The Cell and Molecular Biology of Glaucoma: Mechanisms in the Conventional Outflow Pathway

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Glaucoma, a blinding disease that affects millions in the United States, is treatable. Multiple clinical trials involving thousands of patients have repeatedly shown that lowering intraocular pressure (IOP) saves vision in those with glaucoma. In fact, if IOP in those with glaucoma is lowered sufficiently, vision loss dramatically slows—nearly stopping.

IOP is a function of the balance of fluid (aqueous humor) entering and leaving the eye. Although there are very good medications that lower IOP by decreasing fluid production by the inflow pathway and increasing fluid egress via the secondary outflow pathway, unfortunately there is not a daily medication that enhances outflow through the primary outflow route: the conventional pathway.

The conventional outflow pathway is the tissue that becomes diseased and is responsible for ocular hypertension (elevated IOP) in those with glaucoma. Consequently, persons with glaucoma have elevated IOP and higher resistance to outflow than do those of similar age without glaucoma. Although this extra resistance to outflow can be removed surgically to bypass the dysfunctional tissue, a primary focus of glaucoma research is to understand the molecular and cellular mechanisms that control resistance, so that medications can be developed that can lower resistance and return function to the conventional outflow pathway.

The conventional (or trabecular) outflow pathway has a fascinating design (Fig. 1). Fluid flow through the tissue and out of the eye is driven by a pressure gradient across the tissue. Thus, the pressure difference inside (IOP) and outside (episcleral venous pressure [EVP]) the eye moves aqueous humor through an area that functions like a filter positioned in front of a region that acts like a resistor. The cell surfaces in the innermost parts of the trabecular meshwork (TM) tissue act like a filter, removing cell debris, pigment, and reactive oxygen species from aqueous humor before it reaches (and has the opportunity to clog) the resistant part of the pathway located near the inner wall of Schlemm’s canal (SC), called the juxtacanalicular (JCT) region.

Resistance to outflow is generated in the JCT region of the conventional pathway and most likely involves a funneling mechanism. Hence, resistance due to funneling is a function of two parameters: (1) spacing in the JCT due to the interaction between TM cells and inner wall cells of SC and (2) the number of openings (pores) in the inner wall. The theory is that as fluid moves through the TM and approaches the inner wall, it is funneled toward the pores in the inner wall, creating a bottleneck that generates resistance and thus IOP.

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Molecular and Cellular Targets in the Conventional Outflow Pathway

If most of the fluid leaves the eye by way of (and pathology resides in) the conventional outflow pathway, then why is there no daily medication for glaucoma patients that targets conventional outflow? The simple answer is that the conventional outflow system is complicated, with many backup and compensatory mechanisms, keeping IOP within a very narrow range (usually, <2 mm Hg) in most people throughout life. Moreover, it appears that autoregulatory systems in the conventional pathway are geared toward preventing IOP from descending below a critical level, no matter how effective current medications affect inflow and the secondary outflow route in glaucoma patients. Such a design is probably due to a biological tolerance for elevated IOP, but not for reduced IOP, which may destabilize the ocular structures from the visual axis of the eye and disrupt vision.

Why Is a Conventional Outflow Medication Needed?

Plainly, current medications do not lower IOP sufficiently in most people with glaucoma. Thus, having a medication directed toward an underutilized target (conventional outflow) offers the opportunity for added IOP-lowering beyond levels of existing medications. Because nearly all the fluid leaves the eye through the conventional route (up to 90% in older adults), this new class of medication has the potential to lower IOP dramatically. Moreover, the development of medications that target the disease process in the conventional outflow tissue (responsible for elevated IOP) may restore function by increasing fluid flow to cells, stimulating cell division and tissue repair. Compare such a treatment strategy to current medications, which either reduce aqueous humor production or shunt aqueous humor away from the diseased conventional outflow pathway, starving an already compromised tissue of nutrients. An added benefit to restored function of the conventional outflow tissue may be the dampening of the IOP spikes (due to its pressure-dependent nature) that naturally occur throughout the day and are damaging to nerves in the retina.

A less resistant conventional outflow pathway means that IOP will return to normal more quickly after a transient pressure insult, such as eye rubbing, waking up in the morning, or squinting.

How Can Conventional Outflow Be Increased and Function Restored?

Understanding the molecular and cellular mechanisms that control outflow resistance (increase outflow facility) will reveal targets susceptible to drug action. Accordingly, the discovery that mutations in myocilin result in decreased conventional outflow, elevated IOP, and glaucoma (~4% of total) implies that a critical molecular pathway is in control of conventional...
outflow facility. Unfortunately, the function of myocilin and the molecular pathway through which it operates are unknown. The lack of this information is a major barrier that must be remedied to uncover the critical molecular pathway that is affected by myocilin dysfunction and in turn affects outflow facility and thus IOP. In any case, at least four different general ways are known to increase conventional outflow: The first is to decrease the volume of resident cells; the second is to increase the degradation rate of the extracellular matrix (ECM); the third is to inhibit the contraction of resident cells; and the fourth is to disrupt the funneling effect in the JCT.

Cell Volume

Decreasing the volume of cells that populate the JCT region of the conventional outflow pathway, where passageways for fluid egress are narrow (1–5 μm) is one way to increase outflow facility. Thus, experimentally perfusing the outflow pathway of donor eyes in the laboratory with hypertonic medium pulls water out of cells, decreases their volume, widens flow pathways, and increases outflow facility. Similarly, if Na/K/2Cl transporter activity is inhibited upon addition of bumetanide or by removing chloride from the perfusion medium, cell volume decreases and outflow facility increases. Finally, perfusion of eyes with drugs that activate the BK(Ca) channel also increases outflow facility, probably by decreasing the volume of outflow cells.

