The Role of SPARC in Trabecular Meshwork Extracellular Matrix Turnover and IOP Regulation

Developing an understanding of this matricellular protein may reveal molecular targets for the therapeutic treatment of primary open-angle glaucoma.

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Sometimes, I envy my retinal colleagues for the remarkable advances that have emerged over the past decade in targeted therapy for proliferative vascular diseases. By interrupting the molecular cascade triggered by vascular endothelial growth factor, these therapies have provided an elegant treatment with few complications and side effects relative to the laser therapies that, while effective, destroyed both tissue and sight. The development of these drug treatments required careful basic research to elucidate the pathogenesis of disease and the molecular pathways involved to discover the targets for therapy. Perhaps hope is on the horizon for glaucoma treatment. The work of investigators such as Douglas Rhee, MD, and his colleagues may help us understand why primary open-angle glaucoma (POAG) develops at a molecular level. Their cutting-edge research on secreted protein acidic and rich in cysteine (SPARC), a glycoprotein found in the trabecular meshwork (TM) that appears to be important in extracellular matrix (ECM) turnover and IOP regulation, may expose a key to the pathogenesis of POAG. This understanding could reveal molecular targets for therapy. One day, I hope that we will be able to treat the underlying cause of increased resistance to outflow and elevated IOP instead of just trying one more technique to make a bypass hole in the eye. This month's "Peer Review" column describes the work of Dr. Rhee and his colleagues. Let us all hope they succeed!
—Barbara Smit, MD, PhD, section editor

Elevated IOP in eyes with POAG is caused by poor aqueous humor drainage and can lead to visual field loss due to progressive optic nerve damage.[1] The only rigorously proven treatment for POAG is to lower IOP.[2,3] Thus far, single gene mutations account for less than 10% of POAG cases, with the other 90% likely having polygenic origins.[4] Elucidating the molecular underpinnings of IOP regulation is crucial to the quest for new therapeutic targets. We believe that rigorous investigation of aberrant tissue remodeling and outflow resistance in the TM will yield new treatment targets that will directly interrupt the glaucomatous disease process.

Anders Bill, MD, PhD, showed that 80% to 90% of aqueous outflow occurs through the TM, or conventional pathway, with the remaining 10% to 20% occurring

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through the ciliary body face, or alternative pathway.[5] The juxtacanalicular (JCT) region, which includes the inner wall of Schlemm canal and underlying JCT TM, is thought to be the region where the regulation of aqueous humor outflow takes place.[6] The JCT region is an amorphous layer composed of endothelial cells and ECM. Under conditions of elevated IOP, the JCT region has the highest resistance to outflow.[7] The JCT region is not a static structure. Rather, it is continuously undergoing movement, and the ECM within it is constantly being remodeled.

WHY ECM AND SPARC?

Primary Pathophysiologic Event in POAG

The regulation of IOP in the JCT region is likely a complex system with multiple tissue responses to compensate for numerous physiologic stressors and perturbations. Some processes have been shown to influence IOP such as the regulation of ECM homeostasis,[8-13] modifications in the actin cytoskeleton and cellular tone of the JCT TM and inner wall of Schlemm canal cells,[14] and changes in the number of transcellular pores through the inner wall of Schlemm canal cells.[15]

Eyes with POAG have higher amounts of ECM structures called *sheath-derived* (SD) *plaques* within the JCT region compared with age-matched patients without glaucoma. SD plaques are complex structures composed of elastin, collagens, and various proteoglycans. The increase in number of SD plaques is evident both in treated postmortem samples and in untreated eyes.[16,17] The observation of increased SD plaques in untreated eyes indicates that this finding is not the artifact of the long-term use of medication but likely a primary (ie, causative) issue. SD plaques are seen in nonglaucomatous eyes, and they increase with age.[16] The rise in SD plaques with glaucoma, however, suggests a pathophysiologic change associated with increased resistance to outflow and the development of glaucoma. Furthermore, the composition of glycosaminoglycans in eyes with POAG is different than in nonglaucomatous eyes: specifically, there is a shift in the proportion of hyaluronic acid in nonglaucomatous eyes to chondroitin sulfate.[18]

