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Commentary

Primary Open-Angle Glaucoma

A Transforming Growth Factor-β Pathway–Mediated Disease

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Glaucoma is a leading cause of blindness in the world, affecting almost 5% of persons >70 years.[1] It is a progressive neurological disease of the retina characterized by optic nerve head remodeling, loss of optic nerve axons, and loss of retinal ganglion cells. These effects cause a slow progressive decline of vision, starting in the periphery, moving over time toward central vision, and resulting in complete vision loss. Although family history, ethnicity, and age increase the chances of glaucoma, the most significant risk factor is an elevated intraocular pressure (IOP).[2] Therapies for glaucoma target increased IOP, such that by lowering the IOP, it is possible to slow the progression of glaucoma.

The causes of increased IOP have been a challenge to understand, especially when the most common form of primary glaucoma has an open angle with no visible obstruction of aqueous outflow. The increase in IOP of primary open-angle glaucoma (POAG) is associated with an increase in aqueous outflow resistance in the trabecular meshwork. Many descriptive observations have been made of trabecular meshwork cells and matrix protein deposits, but only a few have compared healthy with glaucomatous eyes. One of the most important differences between healthy and glaucomatous eyes is the marked increase of active transforming growth factor (TGF)-β2 in the aqueous humor of eyes with a high IOP.[3]

A Role for CTGF in Glaucoma

In this issue of *The American Journal of Pathology*, Junglas et al[4] present a link between the descriptive changes induced by TGF-β2 and the biophysical and molecular changes in trabecular meshwork cells that are associated with generation of a high IOP. They used transgenic techniques to overexpress the TGF-β2–induced connective tissue growth factor (CTGF) in the eyes of mice. The eyes of these mice develop a high IOP and retinal ganglion cell axon loss, just as in human POAG. They also demonstrate CTGF-mediated POAG-associated morphological changes in trabecular meshwork cells. The authors provide one of the few mouse models that can be used to study the early stages of POAG and demonstrate a central role for CTGF in regulating aqueous outflow resistance by trabecular meshwork cells.

The matricellular protein CTGF is usually expressed in wounded tissues,[5] but in the eye it is constitutively expressed by the trabecular meshwork cells.[6] CTGF induces fibronectin, collagen production, and actin stress fibers. The major regulators of CTGF expression are TGF-β and mechanical stress.[7] Although mechanical stress induction of CTGF is dependent on RhoA/Rho kinase,[8] the kinase also interacts with the TGF-β signaling pathway.[9] This suggests that, in addition to promoting RhoA-dependent actin assembly, mechanical stress activates a second pathway through the TGF-β SMADs, with the two pathways converging on CTGF. Under healthy conditions, there is constitutive expression of CTGF and normal mechanical stress in the trabecular meshwork, suggesting the existence of a mechanism to maintain a homeostatic level of CTGF and actin stress fibers in the cells, which is necessary to generate an outflow resistance. It also means that this process must function in a narrow range to produce the right amount of resistance to generate an IOP that is not pathological. This possibility is supported by the increase in outflow when healthy eyes are treated with Rho kinase inhibitors, which lower the IOP and aqueous outflow resistance across the trabecular meshwork.[10,11]

In the article by Junglas et al,[4] they approach the question of CTGF activity in POAG partly by generating transgenic mouse lines expressing CTGF under the

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β B1-crystallin (*CRYBB1*) promoter. In these mice, CTGF is constitutively expressed by lens cells and released into the aqueous humor. The transgenic strains that moderately express constitutive amounts of CTGF display progressive increases in fibronectin deposits around Schlemm's canal and in the iris and ciliary body. There is an increase in α-smooth muscle actin fibers in the trabecular meshwork and an increase in IOP with associated loss in optic nerve axons. The ultrastructure of the trabecular meshwork shows bundles of microfilaments of α-smooth muscle actin under the cell membranes, trabecular meshwork cell contraction that would cause increased aqueous outflow resistance, and increased IOP. This contraction is reversed with Rho kinase inhibitor treatment, resulting in reduction of IOP in the treated CTGF-transgenic mice. The transgenic mice with POAG described at the end of the article by Junglas et al,[4] combined with the results of *in vitro* CTGF treatment of trabecular meshwork cells and *in vivo* adenovirus gene delivery of CTGF into the anterior chamber, support a central role for CTGF in mediating the increase in aqueous resistance across the tubercular meshwork. This can be further defined at the cellular level as a CTGF-mediated increase in actin stress filaments and integrin-focal contacts and at the biochemical level of Rho kinase activity that mediates the contraction or tone of the tubercular meshwork cells.

