

## New Insights Into Pathophysiological Mechanisms Regulating Conventional Aqueous Humor Outflow

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**Key Words:** *intraocular pressure; trabecular meshwork; conventional aqueous humor pathway.*

**Summary.** *The aim of the article was to overview the pathophysiology of the conventional outflow pathway, trabecular meshwork, and intraocular pressure and to discuss the options of future glaucoma treatment directed to improvement in aqueous outflow. The literature search in the Medline, Embase, and Cochrane databases from April to May 2012 was performed; a total of 47 articles analyzed. The diminished conventional pathway may be altered by several pathophysiological mechanisms like TM obstruction caused by transforming growth factor- $\beta$ 2, clastic nondeformable cells, macrophages leaking from hypermature cataract, iris pigment, lens capsular fragments after YAG-laser posterior capsulotomy, proteins and their subfragments. It is known that trabecular meshwork contraction reduces outflow, and the actomyosin system is directly linked to this mechanism. New glaucoma drugs are still under investigation, but it is already proven that agents such as latrunculin-B are effective in improving aqueous drainage. Selective Rho-associated coiled coil-forming protein kinase inhibitors have been shown to cause a significant improvement in outflow facility and may become a new option for glaucoma treatment. Caldesmon negatively regulates actin-myosin interactions and thus increases outflow. Stem cells may replace missing or nonfunctional trabecular meshwork cells and hopefully will bring a new treatment solution.*

*Pathophysiological mechanisms regulating conventional aqueous humor outflow are still not fully understood and require further investigations. Future treatment decisions should be directed to a specific mechanism regulating an elevation in intraocular pressure.*

### Introduction

Intraocular pressure (IOP) is the main risk factor in the prevalence, incidence, and progression of glaucoma (1). Glaucoma is the second leading cause of blindness after cataract and the third cause of visual impairment following refractive errors and cataract worldwide (2). Appropriate secretion and regulation of aqueous humor is essential for normal eye function. Increased aqueous humor secretion and an abnormal outflow rate generate increased IOP – the only known treatable risk factor for glaucoma.

At the current stage, most drugs to treat glaucoma are designed to decrease aqueous formation or increase uveoscleral outflow. However, conventional and main aqueous outflow occurs via the trabecular meshwork (TM) and the Schlemm's canal (SC). An elevation in IOP is directly related to increased resistance in aqueous humor outflow, which in turn is related to increased TM stiffness (3). Other mechanisms such as TM and SC contractility and obstruction, which might interfere with aqueous humor outflow, are not fully understood. Comprehensive knowledge about aqueous humor outflow including histological and cellular researches of outflow pathways is important for the development of new

glaucoma drugs.

The aim of this article is to overview the reasons leading to the disturbances of aqueous humor outflow and to discuss the options of future treatment.

### Outflow Pathways

The conventional drainage route of aqueous humor occurs via the TM, SC, collector channels, and aqueous veins and then streams into episcleral venous circulation. The TM is divided into uveal, corneoscleral, and juxtacanalicular meshwork (4). The TM consists of collagen beams, which are covered by endothelial cells, and spaces between beams are filled with the extracellular matrix (ECM) (5). TM cells are unique and perform a variety of functions, including phagocytosis, migration, elaboration of metabolic, lysosomal, and matrix-degrading enzymes, and production of ECM elements. They are essential for the maintenance of the normal aqueous humor outflow system (6). Disturbances in vitality and the functional status by genetic predisposition, aging, or other alterations in the TM may result in the obstruction of aqueous outflow, leading to IOP elevation and glaucoma (7). Fluid via the unconventional route penetrates between ciliary muscle bundles, suprachoroidal space and leaves the eye through the sclera. Uveoscleral outflow is relatively IOP independent in contrast to trabecular outflow, except when very low pressures are present. Some researchers consider uveoscleral outflow analogous

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to the lymphatic drainage of tissue fluid in other organs, since the fluid may be drawn osmotically into the veins and may mix with tissue fluid from the ciliary muscle, ciliary processes, and the choroid (4). However, according to Yücel, there is a separate “uveolymphatic” pathway, and it is possible that prostaglandin analogues lower IOP through this pathway (8).

