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Biomechanics of Schlemm's canal endothelium and intraocular pressure reduction

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ABSTRACT

Ocular hypertension in glaucoma develops due to age-related cellular dysfunction in the conventional outflow tract, resulting in increased resistance to aqueous humor outflow. Two cell types, trabecular meshwork (TM) and Schlemm's canal (SC) endothelia, interact in the juxtacanalicular tissue (JCT) region of the conventional outflow tract to regulate outflow resistance. Unlike endothelial cells lining the systemic vasculature, endothelial cells lining the inner wall of SC support a transcellular pressure gradient in the basal to apical direction, thus acting to push the cells off their basal lamina. The resulting biomechanical strain in SC cells is quite large and is likely to be an important determinant of endothelial barrier function, outflow resistance and intraocular pressure. This review summarizes recent work demonstrating how biomechanical properties of SC cells impact glaucoma. SC cells are highly contractile, and such contraction greatly increases cell stiffness. Elevated cell stiffness in glaucoma may reduce the strain experienced by SC cells, decrease the propensity of SC cells to form pores, and thus impair the egress of aqueous humor from the eye. Furthermore, SC cells are sensitive to the stiffness of their local mechanical microenvironment, altering their own cell stiffness and modulating gene expression in response. Significantly, glaucomatous SC cells appear to be hyper-responsive to substrate stiffness. Thus, evidence suggests that targeting the material properties of SC cells will have therapeutic benefits for lowering intraocular pressure in glaucoma.

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1. Introduction

The elevated intraocular pressure (IOP) that is associated with primary open-angle glaucoma (POAG) is caused by an increased resistance to the outflow of aqueous humor from the eye through the conventional[2] outflow pathway (Grant, 1951). In spite of over 140 years of investigation (Leber, 1873), the precise cause of this increased outflow resistance remains elusive. Interestingly, most treatments for glaucoma focus on diminishing the rate of aqueous humor formation or altering the outflow path. These treatments lower IOP and thereby slow the progression of ganglion cell damage and associated vision loss, but in most cases do not stop it (Hattenhauer et al., 2000, 1998; Leske et al., 2003; Nouri-Mahdavi et al., 2004). Remarkably, there is currently no drug treatment in clinical use that directly targets the increased flow resistance that is a central characteristic of ocular hypertension in glaucoma, mainly because the mechanism(s) of increased flow resistance remain obscure.

Logically, the primary pathology underlying increased outflow resistance might be cellular, extracellular, or some combination of the two. Cellular contributions might include altered hydraulic conductivity of the endothelial lining of Schlemm's canal (SC), and extracellular contributions might include increased extracellular matrix in the juxtacanalicular tissue (JCT) or altered basement membrane beneath SC cells; however, comparisons of the outflow pathways of glaucomatous and age-matched normal eyes have found only subtle structural differences. Specifically, in glaucoma, there is an accelerated loss of trabecular meshwork (TM) cells that is limited to the inner region of the conventional outflow pathway (Alvarado et al., 1981, 1984) and cytoskeletal changes in the actin architecture of JCT-TM[3] and SC cells (Read et al., 2007). Additionally, there is an accumulation of a "sheath derived plaque material" in the JCT of glaucomatous eyes (Alvarado et al., 1986; Lutjen-Drecoll et al., 1981), but this accumulation has been shown to have negligible hydrodynamic consequence (Alvarado et al., 1986) (Murphy et al., 1992). There is little other morphological evidence of increased extracellular matrix or altered basement membrane composition in glaucomatous eyes compared to age-matched controls.

At the level of SC, however, several changes in glaucomatous eyes have been observed with the potential to be a significant contributor to the increased outflow resistance. The dimensions of the lumen of SC are smaller in glaucomatous eyes and these changes correlate with outflow resistance (Allingham et al., 1996). Herniations of the inner wall and JCT tissue into collector channels are more frequently observed in glaucomatous eyes than age-matched non-glaucomatous eyes (Gong et al., 2007; Hann et al., 2014). There is also a reduced pore density in the inner wall endothelium of SC comparing normal to glaucomatous eyes (Allingham et al., 1992; Johnson et al., 2002) that is potentially quite important. Collectively, these data point to dysfunction at the level of the inner wall of SC in glaucoma.

In this article, we review recent evidence that increased stiffness of SC endothelial cells is responsible for the elevated outflow resistance and IOP characteristic of glaucoma; we also present data showing that drugs that change cell stiffness also alter outflow resistance. By understanding the coupling between biomechanics and flow through the inner wall endothelium, we outline opportunities to exploit cell biomechanics as a targeted approach to reduce IOP at the site of outflow resistance regulation.

2. The inner wall endothelium of Schlemm's canal experiences a unique biomechanical environment

The typical pressure loading on vascular endothelia generates a pressure gradient in the *apical to basal* direction. The basement membrane and other tissues underlying vascular endothelia amply support the transcellular pressure drop generated by this gradient, and thus the vascular endothelial cells themselves do not have to support the associated radial and circumferential stresses. This is not the case for the endothelium of SC, where the SC cells themselves must support a "backwards" *basal to apical* pressure gradient associated with fluid flowing into the SC lumen, which tends to push SC cells off their supporting basement membrane (Fig. 1). While terminal or capillary lymphatics also are exposed to such an adverse pressure gradient, the endothelial linings of these lymphatics are not sealed completely by tight junctions (Raviola and Raviola, 1981; Swartz, 2001), and thus little pressure difference needs be supported by the lymphatic endothelial cells themselves (Ramos et al., 2007; Zweifach and Lipowsky, 1984).

Under physiological conditions, the basal-to-apical pressure drop between intraocular pressure and episcleral venous pressure deforms SC cells to create large dome-like outpouchings into the SC lumen, the so-called "giant vacuoles" (Fig. 1) (Holmberg, 1959). Despite this deformation, the inner wall endothelium remains continuous to preserve the blood-aqueous barrier that prevents plasma entry into the anterior chamber, helping maintain ocular immune privilege (Streilein, 1996). The SC cells appear to be particularly well adapted to function within this biomechanical environment. Forces arising from the pressure gradient are transmitted through the cell to neighboring cells and extracellular matrix via adhesive and elastic tethers (Grierson et al., 1978; Overby et al., 2009; VanderWyst et al., 2011). SC cells must support this transmitted load and do so primarily through the stiffness of their cytoskeleton and contractile machinery.

Importantly, SC cells are also highly contractile, and contraction of these cells causes a significant increase in their stiffness (Zhou et al., 2012). Remarkably, the dynamic range of contractility of an SC cell is similar to that of a smooth muscle cell (Zhou et al., 2012), ideally suited for a lively environment. However, the cytoskeletal architecture of SC cells is more similar to an endothelial than a

[2] There is also an unconventional outflow pathway that carries a fraction of the total aqueous humor outflow. While this pathway is important in the treatment of glaucoma (as an alternate outflow pathway), there is little evidence that it is involved in the pathogenesis of ocular hypertension characteristic of glaucoma.

[3] There are, at least, two morphologically and functionally distinct populations of TM cells in the conventional outflow tract. Beam TM cells are endothelial like cells that cover TM cords, lamellae and plates as monolayers, maintaining these structures and also filtering aqueous humor by phagocytosis as it enters the outflow tract. JCT-TM cells are fibroblastic appearing cells in the JCT, functioning to regulate extracellular matrix content and interact with inner wall of SC cells to regulate outflow resistance.