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Review Series

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Fibrosis and diseases of the eye

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Introduction

To see well, we must maintain a clear visual axis and normally functioning cellular phototransduction. Light entering the eye passes through the cornea (the major refractive surface), the lens, the vitreous (gel in the posterior chamber of the eye), the inner retina, and, finally, into the photoreceptors of the outer retina (Figure 1). These photoreceptors are the site at which photons of light are converted into electrical signals that are transmitted to the visual cortex of the brain by a complex series of synaptic transmissions (Figure 1). To maintain a visual axis through which light can pass undisturbed, a highly ordered tissue structure is required. Any disturbance in normal cell-cell relationships can lead to biological malfunctioning and/or diffraction, absorbance, or reflection of photons, resulting in disturbed or diminished vision.

Homeostasis of the eye, as in tissues elsewhere in the body, depends on the presence of normal vasculature, ECM, and various cell types. If homeostasis is disturbed by infection, inflammation, or metabolic disease, visual function becomes impaired. The end result of these conditions is often fibrosis. In the CNS, of which the retina is a part, such wound-healing responses and associated fibrosis are mediated by glial cells, which perform functions in the CNS similar to those performed by fibroblasts in the rest of the body. Therefore, gliosis is frequently used to describe the glial cell–mediated wound-healing response observed in the CNS, much as fibrosis (which is fibroblast mediated) is used to describe similar processes in non-CNS tissues. In the skin, fibrosis can lead to a cosmetic blemish in the form of a scar; in the eye this can have disastrous consequences for vision — mechanically disrupting the visual axis or sufficiently disturbing the tissue microenvironment such that proper cellular functioning is no longer possible. For example, fibrosis of the cornea can occur after a viral infection, leading to corneal opacification and thereby loss of vision. In the posterior segment of the eye (Figure 1), uncontrolled retinal vascular proliferation, as a result of diabetes-associated retinal hypoxia, can lead to fibrosis and traction retinal detachment, a dreaded complication of advanced diabetic retinopathy (DR). Under the retina, similar fibrosis can occur subsequent to subretinal hemorrhage associated with neovascular age-related macular degeneration (ARMD).

Collectively, these conditions of fibrosis in the eye lead to vision loss in millions of individuals worldwide. In this Review, I discuss the cellular pathophysiology associated with fibrosis in the anterior and posterior segments of the eye (Figure 1), with a focus on the latter. Therapeutic approaches for treating these disorders, based on advances in our understanding of the biological mechanisms underlying these conditions, are reviewed and then discussed in the context of recent novel advances in the area of cell-based therapies.

Nonstandard abbreviations used: ARMD, age-related macular degeneration; CNTF, ciliary neurotrophic factor; DR, diabetic retinopathy; EPC, endothelial progenitor cell; PEX, carboxyterminal, noncatalytic domain of MMP-2; ROP, retinopathy of prematurity; RPE, retinal pigmented epithelium; TIMP, tissue inhibitor of metalloproteinases.

Fibrosis in the eye: general considerations

Fibrosis commonly refers to the response of a tissue to injury. The injury can occur as a result of a mechanical wound or various metabolic malfunctions, including responses to inflammation, ischemia, and degenerative disease. The local response to such injuries includes infiltration by inflammatory cells, neovascularization, altered vascular permeability, proliferation of fibroblasts and fibroblast-like cells, modification of the ECM, and, ultimately, some sort of resolution of the damaged tissue. The CNS is highly specialized in many ways, including the types of inflammatory and wound-healing cells present. Since the retina is part of the CNS, its response to injury utilizes mechanisms very similar to those observed in the rest of the brain; this is true not only for the wound-healing response but also for utilization of migratory cues functional during development of the neuronal and vascular components of this highly organized tissue (1, 2). As discussed below, the response of the anterior segment of the eye to wound healing more closely resembles the response of non-CNS tissues than do such events in the posterior segment or the eye. Therefore, I refer to such wound-healing events in the anterior segment as *fibrosis*, whereas comparable events in the retina are referred to as *gliosis*. Although such distinction is somewhat artificial, it does serve to differentiate between the fibroblasts and glial cells that effect the wound-healing and scar-formation events.

