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Research article

Pressure-induced expression changes in segmental flow regions of the human trabecular meshwork

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ABSTRACT

Elevated intraocular pressure (IOP) is thought to create distortion or stretching of the juxtacanalicular and Schlemm's canal cells and their extracellular matrix (ECM) leading to a cascade of events that restore IOP to normal levels, a process termed IOP homeostasis. The ECM of the trabecular meshwork (TM) is intricately involved in the regulation of outflow resistance and IOP homeostasis, as matrix metalloproteinase (MMP)-initiated ECM turnover in the TM is necessary to maintain outflow facility. Previous studies have shown ECM gene expression and mRNA splice form differences in TM cells in response to sustained stretch, implicating their involvement in the dynamic process of IOP homeostasis. The observation that outflow is segmental around the circumference of the eye adds another layer of complexity to understanding the molecular events necessary to maintaining proper outflow facility. The aim of this work was to identify molecular expression differences between segmental flow regions of the TM from anterior segments perfused at either physiological or elevated pressure. Human anterior segments were perfused in an ex vivo model system, TM tissues were extracted and quantitative PCR arrays were performed. Comparisons were made between high flow and low flow regions of the TM from anterior segments perfused either at normal (8.8 mmHg) or at elevated (17.6 mmHg) perfusion pressure for 48 h. The results are presented here as independent sets: 1) fold change gene expression between segmental flow regions at a single perfusion pressure, and 2) fold change gene expression in response to elevated perfusion pressure in a single flow region. Multiple genes from the following functional families were found to be differentially expressed in segmental regions and in response to elevated pressure: collagens, ECM glycoproteins including matricellular proteins, ECM receptors such as integrins and adhesion molecules and ECM regulators, such as matrix metalloproteinases. In general, under normal perfusion pressure, more ECM genes were enriched in the high flow regions than in the low flow regions of the TM, whereas more ECM genes were found to be enriched in low flow regions of the TM in response to elevated perfusion pressure. Thus it appears that a limited subset of ECM genes is differentially regulated in both high and low flow regions and in response to elevated pressure. Some of these same ECM genes have previously been shown to be involved in the pressure response of stretched TM cells supporting their central role in IOP homeostasis. In general, different ECM gene family members are called upon to produce the response to elevated pressure in different segmental regions of the TM.

1. Introduction

Glaucoma is one of the leading causes of blindness affecting over 67 million people worldwide (Quigley, 1996, 2011). Elevated intraocular pressure (IOP) is the primary risk factor for glaucoma, and is targeted for all current glaucoma therapies. Aqueous humor flows out of the anterior chamber primarily via the conventional outflow pathway through the trabecular meshwork (TM) tissue to Schlemm's canal (SC) and then into the episcleral venous system (Acott and Kelley, 2008; Acott et al., 2014; Brubaker, 1991). IOP is generated primarily by creating resistance to aqueous humor outflow in the TM (Johnson, 2006; Tamm, 2009). This resistance is believed to reside predominantly within the juxtacanalicular (JCT) region of the TM and the inner wall of Schlemm's canal (Acott and Kelley, 2008; Inomata et al., 1972; Johnson et al., 1990; Overby et al., 2009).

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2. Segmental outflow

Aqueous humor outflow has been shown to be segmental in nature around the circumference of the eye. Regions of relatively high, intermediate or mixed, and low flow have been demonstrated in many studies using tracers of different composition and size to visualize the outflow patterns (Buller and Johnson, 1994; Chang et al., 2014; de Kater et al., 1989; Ethier and Chan, 2001; Hann et al., 2005; Keller et al., 2011; Vranka et al., 2015). In addition, non-uniform patterns of aqueous outflow have been demonstrated in many different species including human, monkey, mouse, porcine, and bovine eyes (Battista et al., 2008; Lu et al., 2011; Swaminathan et al., 2013; Vranka et al., 2015; Zhu et al., 2010, 2013). Segmental flow patterns have also been detected in the TMs from glaucomatous human eyes (de Kater et al., 1989). Only recently have studies started to shed light on molecular differences of segmental flow regions. We demonstrated that versican expression levels are inversely correlated with segmental flow regions across the TM (Keller et al., 2011). The matricellular protein SPARC displays segmental variations in expression (Vranka et al., 2013) and SPARC-null mice showed a more uniform pattern of outflow than do wild-type mice (Swaminathan et al., 2013). Our most recent study has suggested that segmental regions differ in their molecular composition, but it is not known how this may affect outflow resistance (Vranka et al., 2015). In addition to expression differences, morphological differences also exist coincident with regions of non-uniform outflow. SC cells along the inner wall have micron size transendothelial pores that allow fluid flow through or between inner wall cells (Bill and Svedbergh, 1972; Ethier, 2002; Ethier et al., 1998; Johnson et al., 1990, 2002; Tamm, 2009). There are two types of pores in SC cells: intracellular pores (I-pores) and border pores (B-pores). A recent study has shown a positive association of B-pores and high flow regions of the JCT and the inner wall endothelium of SC, suggesting that pores correlate with outflow segmentation (Braakman et al., 2015). Of course, pores could be an indicator of flow regions, or they could influence flow regions. In spite of these observations, the broader implications of segmental outflow on outflow resistance are poorly understood.