Contractility

TM tissue and cells of the conventional outflow pathway are under constant tension. Hence, contraction of TM cells results in decreased outflow facility, whereas relaxation of the cells has an immediate and opposite outcome. Maximum effects of TM relaxation on outflow facility can be observed after treatment with actin-depolymerizing drugs such as latrunculin. Robust, but more modest, effects on outflow facility are observed after treatment of outflow cells with drugs that target part of the contractile machinery, such as rho kinase, an enzyme that regulates cellular contraction. TM contractility can also be modified by cell surface receptor activation. Thus, activation of S1P2 receptors contracts the TM and decreases outflow facility, whereas stimulation of prostaglandin EP4 or FP receptors (or antagonizing S1P2 receptors) relaxes the TM and increases outflow facility. Significantly, pharmaceutical companies are currently pursuing commercialization of drugs that target the contractile nature of the conventional outflow tissues, including drugs that inhibit rho kinase.

Extracellular Matrix

ECM material in flow pathways is thought to be essential for the generation of outflow resistance that induces IOP. The importance of the ECM in outflow facility regulation was initially demonstrated after perfusion of human eyes with purified enzymes that degrade the ECM and increase outflow facility—a finding that has clinical relevance to the primary mechanism of action of laser trabeculoplasty (the first-line invasive treatment for elevated IOP in glaucoma). Thus, laser trabeculoplasty results in the generation of the inflammatory mediators IL-1β and TNFα, which work synergistically to increase production of matrix-degrading enzymes (metalloproteinases) by TM cells to increase outflow facility. Similarly, the first-line medical treatments for elevated IOP, prostaglandin F2 drugs, owe a minor portion of their effects to alterations in the ECM of the conventional outflow. (Their primary site of action is the secondary pathway.) Medications are currently in development that target receptors that affect ECM turnover in the conventional pathway, such as adenosine A1 agonists.

Although alterations in TM contractility result in immediate effects on outflow facility, changes in the ECM composition of the conventional pathway gradually influence outflow facility over time. The slow onset is most likely due to the time necessary to change the expression profile for ECM materials and enzymes and the time needed for remodeling to occur. For example, perfusion of human eyes with media containing the cytokine TGFβ2 (elevated in the aqueous humor of glaucoma patients) results in a gradual, but sizable, decrease in outflow facility (increased IOP), requiring 5 days to reach maximum consequence. Similar effects on outflow facility are observed 2 days after treatment of human eyes with soluble frizzled-related protein (sFRP). Since sFRP is a physiological antagonist to the Wnt signaling pathway, these results suggest (1) that an endogenous level of Wnt molecules is present in conventional outflow tissue, regulating outflow facility, and (2) that stimulation of the Wnt pathway may increase outflow facility.

In addition to the generation of resistance to total outflow, the ECM appears to control patterns of fluid flow through the conventional tissues. Flow patterns through the conventional outflow pathway are nonuniform, with some areas having high flow and others having limited flow. Thus, there appears to be a reserve available to increase total outflow. Recent data support the idea that there is a mechanism that alters flow patterns and tissue usage based on demand. Results show that passageways for aqueous humor in the conventional outflow tissue change dynamically, probably because of focal areas of matrix enzyme activity mediated by podosomes and invadopodia.

Funneling

Another way that conventional outflow facility can be increased is by interference with the funneling mechanism that creates resistance in the JCT. As mentioned before, the two parameters that are essential for proper funneling and thus are targets to disrupt it are (1) the separation distance between TM cells in the JCT and the inner wall of SC and (2) the number of pores in the inner wall. Relaxation of the TM may increase outflow facility by increasing the separation distance between the TM and the inner wall of SC and increasing access of the aqueous humor to the inner wall. For example, treatment of
eyes with the kinase inhibitor H7 results in a relaxed TM, disappearance of funneling flow patterns (indicated by gold tracer), and increased outflow facility. Another way to disrupt funneling is by changing the number of pores in the inner wall. Thus, blocking pores at the inner wall with a flow tracer, like cationic ferritin, causes a dramatic and immediate decrease in outflow facility. Alternatively, opening more pores with drugs that induce disassembly of cell–cell junctions of the inner wall of SC may also disrupt funneling. Hence, nitric oxide likely increases outflow facility by increasing openings in the inner wall but also by its effects on relaxation of the TM.

**SUMMARY: KEY NEEDS AND OPPORTUNITIES**

Although the four processes that affect conventional outflow have been separated in this article for simplicity of presentation, physiologically they are interdependent. For example, the amount and type of ECM in the conventional outflow pathway affects the contractility status of the TM cells. Similarly, proper funneling is dependent on spacing parameters in the JCT that are affected by ECM content, TM cell contractility, and cell volume. Although the regulation of outflow facility is complicated, we have made great strides in better understanding the molecular and cellular processes that establish a set point for IOP and now have several molecular candidates for drug development. That said, we still have more to learn before medications can be developed for glaucoma patients that (1) clear debris that has accumulated in the conventional outflow pathway over time and (2) restore function to the tissue that dampens transient IOP spikes and establishes a new, lower IOP level for the long term.

**References**