Anterior segment fibrotic diseases of the eye

Two major diseases of the anterior segment of the eye leading to visual loss are corneal opacification and glaucoma. In glaucoma, there is progressive loss of ganglion cells of the nerve fiber layer; this results in degeneration of the neuronal tracts through which efferent signals travel from the retina to the visual cortex (3). Typi-

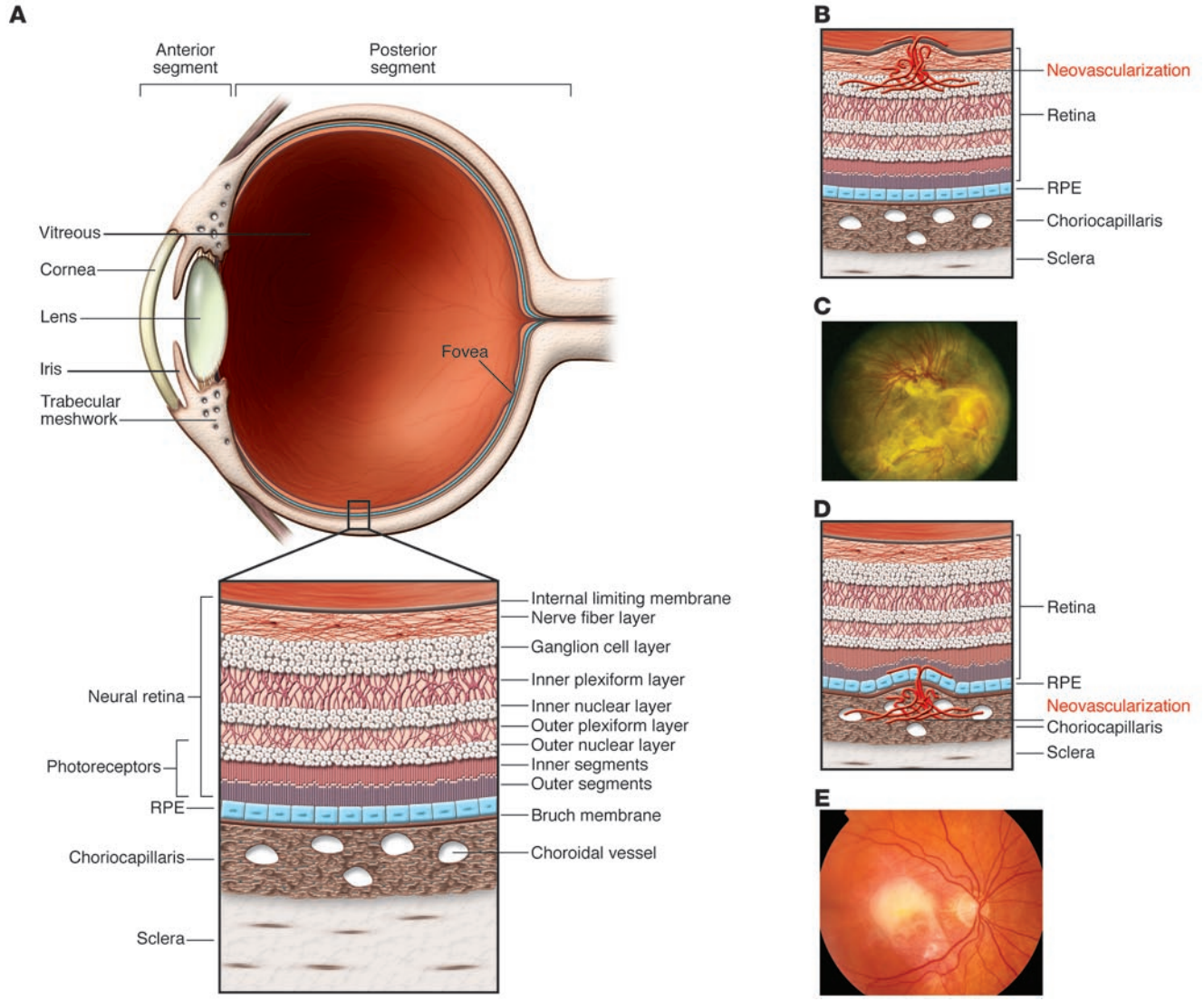

Figure 1
Schematic representation of the eye and principal types of retinal neovascularization and fibrosis/gliosis. (**A**) The anterior segment of the eye, consisting primarily of the cornea and iris, is separated from the posterior segment by the lens. The posterior segment consists primarily of the vitreous and the retina. (**B**) The retina is a highly ordered, multilayered structure that is richly vascularized. Ischemic retinopathies, such as DR, can lead to ischemia and neovascularization on the surface of the retina. (**C**) In extreme cases, associated gliosis can lead to tractional retinal detachments. Reproduced with permission from the American Academy of Ophthalmology (122). (**D**) ARMD can be associated with subretinal neovascularization originating from the choriocapillaris, and this can lead to subretinal hemorrhage and fibrosis (**E**).