#### ***Influence of Age on Trabecular Meshwork***

Normally TM endothelial cellularity diminishes with aging, while in primary open-angle glaucoma (POAG) patients, cell death is considered to be an early event (9). The glaucomatous TM has a different structure: it has a decreased cell number (10) and an increased amount of ECM and tends to form plaque-like substances (11). Grierson and Howes estimated that the whole meshwork contained 763 000 cells at the age of 20, and this number decreased to 403 000 cells by the age of 80 (12). The mechanism of cell reduction is still not fully understood, but possibly phagocytosis, cell migration, cell death, or wear-and-tear mechanisms could determine changes in cellularity (13). Baleriola et al. reported a tendency for more apoptotic cells to accumulate in the TM in patients with POAG than in patients with primary angle-closure glaucoma. Their observations suggested that apoptosis could be one of the mechanisms by which trabecular cells die in case of glaucoma (14). Rohen et al. stated that trabecular hypoperfusion due to glaucoma could cause TM cell apoptosis (15). Grierson et al. found that cell death could be caused by mechanical stress (16). Morphologic studies have also revealed thickened basement membranes and the accumulation of plaque-like material blocking the extracellular space in the TM and long spacing collagens in the TM of aged eyes (17, 18). Lütjen-Drecoll et al. found that plaque-like material was unequally distributed in the SC, and IOP alone probably was not responsible for new plaque formation (11). Gonzalez et al. noticed that mechanical stresses, oxidative challenges, or other insults and injuries may also contribute to the dysfunction of TM or cell death (19). Enzymes like catalase, glutathione reductase, superoxide dismutase, and glutathione peroxidase protect against oxidative stress and are found in the TM. It has been reported that in TM tissue, the activity of specific superoxide dismutase declines with aging, but not the activity of catalase (20). However, Russel and Johnson did not find such an age-related change (21). TM cells also synthesize a specific set of proteins, such as  $\alpha\beta$ -crystallines, that may act as molecular chaperones to prevent oxidative or heat shock damage (22). Oxidative stress that exceeds the capacity of TM cells for detoxification is involved in damaging the cells and alteration of aqueous humor outflow (23). According to Mochizuki et al., oxidative stress stabilizes cytokine IL-6

and IL-8 mRNA in human TM cells, which may contribute to the progression of glaucoma (24).

#### ***Trabecular Meshwork Outflow Obstruction***

There is an increase of ECM in the inner wall of the SC and in the cribriform region of the TM, leading to the thickened TM lamellae in eyes with glaucoma (11, 25). These processes can influence the passage of the flow or partly obliterate the SC. Transforming growth factors TGF- $\beta$ 1 and TGF- $\beta$ 2 are constituents of human aqueous humor, where they are largely present in a latent form that needs activation. Thrombospondin-1 is an ECM matrix-cellular protein, which activates a latent TGF- $\beta$  form. These growth factors induce the expression of  $\alpha$ -smooth muscle actin and actin stress fibers in cultured trabecular meshwork cells (26). Presumably, this determines a contractile state. It is also known that TGF- $\beta$ 2 can increase the production of ECM in the TM and possibly elevates IOP. According to several studies, TGF- $\beta$ 2 is increased in eyes with glaucoma (27, 28) and thus may reduce outflow facility (29). This factor decreases the activity of matrix metalloproteinases (MMPs), in this way possibly increasing ECM quantity in the TM (30). Borisuth et al. mentioned that TGF  $\beta$ 2 acted like an inhibitor of the proliferation and motility of TM cells in vitro and hereby decreased the cellularity of the TM (31). Gottanka et al. added that TGF  $\beta$ 2 was found in the inner SC wall as well (29). According to Bhattacharya et al., TGF  $\beta$ 2 enhances the production of cochlin, but its function in the TM is still unknown (32). It has been confirmed that cochlin is present only in the glaucomatous tissues and is important in early events and even may lead to glaucoma. Recent data have suggested that cochlin contributes to elevated IOP in POAG (33, 34) by altering physiological mechanisms within the TM such as cell aggregation, mucopolysaccharide-associated deposition and obstruction of the aqueous humor circulation (35). Endothelial TM cells produce mucopolysaccharides that are important constituents of the ECM. Mucopolysaccharides control several functions of macrophages. Abnormal mucopolysaccharide levels disrupt the self-cleaning process resulting in large changes in aqueous humor outflow and, subsequently, in the elevation of IOP (36).