Regulation of the TGF-β2 Signaling Pathway

The production of TGF-β2 in the eye is constitutive and considered to be from the iris and ciliary body cells[12–14]; however, TGF-β2 is not released by cells in an active form.[3,15] The process of producing and secreting TGF-β results in the release of latent TGF-β, consisting of mature TGF-β noncovalently bound to the TGF-β proprotein called latency-associated peptide (LAP). This small latent TGF-β complex is bound through disulfide links between the LAP and a second separately produced protein called the latent TGF-β–binding protein to make the large latent TGF-β complex. TGF-β activity is blocked when it is bound to LAP, with or without the latent TGF-β–binding protein. It is this large TGF-β complex that is held in the extracellular matrix that sequesters TGF-β until released by proteases that cleave the latent TGF-β–binding protein. When released, it is still in an inactive form of TGF-β bound to LAP in the small latent TGF-β complex. Therefore, an additional activation step is needed to free the active form of TGF-β to bind its receptors.

TGF-β activation can be achieved through matrix metalloproteases or tissue plasmin that cleaves the LAP to release TGF-β. This can be facilitated by LAP binding to the integrin $\alpha_v\beta_8$. In addition to the enzymatic activation, there are conformational mechanisms of activating TGF-β. When the latent TGF-β complex binds to thrombospondin-1 or integrin $\alpha_v\beta_6$, a change in the structural conformation of LAP releases active TGF-β. However, integrin-induced activation of TGF-β2 is not possible because the TGF-β2 LAP lacks the necessary Arg-Gly-Asp motif to bind. Therefore, the finding of active TGF-β2 in the aqueous humor means that it must result from increased levels of proteases within the anterior chamber. In addition to activating the expression of CTGF, TGF-β is a potent mediator of fibrosis and a regulator of the cell cycle and apoptosis. This is mediated by TGF-β receptors activating the intracellular SMAD and DAXX pathways.[16,17] Because of the wide range of cellular activities regulated by TGF-β, and the fact that almost all cells have receptors for TGF-β, its activation is highly regulated. Therefore, the heightened level of active TGF-β2 in the aqueous humor of eyes with POAG is important, with the consequence of mediating cellular changes throughout the anterior chamber, including the trabecular meshwork cells.

Future Implications

The ocular CTGF-overexpressing transgenic mice presented by Junglas et al[4] as a model to study POAG provide the means to test new therapies in reducing IOP. Although this mouse model of POAG will greatly advance the understanding of the biochemical, cell biological, and pathological characteristics of POAG, it is not clear if it is part of the early stages of glaucoma. The finding that the CTGF transgenic mice exhibit the same morphological and pathological characteristics as POAG argues strongly for a central role of CTGF in trabecular meshwork cell biological characteristics. However, does this also raise questions regarding the role of active TGF-β2? The simplest answer is that active TGF-β2 initiates an overexpression of CTGF that leads to the physical changes of the trabecular meshwork cells, resulting in increased aqueous outflow resistance and IOP. Then, localized production and activation of TGF-β by the trabecular meshwork cells, along with aqueous humor active TGF-β, may sustain the elevated levels of CTGF and the progressive changes in the trabecular meshwork. These changes increase the mechanical stress on the trabecular meshwork cells, leading to further production of CTGF and increased IOP. In this way, it is a self-sustaining cycle of ever-worsening pathological characteristics and one that can be relieved through Rho kinase inhibitors.