tually, complete loss of vision. Although increased intraocular pressure can occur from either increased production of intraocular fluid or increased resistance to outflow, it is more commonly believed that progressive fibrosis of the tracts through which the intraocular fluid leaves the eye (called the trabecular meshwork) accounts for most of the damage that causes glaucoma. Increased understanding of the molecular basis for malfunctioning of the trabecular meshwork (4) (in particular, the aberrant production of ECM components) and of the fibrosis associated with increased resistance to outflow, holds promise for developing therapeutics for this relentlessly progressive disease (5).

which light must pass before entering the rest of the eye. The cornea is covered externally by a stratified nonkeratinizing epithelium and internally by a single layer of transporting endothelium with multiple orthogonal arrays of collagen in between. It is normally avascular due to the high concentration of soluble VEGFR-1 (6) and is surrounded by a transitional margin, the corneal limbus, within which resides nascent endothelium and corneal epithelial stem cells (7), which have high potential for therapeutic value (8). Diseases of the cornea can be genetic (e.g., inherited dystrophies) or acquired secondary to infection (e.g., herpetic keratitis) or inflammation (e.g., pterygia). Elastoid degeneration of the conjunctiva,

induced astigmatism and/or obstruction of the visual axis and would be amenable to topically applied inhibitors of fibrosis and/or angiogenesis (9). The final common events in all of these diseases are often inflammatory changes associated with neovascularization, tissue edema, and, ultimately, fibrosis of the corneal stroma, which leads to opacification and decreased vision (10). Nearly 20 years ago, penetrating keratoplasty (or corneal transplants) changed the uniformly dismal prognosis for patients with opacified or failed corneas; in a substantial percentage of patients undergoing this procedure, if there are no other associated abnormalities, the visual axis is cleared and vision is restored. Despite advances in the use of antiinflammatory drugs, antibiotics, and hypertonic solutions to reduce corneal edema associated with the immune response to the transplant, there is a substantial failure rate, typically due to recurrent opacification. Recent advances in corneal limbal stem cell biology hold the promise of reducing the failure rate for this procedure (11).

Posterior segment fibrotic diseases of the eye

General comments. The posterior segment of the eye consists of structures behind the lens; the interior of the back of the eye is filled with vitreous, a viscoelastic material consisting largely of water, collagen, and hyaluronic acid (12). The vitreous serves as a shock absorber, among other things, for the retina, the most posterior tissue in the eye. In addition, the vitreous can provide scaffolding over which glial and endothelial cells migrate from their normal intraretinal position anteriorly over the retinal surface and/or into the vitreous in certain disease states (e.g., diabetes, proliferative vitreoretinopathy, retinopathy of prematurity [ROP]). The retina consists of multiple layers of neurons, blood vessels, ECM, and various resident and transient cells such as glial cells and monocytes. The vascular supply of the retina consists of the retinal blood vessels (found in three layers on the innermost portion of the retina) and the choriocapillaris (a rich vascular plexus found in the outermost portion of the retina). The photoreceptors are in the outermost portion of the neurosensory retina and rest on a monolayer of cells, the retinal pigmented epithelium (RPE), discussed further below. The RPE rests on a collagenous basement membrane (Bruch membrane), and directly beneath this structure flows the choriocapillaris, providing blood supply for the outer third of the retina. Although there is a blood-retina barrier and relative immune privilege in this part of the eye, normal inflammatory responses to irritation and hypoxia can be quite robust and can lead to much of the pathology observed in diseases that decrease vision (Figure 1).

