rescue.⁴⁶

CONCLUSIONS

Although significant progress has been made in developing pharmacological agents and surgical procedures that can significantly enhance our abilities to control IOP, the vision loss associated with glaucoma remains a significant problem. Recent advances in cell-based progenitor and stem-cell-based therapies hold the possibility of providing replacement cells for those damaged by glaucoma. Cell-based approaches for drug delivery and paracrine trophic rescue similarly have potential as therapeutic options in the treatment of glaucoma. As we gain more insight into the molecular pathology underlying the disease itself and the cellular response to alterations in normal IOP, we will be better able to apply advances in cell and molecular biology, as well as stem cell biology, to the understanding and treatment of these visually devastating diseases.

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