Understanding Trabecular Meshwork Outflow

Its role in modulating aqueous humor outflow and IOP.

BY SWARUP S. SWAMINATHAN; DONG-JIN OH, PhD; MIN HYUNG KANG, PhD; RAMEZ I. HADDADIN, MD; GUADALUPE VILLARREAL Jr, MD; MARC TÖTEBERG-HARMS, MD; AYAN CHATTERJEE; AND DOUGLAS J. RHEE, MD

Aqueous humor outflow occurs through two routes, the conventional and uveoscleral pathways. The conventional pathway is responsible for approximately 85% of aqueous outflow. The primary constituent is the trabecular meshwork (TM), which consists of seven to eight layers of cellular beams surrounded by extracellular matrix (ECM) in human eyes. The aqueous humor traverses the superficial portions of the TM, known as the corneoscleral TM, to reach the juxtaocular connective tissue (JCT) TM, which is an amorphous layer of cells interspersed amongst ECM. The aqueous humor subsequently passes into Schlemm canal and enters the episcleral venous system.

The TM overall and especially the JCT TM are the anatomic location of the highest amount of outflow resistance in the conventional pathway. TM outflow is primarily mediated by two forces: (1) alterations in the ECM-surrounding cells and (2) alterations within the cells of TM and Schlemm canal’s inner wall that modulate cellular contractility and tension.

PARACELLULAR OUTFLOW
Numerous studies have demonstrated the impact of increased cellular stiffness on outflow resistance—specifically, an elevation in the number of actin stress fibers, actomyosin fiber bundles, and cross-linked actin

networks within the cells of the TM and inner wall of Schlemm canal.\textsuperscript{4,5} Multiple compounds inhibit or hinder such changes. Latrunculins, molecules that disrupt actin filaments, cause TM cells to shrink and retract, thereby decreasing IOP.\textsuperscript{6} Ethacrynic acid, a compound that inhibits the formation of cytoskeleton, alters cellular shape by disrupting actin networks, leading to greater aqueous flow around cells and reducing IOP.\textsuperscript{7} The intracellular enzyme Rho regulates these intracellular changes in addition to alterations in cell-ECM interactions.\textsuperscript{5,8,9} If either Rho or a related enzyme, Rho kinase, is inhibited, TM cells relax significantly, increasing outflow and decreasing IOP (Figure 1).\textsuperscript{5,10} This discovery led to the development of Rho kinase inhibitors, a novel class of glaucoma medications currently being evaluated in clinical trials. If approved, these drugs will be the first to act directly on the TM to increase aqueous outflow since the release of pilocarpine in the 1870s.

**ALTERING ECM**

Changes in the ECM may also alter aqueous outflow. According to multiple research groups, ECM or ECM-modulating proteins appear to be crucial to mediating outflow. For example, gremlin mediates the effects of transforming growth factor-ß2 (TGF-ß2) on ECM deposition,\textsuperscript{11} whereas cochlin is an ECM protein that appears to sense shear stress in the TM.\textsuperscript{12} Myocilin influences interactions between TM cells and surrounding ECM,\textsuperscript{13} and sFRP-1 indirectly modulates ECM protein synthesis.\textsuperscript{14} Matricellular proteins are secreted and modulate ECM organization and the interaction between TM cells and the ECM. This family of proteins includes SPARC and thrombospondin-1 and 2, which have essential roles in the regulation of IOP and aqueous outflow.\textsuperscript{15,16} Both proteins appear to affect collagen fiber formation, which may alter the nature of the ECM around TM cells and change outflow.

Other ECM-related alterations in glaucomatous eyes have been found. TGF-ß2, an essential molecule promoting tissue growth, is elevated in the aqueous humor of glaucomatous eyes.\textsuperscript{17,18} Studies have demonstrated that, when TGF-ß2 is overexpressed in cadaveric human eyes, ECM deposition increases within the TM and reduces the amount of aqueous outflow through the TM.\textsuperscript{19,20} Various proteins, including connective tissue growth factor and SPARC, are thought to mediate the TGF-ß2–driven increase in ECM.\textsuperscript{21,22} In addition, protein aggregates known as *sheath-derived plaques* have been found in the JCT TM of glaucomatous eyes.\textsuperscript{23} These aggregates contain several ECM proteins, includ-
ing elastin, collagen, and various proteoglycans. Studies have also shown that either increasing ECM production or decreasing ECM degradation increases IOP. When SPARC is overexpressed in perfused human eyes, the concentration of certain metalloproteinases (which catalyze the enzymatic degradation of ECM proteins) is decreased, whereas their inhibitors are upregulated.24 Metalloproteinases also play a role in reducing IOP via ECM degradation when activated by the adenosine receptor.25 Agonist compounds for this receptor are currently in clinical trials as potential therapeutic agents.

Aqueous outflow does not occur consistently throughout all 360° of the TM. Rather, outflow occurs only in certain sections of the TM, a concept referred to as **segmental flow**.16,26,27 In mice lacking SPARC, IOP is decreased, and a reduction in IOP is correlated with an increase in the amount of area utilized for outflow (Figure 2).16,28 It appears as though the greater the available area for outflow through the TM, the lower the IOP. Segmental flow may explain why multiple iStent Trabecular Micro-Bypass Stents (Glaukos Corporation) are often required to achieve a substantial reduction in IOP in glaucomatous eyes.

**FINAL THOUGHTS**

The TM has been the recent focus of surgical innovation such as ab interno trabeculotomy (Trabectome; NeoMedix Corporation) and the iStent. Results thus far have been limited. Further elucidating the mechanisms of TM outflow such as segmental outflow will be essential to identifying the pathophysiologic basis of primary open-angle glaucoma as well as to increasing the success rates of TM bypass procedures. Although investigators have begun to explain outflow physiology in the nonglaucomatous eye, the molecular pathways responsible for the pathologic changes leading to ocular hypertension and subsequent glaucoma remain elusive. Numerous research groups, including the authors’, have aimed at therapeutically inhibiting this disease process. There will be more soon!

Ayan Chatterjee is a medical student at the Perelman School of Medicine at the University of Pennsylvania in Philadelphia.

Ramez I. Haddadin, MD, is a cornea fellow at the Massachusetts Eye and Ear Infirmary in Boston.

Min Hyung Kang, PhD, is a research scientist at Case Western Reserve University in Cleveland.

Dong-Jin Oh, PhD, is an assistant professor at Case Western Reserve University in Cleveland.

Douglas J. Rhee, MD, is the chair of the Department of Ophthalmology and Visual Sciences at Case Western Reserve University in Cleveland. He is an ad hoc consultant to AqueSys, Inc., and Glaukos Corporation. Dr. Rhee may be reached at (216) 844-8590; doug-rhee@aol.com.

Swarup S. Swaminathan is a medical student in the Harvard-Massachusetts Institute of Technology Division of Health Sciences & Technology at Harvard Medical School in Boston.

Marc Töteberg-Harms, MD, is a clinical and basic research glaucoma fellow at Case Western Reserve University in Cleveland.

Guadalupe Villarreal Jr, MD, is a resident at the Wilmer Eye Institute of Johns Hopkins University in Baltimore.

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