TM cells act like a self-cleaning filter and have a “cleaning” function as well. In most POAG cases, this function is insufficient or inadequate (37). Normally erythrocytes and other deformable cells can easily pass through the TM, but clastic or sickled nondeformable cells could be trapped. Moreover, TM obstruction can be caused by leaking macrophages from hypermature cataract, spontaneously liberated pigment from iris or iatrogenically after laser iridotomy, lens capsular fragments after YAG-laser posterior capsulotomy (38), and viscoelastic ma-

terials after intraocular surgery. Proteins and their fragments can also obstruct TM, but they are essential for maintaining the normal TM resistance (39).

#### **Other Reasons of Increased Trabecular Outflow Resistance**

It is known that the TM has contractile properties, and the contraction of TM is associated with reduced outflow (5). Tian et al. stated that outflow resistance was influenced by the contractility of the actomyosin system in TM cells or inner wall endothelium of the SC (5). The actomyosin system consists of actin microfilaments with associated proteins. It has a definite structure in TM and SC cells. TM cells exhibit 3 major types of cytoskeletal elements: actin filaments, microtubules, and intermediate filaments, vimentin and desmin (40). The adhesion to the ECM or neighboring cells has multiple effects on cell shape, volume, and contractility (5). These changes in TM and/or SC cells can alter outflow by increasing resistance; in addition, the amount and composition of ECM can be changed. Rao et al. and Rosenthal et al. reported the TM to be a smooth muscle-like tissue (41, 42). The contraction and relaxation of smooth muscle are known to be regulated through the phosphorylation of myosin light chain (MLC) and controlled by MLC kinase and phosphatase. MLC phosphorylation promotes cellular contraction while dephosphorylation leads to relaxation. Tian et al. reported that changes in TM cells can be modulated directly by actin-disrupting agents or indirectly by the inhibition of specific protein kinases (5). Changes in the actin cytoskeleton may also result in cell swelling or shrinkage (43). Relaxation of the TM and the SC increases the area available for fluid drainage.

#### **New Treatment Options**

**Latrunculins.** Recent studies have showed that potent actin-disrupting agents like latrunculins sequester monomeric G-actin and can lead to the massive destruction of filamentous actin and the disorganization of cytoskeleton in the TM and inner wall cells of the SC (44, 45). There are 2 common types of latrunculins: A and B (LAT-A and B). Peterson et al. reported that LAT-B was 10 times more potent in reducing outflow resistance than LAT-A (46, 47) and had fewer and milder adverse effects (48). Electron microscopy of live monkey eyes indicated that LAT-B expanded the juxtacanalicular space, increased drainage surface and enlarged volume, which may be helpful for a drug to reduce outflow resistance. The expansion of the juxtacanalicular space is probably due to inhibition of the TM cell contractility by LAT-B (49). Sabanay et al. suggested that the effect of LAT-B on the TM might be more significant in the glaucomatous eyes with elevated IOP comparing to healthy eyes. Since LAT-B effectively improves

drainage and does not significantly alter the corneal endothelium, it could be used as potential future antiglaucomatous therapy (49). However, Tian et al. mentioned about possible corneal toxicity of LAT-B at higher concentrations (5).