Most diseases that lead to vision loss in industrialized nations do so as a result of abnormalities in the retinal or choroidal vasculature. These diseases, characterized by macula edema, retinal and vitreous hemorrhage, and fibrovascular scarring, include ARMD, DR, ROP, and neovascular glaucoma. The final common pathophysiological denominator in all of these diseases is the retinal response to injury, with chronic wound healing leading to fibrosis. Although the underlying principles of wound healing in other tissues apply to this process in the eye, it is the uniqueness of the cellular composition and anatomical structure of the retina that makes this normal biological process so potentially devastating to vision. The photoreceptors are located in the outermost portion of the neurosensory retina, just anterior to the RPE and

ler-glial cells and their processes (Figure 1). For light to hit the photoreceptors in an undisturbed manner such that visual images can be formed, it is important that the highly organized architecture of the retina is preserved. When abnormal blood vessels form in response to inflammatory or hypoxic stimuli, they can leak fluid, causing retinal thickening and edema, and/or bleed, leading to fibrovascular proliferation and tractional retinal detachment. The following discussion focuses on the unique aspects of wound healing, fibrosis, and scar formation as it occurs in the posterior segment of the eye.

Fibrovascular scarring and gliosis in the retina. In simplest terms, fibrovascular scarring is a consequence of the underlying inflammatory or hypoxia-driven neovascularization and its associated fibrosis. Therefore, prevention of the primary vascular abnormality is the most appropriate therapeutic target to preserve retinal structure and function. To understand fibrosis and its consequences in the back of the eye, understanding the unique aspects of retinal fibrosis is necessary. Glial cells are the CNS counterparts of peripheral fibroblasts, with several key distinctions, and are therefore the primary participants in the formation of fibrotic scars in response to retinal injury and disease. In addition to their fibrotic tendencies, glial cells also perform a myriad of supportive functions for the neurons with which they are intimately associated. In the retina, this trophic relationship to neurons is extended to the vascular endothelium, with which certain glia are intimately associated in both developing and mature tissue. For example, activated astrocytes form the template over which retinal vascular endothelial cells migrate during formation of the superficial vascular plexus (1, 13); disturbances in the number or distribution of these cells disrupts the normal development of the retinal vasculature (14). Glial cells of the retina include the resident immune cells, microglial cells, and two types of macroglial cell, the astrocyte and the retina-specific Mueller-glial cell (15). Two broad categories of disease account for most of the conditions that lead to fibrovascular scarring in the retina and its associated vision loss — inflammatory diseases (e.g., ARMD) and ischemic diseases (e.g., DR).

Subretinal fibrosis: ARMD. The leading cause of vision loss in Americans over the age of 65 is ARMD; 12–15 million Americans over the age of 65 have this disease and 10%–15% of them will lose central vision as a direct effect of choroidal (subretinal) neovascularization and fibrosis. Clinically, most of these individuals develop atrophic changes in the RPE, which performs a myriad of functions associated with normal photoreceptor functioning (16) and is the cellular interface between the underlying choriocapillaris and the outermost portion of the neurosensory retina, the photoreceptors. As the RPE ages or becomes diseased, it can function improperly, and a build-up of subretinal deposits, called *drusen*, accumulate. These drusen contain, among other things, angiogenic lipids and damaged proteins (17). RPE dysfunction and the accumulation of drusen can lead to thickening of Bruch membrane, and the accumulation of angiogenic drusen associated with this fibrosis can lead to decreased diffusion of oxygen from the choriocapillaris to the photoreceptors, further exacerbating conditions that can lead to choroidal neovascularization. Once these new abnormal blood vessels begin to grow in the subretinal space, they often hemorrhage, leading to further wound-healing responses and, ultimately, to subretinal fibrosis (Figure 1, D and E). Needless to say, local destruction of photoreceptors, the RPE, and choroidal blood ves-