Experimental evidence shows that, in nonglaucomatous eyes, altering ECM homeostasis either by increasing its production or by slowing its turnover alters IOP and that alterations of the JCT ECM constitute primary pathophysiologic events. Understanding the regulation of ECM turnover will be critical to understanding IOP regulation. We therefore have focused our attention on the genes involved in ECM turnover to help explain the development of glaucoma and to look for potential therapeutic targets.

Aberrant Tissue Remodeling in Nonocular Tissues

Matricellular proteins are nonstructural secreted glycoproteins that facilitate cellular control over the surrounding ECM. SPARC is the prototypical matricellular protein. It is generally associated with increased fibrosis and aberrant tissue remodeling processes such as those found in systemic sclerosis, renal interstitial fibrosis, hepatic fibrosis, and pulmonary fibrosis. SPARC is widely expressed in human ocular tissues and is produced by lenticular cells, retinal pigment epithelieal cells, and corneal epithelial cells as well as by TM and ciliary body smooth muscle cells.[19,20]

REGULATORY ROLE IN IOP

We have shown that SPARC is highly expressed by TM cells and is present in very high levels within the JCT region.[20] In normal TM tissue, SPARC is one of the most highly expressed genes.[21] In TM endothelial cells, SPARC is one of the most highly upregulated genes in response to physiologic mechanical stretching, and it is likely important to the baseline function of the TM.[22]

The ability to create null or knockout mice (ie, mice that do not express a single or multiple gene after birth) has become a very powerful tool with which to study the functional role of a particular gene of interest. We have found that SPARC-null mice have a 15% to 20% lower IOP than their wild-type counterparts as a result of increased aqueous drainage.[23] We made this observation using mice with comparable central corneal thicknesses and no gross changes in the overall architecture of anterior chamber tissue.

We have identified two significant upstream regulators of SPARC, transforming growth factor-β2 (TGF-β2) and the microRNA family miR-29. TGF-β2 is greatly increased in the aqueous humor of patients with POAG compared with age-matched controls. Numerous studies implicate its role in the pathogenesis of POAG.[24] We have shown that TGF-β2 upregulates SPARC expression in primary cultured human TM cells[25] and that the reverse, SPARC modulation, has no effect on TGF-β2,[26] indicating that SPARC lies downstream of TGF-β2. MicroRNAs are small, single-stranded RNAs that modulate the posttranscriptional expression of genes. In particular, the miR-29 fam-

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ily is a mediator of tissue fibrosis in nonocular human tissues. We demonstrated that all three members of the miR-29 family are expressed in human TM and have an inhibitory effect on SPARC.[27] As we learn how SPARC modulation affects TM ECM and IOP and the pathway by which SPARC is regulated, we will seek points for possible therapeutic intervention in the pathway.

FUTURE DIRECTIONS

We hope both to broaden our understanding of the effects of the matricellular protein family and to focus on the molecular mechanisms by which SPARC exerts its influence on the ECM and IOP. We have investigated the IOP of mice that do not express two other matricellular proteins, hevin (which shares more than 60% homology with SPARC) and osteopontin. Deletions of neither protein seemed to affect the IOP compared with their wild-type counterparts.[28-30] These results support our hypothesis that SPARC is specifically involved in IOP regulation relative to other matricellular proteins.

We have presented preliminary evidence showing that overexpression of SPARC by TM cells increases IOP in perfused human anterior segments isolated from non-glaucomatous eyes.[31] This elevation of IOP coincides with an increase in fibronectinas well as collagens I and IV within the JCT region. This effect is mediated by TM cells. Further work will be directed toward elucidating in more detail how SPARC modulates ECM homeostasis.