**Protein Kinase Inhibitors.** Small guanosine 5'-triphosphatases (GTPases) of the Rho family are important regulators of actin cytoskeletal dynamics (50). Rho kinase stimulates myosin II activity by MLC inhibition and myosin regulatory light chain phosphorylation. This makes actomyosin bundles to contract and generate strong tensile forces (51). A Rho kinase inhibitor (Y-27632) promotes reversible cell shape changes and causes a decrease in actin stress fibers, focal adhesions, and protein phosphotyrosine staining in human TM and SC cells (52). A specific inhibition of Rho kinase activity in the TM by dominant negative Rho expression increases outflow facility in organ cultured human eye anterior segments (53). Recently, Kameda et al. have described a disrupting effect of Rho-associated coiled coil-forming protein kinase (ROCK) inhibitor, Y-27632, on protein tight junctions and thus a positive effect on outflow (54). A clinical study performed by Tanihara et al. with 45 healthy volunteers demonstrated that the IOP-lowering efficacy of topical administration of a selective ROCK inhibitor (SNJ-1656) was significant (55). No systemic adverse events were observed, but transient ocular hyperemia occurred. Other authors are still arguing about possible ocular surface toxicity (55). Hyperemia may be the result of relaxation of the blood vessels because ROCK inhibition induces smooth muscle relaxation. Therefore, it is hoped that along with the existing pharmaceutical strategies to treat ocular hypertension, suitable ROCK inhibitors with adequate efficacy may become available to treat ocular hypertension and glaucoma (56, 57).

**Caldesmon.** Numerous studies have shown that the outflow facility is modified via alterations in cell-matrix and/or cell-cell adhesions (50). For example, Bill et al. have reported that the depletion of extracellular calcium, by dissociating cell-cell junctions, decreases outflow resistance (58). Caldesmon is a modulating protein that causes TM relaxation and negatively regulates the actin-myosin complex. If caldesmon is overexpressed, actin is detached from myosin, consequently focal adhesions are lost, and/or adherens junctions in TM cells are disrupted. Furthermore, the exoenzyme C3 transferase inhibits Rho-GTP and thus disrupts the actin-myosin complex, and hereby the Rho cascade is blocked (5). The development of new glaucoma drugs could be based on the mechanism of action of caldesmon.

**Stem Cells in Trabecular Meshwork.** Currently stem cells are under investigation because of promising future results. Stem cells could be used as a

replacement of differentiated old, damaged, or non-functioning cells (59). More than 30 years ago, Raviola identified unusual cells in the anterior end of the TM, beneath the Schwalbe's line (60). According to Kelly et al., this region containing "stem-like cells" was entitled as the insert area (61). Acotts et al. performed the study suggesting that the inserted cells served as a source for TM cell renewal indicating that they might be stem cells (62). More recent studies identified a new type of cells—novel cells. They differ in morphology, ultrastructure, and growth patterns from other TM or local cells (63). Cells described by Raviola, inserted and novel cells appeared to be the same cell type and have stem-like properties (60). Adult stem/progenitor cells might be cultivated ex vivo and then replaced instead of nonfunctional TM cells in glaucomatous eyes and hereby preventing further optic nerve damage (61). Hopefully, it could be a new solution in glaucoma management.

### Concluding Remarks

Currently, glaucoma treatment is focused on risk factors but not on the etiology and pathophysiology of the disease. Lately, researchers are trying to elucidate the reasons of the diminished conventional pathway. Several pathophysiological mechanisms, such as TM obstruction caused by TGF- $\beta$ 2, have been elucidated, which increases the production of

ECM and decreases the proliferation and cellularity of TM cells. Phagocytic TM activity is still under research and it is known to be associated with cell death. As noted before, increased TM outflow resistance is linked to TM contractile properties. Other pathophysiological mechanisms are still not fully understood and require further investigations for clarification. Studies have gathered significant evidence suggesting the effectiveness of ROCK inhibitors, which are a potential option for future treatment. Upcoming studies will show if caldesmon overexpression gives any benefits in gene therapy, but its positive effect on cytoskeletal regulation in TM cells has already been confirmed. Stem cells might replace missing or nonfunctional TM cells, which could provide long-term solution for glaucomatous patients. Future treatment decisions should be directed to a specific mechanism of IOP increase, and more potent ocular pharmacological agents should be chosen for individual patient's management.

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### Statement of Conflict of Interest

The authors state no conflict of interest.

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