3. Extracellular matrix of the TM and IOP homeostasis

The probable primary site of outflow resistance is located within the deepest portion of the JCT and Schlemm's canal inner wall basement membrane (Acott and Kelley, 2008; Ethier, 2002; Johnson, 2006; Stamer and Acott, 2012). The extracellular matrix (ECM) of the TM is thought to play a significant role in modulating aqueous humor outflow resistance, since modulating or disrupting it has been shown to have a direct effect on outflow resistance (Acott and Kelley, 2008; Bradley et al., 1998; Keller et al., 2009b, 2008, 2011). Ongoing ECM turnover, initiated by MMPs in the TM, is necessary to maintain outflow facility, and inhibition of endogenous MMPs decreases outflow facility (Bradley et al., 1998; Keller et al., 2009b). IOP homeostasis is the term that refers to the corrective adjustments of the outflow resistance in response to sustained pressure that serve to maintain IOP within a narrow range of acceptable levels (Acott et al., 2014). Mechanical stretching of TM cells, as well as increased perfusion pressure in anterior segments, triggers numerous changes in ECM protein and gene expression levels of the TM at times compatible with ECM remodeling (Keller et al., 2007; Okada et al., 1998; Vittal et al., 2005; Vittitow and Borras, 2004; WuDunn, 2001).

4. Molecular components of segmental flow

One of the goals of a newly published study was to correlate patterns of ECM gene expression with high and low flow regions of the TM in human anterior segments perfused at physiological pressure in organ culture (Vranka et al., 2015). Standard physiological flow rates were in the range of 1–9 μl/min when perfused at physiologic pressure of 8.8 mmHg, which is similar to normal physiological rate and pressure (minus episcleral venous pressure) *in vivo*. A number of ECM and adhesion genes were shown to be differentially expressed in high and low flow regions of the TM when perfused at normal, physiological pressure, or what we call "1x" perfusion pressure. Here we show the table of actual fold change expression differences in segmental regions of the TM at physiologic (1x) pressure perfusion (Table 1A). When perfusion pressure is doubled to 17.6 mmHg we call this elevated perfusion pressure or "2x" perfusion pressure mimicking elevated pressure conditions *in vivo*. Here we compare gene differences in segmental regions of the TM from anterior segments perfused at elevated (2x) pressure for 48 h (Table 1B). The methods used herein are as follows: perfusion of human anterior segments in organ culture, labeling of anterior segments by perfusion with fluorescent tracers to identify high and low flow regions of the TM, followed by TM dissection, RNA isolation and quantitative PCR arrays to analyze ECM and cell adhesion gene expression levels, as described previously (Vranka et al., 2015). Table 1 shows fold change values of differentially expressed genes in high flow (HF) regions in comparison with low flow (LF) regions when anterior segments were perfused at either normal (1x) pressure (Table 1A) or at elevated (2x) pressure (Table 1B). Biologically significant fold change values were determined semi-arbitrarily to be those that were either greater than 1.5, representing genes enriched in high flow regions (genes upregulated), or less than 0.5, representing genes that were enriched in low flow regions (genes down regulated). The genes in Table 1 are grouped according to function and then ranked according to the genes with the largest fold change to genes with the smallest fold change in each functional group.

Many genes from multiple functional groups were preferentially expressed in HF regions compared with LF regions under normal pressure conditions (Table 1A). For example in the collagen group of genes, COL1A1 was upregulated or enriched in high flow regions at normal perfusion pressure with a fold change value of 6.14, whereas COL15A1 was down regulated or enriched in low flow regions with a fold change value of 0.28. Several other collagen genes were enriched only in high flow regions such as COL6A2, COL6A1, COL4A2, and COL16A1. A similar trend was seen with the ECM receptors group where all the genes listed in Table 1A except ITGB4 were found to be enriched in HF regions at normal pressure. This includes several integrins, catenins and the cell adhesion molecules VCAM1 and NCAM1. In the ECM regulators group of genes matrix metalloproteinases (MMPs) 1, 2, 3, and 12 were all enriched in high flow regions, whereas MMP16 and ADAMTS8 are enriched in low flow regions at normal perfusion pressure. The endogenous MMP inhibitor TIMP1 was enriched in high flow regions at normal pressure. Fewer genes across all of the functional groups were enriched in the low flow regions at normal perfusion pressure and included laminins (LAMC1, LAMA3, and LAMB3), ITGB4 and osteopontin (SPP1) suggesting that the high flow regions are more active in terms of ECM gene expression at normal physiologic pressure in order to properly maintain outflow resistance.

In contrast, when comparing high flow regions to low flow regions from TMs perfused at elevated (2x) pressure (Table 1B), far fewer ECM genes were found to be enriched in the high flow regions, namely ITGAV, VCAM1 and THBS2, while many more genes were enriched in the low flow regions. The collagen gene, COL14A1, and the laminins, LAMB1, LAMA1, and LAMB3 were all enriched in low flow regions. Additionally, MMPs 1, 14, and 11, were enriched in low flow regions at elevated perfusion pressure, as were their

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