In the transgenic mouse model, a Rho kinase inhibitor is effective in transiently relaxing the trabecular meshwork cells and lowering the IOP in the presence of sustained elevated levels of CTGF. If it is a TGF-β–mediated cycle, then TGF-β antagonists should be tested.[18] It would be interesting to know whether there is an increase in TGF-β expression and activation in the trabecular meshwork of the CTGF-overexpressing mouse eyes. Also, could antagonists of TGF-β signaling neutralize CTGF production?[19] Positive answers would suggest that the process of trabecular meshwork changes leading to POAG is mediated by a self-sustaining cycle of pathological expression of active TGF-β, CTGF, and Rho kinase activity. This further suggests that, before the disease progresses with extracellular plaques of fibrillar material and neurological damage, there may be a simple growth factor loop by which therapeutic intervention is the most effective. In this scenario, the progressive

disease of the transgenic CTGF mice will provide the most benefit. However, this still leaves the following question: What is the initiating event that would induce a pathogenic state in the trabecular meshwork cells leading to POAG?

The expression of CTGF in trabecular meshwork cells[6] and TGF-β2 in aqueous humor[12–14] is constitutive with CTGF at a low level of expression and with latent TGF-β2. In glaucomatous eyes, there are elevated levels of activated TGF-β2.[3] Therefore, under healthy ocular conditions, there is a sustained level of CTGF that is likely induced by a steady-state rate of TGF-β2 activation in the trabecular meshwork to maintain a healthy level of aqueous outflow resistance. This means that there should be a healthy homeostatic set point for TGF-β2 activation and CTGF expression. Therefore, POAG may be the result of an initiated process in eyes that have a higher homeostatic set point for TGF-β2 activation. This could be the result of trauma or genetic predisposition. It may also be something that happens often within the eye. The difference between pathological characteristics and maintaining health is how the trabecular meshwork cells respond and how retinal ganglion cells survive under a high IOP. There are several prevalent gene mutations associated with POAG, but most are carried by <20% of patients with POAG. These mutations are in genes for a limited set of proteins that modulate mitochondrial function, protein transport, neurite outgrowth, and neuroprotection.[20]

Junglas et al[4] note that only transgenic mice with moderate constitutive expression of CTGF express the pathological characteristics of POAG. Others have shown that overexpression of active TGF-β1 or TGF-β2 in the eye by adenoviral gene delivery into the anterior chamber causes fibrosis and closing of the angle, which are not characteristics of POAG.[3] Therefore, POAG is not caused by extremes in active TGF-β nor in CTGF production but rather by elevated levels that could well be the result of subtle shifts in activation and production. If not corrected, there would be induction of a progressive change in the trabecular meshwork leading to a high IOP and retinal ganglion cell death. Therefore, the potential exists that the initiating events of POAG are low levels of abnormal TGF-β activation that induce pathways that include increased CTGF production. Because the animal model is based on genetic overexpression of CTGF and not dependent on TGF-β2 induction, the possibility still exists that, in POAG, factors other than TGF-β2 could be involved in the up-regulation of CTGF in the trabecular meshwork cells.

Concluding Remarks

Although not all animal models of disease can replicate all aspects of a human disease, the more models that are generated, the more insight into different stages of disease can be achieved. In some cases, this may lead to rejecting hypotheses regarding disease causation or may simply not answer such questions. Junglas et al[4] have generated a mouse model and provided ample experimental results to support calling it a mouse model of POAG. In addition, their mouse model could benefit the study of other types of human glaucoma associated with up-regulation of CTGF, such as pseudoexfoliation glaucoma.[21,22] They present detailed descriptions of the morphological and pathological changes expected in a mouse model of POAG. They demonstrate the role of CTGF in mediating the changes in trabecular meshwork cells that increase aqueous outflow resistance in POAG. Finally, this argues for an important role of TGF-β–mediated pathways in the pathological features of glaucoma.[3]

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