We are also attempting to clarify the regulatory mechanisms that lie upstream of SPARC. We have presented preliminary evidence identifying the specific signaling pathways that underlie the effects of TGF-β2 on SPARC.[26,32]

Our initial work with SPARC-null mice implicates an increase in aqueous drainage as the physiologic mechanism of the lower IOP, and we are working to clarify this observation. Most recently, by injecting fluorescent microbeads into the anterior chambers of SPARC-null and wild-type mice, we have found preliminary evidence that the transgenic deletion of SPARC results in outflow over a greater area of the TM than the typical segmental outflow.[33] SPARC overexpression may somehow alter the distribution of outflow channels and thus change the cross-sectional area available for outflow.

An understanding of the molecular pathogenesis of POAG will be critical to researchers' ability to devise new and more targeted treatment strategies in the coming years. A greater knowledge of matricellular protein function may help elucidate potential therapeutic targets for IOP reduction. ■

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1. Larsson LI, Rettig ES, Brubaker RF. Aqueous flow in open-angle glaucoma. *Arch Ophthalmol*. 1995;113(3):283-286.
2. The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. The AGIS Investigators. *Am J Ophthalmol*. 2000;130(4):429-440.
3. Heijl A, Leske MC, Bengtsson B, et al. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Arch Ophthalmol*. 2002;120(10):1268-1279.
4. Allingham RR, Liu Y, Rhee DJ. The genetics of primary open-angle glaucoma: a review. *Exp Eye Res*. 2009;88(4):837-844.
5. Pederson JE, Gaasterland DE, MacLellan HM. Uveoscleral aqueous outflow in the rhesus monkey: importance of uveal reabsorption. *Invest Ophthalmol Vis Sci*. 1977;16(11):1008-1017.
6. Bill A. Editorial: the drainage of aqueous humor. *Invest Ophthalmol*. 1975;14(1):1-3.
7. Seiler T, Wollensak J. The resistance of the trabecular meshwork to aqueous humor outflow. *Graefes Arch Clin Exp Ophthalmol*. 1985;223(2):88-91.
8. Knepper PA, Farbman AI, Telser AG. Exogenous hyaluronidases and degradation of hyaluronic acid in the rabbit eye. *Invest Ophthalmol Vis Sci*. 1984;25(3):286-293.
9. Barany EH, Scotchbrook S. Influence of testicular hyaluronidase on the resistance to flow through the angle of the anterior chamber. *Acta Physiol Scand*. 1954;30(2-3):240-248.
10. Bradley JM, Vranka J, Colvis CM, et al. Effect of matrix metalloproteinases activity on outflow in perfused human organ culture. *Invest Ophthalmol Vis Sci*. 1998;39(13):2649-2658.
11. Keller KE, Aga M, Bradley JM, et al. Extracellular matrix turnover and outflow resistance. *Exp Eye Res*. 2009;88(4):676-682.
12. Fuchshofer R, Tamm ER. Modulation of extracellular matrix turnover in the trabecular meshwork. *Exp Eye Res*. 2009;88(4):683-688.
13. Liton PB, Gonzalez P, Epstein DL. The role of proteolytic cellular systems in trabecular meshwork homeostasis. *Exp Eye Res*. 2009;88(4):724-728.
14. Tian B, Gabelt BT, Geiger B, Kaufman PL. The role of the actomyosin system in regulating trabecular fluid outflow. *Exp Eye Res*. 2009;88(4):713-717.
15. Overby DR, Stamer WD, Johnson M. The changing paradigm of outflow resistance generation: towards synergistic models of the JCT and inner wall endothelium. *Exp Eye Res*. 2009;88(4):656-670.