Table 1
Molecules with angiogenic activity in the eye

Name	General angiogenic activity	Angiogenic activity in the eye	Clinical use
Angiogenin	Increases EC proliferation Promotes tubular organization in vitro	Increased in vitreous of patients with PDR and PVR	Possible tumor prognostic marker
Angiopoietin-1	Stabilizes neovessels Matures neovessels	Important role during development Important role in pathological NV	Might prevent vessel permeability in the eye
Angiopoietin-2	Can be angiogenic or angiostatic (depends on cofactors)	Can increase ischemia-induced NV Increased in patients with PDR	Under evaluation for potential clinical use
FGFs	Increases angiogenesis in vitro and in vivo	Associated with choroidal and retinal NV	Under evaluation for potential clinical use
IGF-1	Expression correlates with angiogenesis Expression correlates with tumor metastasis	Mediates VEGF-induced NV in ischemic retinopathies	Somatostatin analogs in clinical trials to treat diabetic retinopathy
Integrins	Some (e.g., $\alpha_v\beta_3$, and $\alpha_v\beta_5$) are critical for vessel growth and survival	$\alpha_v\beta_3$ mediates basic FGF-increased angiogenesis $\alpha_v\beta_5$ mediates VEGF-increased angiogenesis	Integrin antagonists are being tested as potent angiostatics
IL-8	Increases EC proliferation Increases angiogenesis	Associated with ischemic retinal NV Associated with inflammation	Under evaluation for potential clinical use
PlGF	Specific modulator of EC response to VEGF during angiogenesis	Increased in human CNV Inhibition increased NV in mouse	Under evaluation for potential clinical use
PDGF-BB	Induces VEGF expression Increases angiogenesis	Might mediate pericyte recruitment Might mediate vascular stabilization	Under evaluation for potential clinical use
TGF-β	Low doses increase angiogenesis High doses decrease angiogenesis Inflammatory	Increases vascular permeability in the retina by increased MMP9	Under evaluation for potential clinical use
TNF-α	Increases EC proliferation Increases growth factor effects High doses decrease angiogenesis	Associated with various ocular diseases with related NV	Infliximab (TNF-α–specific antibody)
VE-cadherin	Critical for EC intracellular adhesion Modulates VEGF activity	Retinal vascular development	Inhibitory antibodies and T2-TrpRS are antiangiogenics for tumors or ocular diseases
VEGF	Critical proangiogenic growth factor	Vascular development, pathological NV	Multiple anti-VEGF treatments in the clinic or clinical trials

NV, neovascularization; PlGF, placental growth factor; PDR proliferative DR; PVR, proliferative vitreoretinopathy, VE-cadherin, vascular endothelial cadherin.

hampered by the fact that rodents do not seem to faithfully mimic the human disease, although transgenic mice have provided some use in this regard (18).

Advances in therapeutic options available to treat neovascular ARMD have provided some benefit to small subsets of patients with this disease (19, 20). Most drugs currently in clinical trials or approved for treating ARMD-associated choroidal neovascularization are directed at inhibiting promoters of angiogenesis, such as VEGF. There is extensive literature covering these approaches, and I refer the reader to several excellent recent reviews (refs. 16, 19). Unfortunately, inhibiting angiogenic cytokines does not address the underlying pathophysiology — ischemia and inflammatory stimuli. Efforts to minimize sub- and epiretinal fibrosis have met with limited success and, in any event, would represent a therapeutic intervention occurring too late to rescue vision, since such scarring would have already led to photoreceptor death.

Epiretinal fibrosis: DR. The leading cause of visual loss for Americans under the age of 65 is diabetes; 6%–8% of the American population is diabetic, and 40,000 patients each year suffer visual loss from complications of the disease, often as a result of retinal edema or neovascularization (21). Virtually every diabetic has

cyte cell death, microaneurysms, intraretinal microvascular abnormalities, altered vascular permeability, and macular edema (22). As the hypoxia increases, neovascularization can occur, leading to intraretinal, subhyaloid (between the retinal surface and posterior vitreous base) and vitreous hemorrhage (Figure 1B). These proliferating blood vessels are accompanied by fibrosis that occurs as a consequence of glial cell activation and proliferation (gliosis) (Figure 1C). As abnormal vessels continue to proliferate on the retinal surface, they can extend into the vitreous and contract, causing traction on the retinal surface and leading to retinal detachment, a dreaded complication of proliferative DR. Retinal neovascularization and associated gliosis and fibrosis are also observed in ROP (23) and as a complication of surgery to treat retinal detachment (24, 25). Surgical intervention and laser obliteration of the peripheral retina (to decrease the metabolic demand and thereby match up supply and demand) are the current treatments and are of limited benefit. Although animal models of ischemic retinopathy have been very useful in helping to develop a better understanding of factors that control retinal vascular proliferation (24, 26), the rodent does not develop the associated preretinal fibrosis, limiting its utility in studying the gliosis observed in the human condition.

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