16. Ueda J, Wentz-Hunter K, Yue BY. Distribution of myocilin and extracellular matrix components in the juxtacanalicular tissue of human eyes. *Invest Ophthalmol Vis Sci*. 2002;43(4):1068-1076.
17. Tektas OY, Lutjen-Drecoll E. Structural changes of the trabecular meshwork in different kinds of glaucoma. *Exp Eye Res*. 2009;88(4):769-775.
18. Knepper PA, Miller AM, Choi J, et al. Hypophosphorylation of aqueous humor sCD44 and primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*. 2005;46(8):2829-2837.
19. Yan Q, Clark JI, Sage EH. Expression and characterization of SPARC in human lens and in the aqueous and vitreous humors. *Exp Eye Res*. 2000;71(1):81-90.
20. Rhee DJ, Fariss RN, Brekken R, et al. The matricellular protein SPARC is expressed in human trabecular meshwork. *Exp Eye Res*. 2003;77(5):601-607.
21. Tomarev SI, Wistow G, Raymond V, et al. Gene expression profile of the human trabecular meshwork: NEIBank sequence tag analysis. *Invest Ophthalmol Vis Sci*. 2003;44(6):2588-2596.
22. Vittal V, Rose A, Gregory KE, et al. Changes in gene expression by trabecular meshwork cells in response to mechanical stretching. *Invest Ophthalmol Vis Sci*. 2005;46(8):2857-2868.
23. Haddadin RI, Oh DJ, Kang MH, et al. SPARC-null mice exhibit lower intraocular pressures. *Invest Ophthalmol Vis Sci*. 2009;50(8):3771-3777.
24. Ochiai Y, Ochiai H. Higher concentration of transforming growth factor-beta in aqueous humor of glaucomatous eyes and diabetic eyes. *Jpn J Ophthalmol*. 2002;46(3):249-253.
25. Oh D, Peck R, Martin J, et al. Effects of dexamethasone and TGF-β2 on SPARC expression in cultured human trabecular meshwork and ciliary body. Paper presented at: The ARVO Annual Meeting; May 1, 2006; Fort Lauderdale, FL.
26. Kang M, Oh D, Rhee D. Transforming growth factor β-2 up-regulates the expression of secreted protein, acidic and rich in cysteine (SPARC) through two distinct signaling pathways in human trabecular meshwork. Paper presented at: The ARVO Annual Meeting; May 4, 2011; Fort Lauderdale, FL.
27. Villarreal G Jr, Oh DJ, Kang MH, Rhee DJ. Coordinated regulation of extracellular matrix synthesis by the microRNA-29 family in the trabecular meshwork. *Invest Ophthalmol Vis Sci*. 2011;52(6):3391-3397.
28. Oh D, Kang M, Choi K, et al. Adenoviral gene transfer of SPARC to human trabecular meshwork elevates intraocular pressure. Paper presented at: The ARVO Annual Meeting; May 4, 2011; Fort Lauderdale, FL.
29. Chowdhury U, Bahler C, Hann C, et al. Additive effect of latanoprost-free acid and diazoxide, an ATP-sensitive potassium (KATP) channel opener, on outflow facility in human anterior segment culture. Paper presented at: The ARVO Annual Meeting; May 4, 2011; Fort Lauderdale, FL.
30. Oh D, Haddadin R, Rhee D. Thrombospondin-1 and -2 knockout mice have lower intraocular pressures. Paper presented at: The ARVO Annual Meeting; May 6, 2009; Fort Lauderdale, FL.
31. Rhee DJ. SPARC, a matricellular protein, regulates intraocular pressure in experimental systems. Paper presented at: The AGS Annual Meeting; March 3, 2012; New York, NY.
32. Kang M, Oh D, Chatterjee A, Rhee D. Testican-1 (SPOCK-1) is differentially expressed in human ciliary body and trabecular meshwork. Paper presented at: The ARVO Annual Meeting; May 8, 2012; Fort Lauderdale, FL.
33. Swaminathan S, Oh D, Kang M, et al. Modulation of aqueous humor resistance and outflow by SPARC. Paper presented at: The ARVO Annual Meeting; May 5, 2011; Fort Lauderdale, FL.