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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.

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Disclosure

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Keywords

Trabecular meshwork; Extracellular matrix; Aqueous humor outflow; Segmental outflow; Intraocular pressure homeostasis

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).

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 nonuniform 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, 2008b, 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 endogenous inhibitors TIMP1 and TIMP2, as well as the matricellular genes CD44 and TNC. VCAM1 and THBS2, both known to be important in cell-cell recognition, were the only genes that were enriched in the high flow regions at both elevated and normal pressure suggesting an important role in maintaining outflow resistance at either pressure, whereas, osteopontin (SPP1) and LAMB3 were the only genes that were enriched in the low flow regions at both pressures. The genes LAMB1, TIMP1, and MMP1 were enriched in high flow regions at normal pressure, but conversely found to be enriched in low flow regions at elevated pressure. Overall, these complex gene expression differences between high and low flow regions suggest an important correlation of ECM genes expressed and functional consequences on outflow resistance per segmental region. It is not clear how high and low flow regions individually contribute to the maintenance of outflow resistance in an individual eye, but it likely involves a complex mechanism of communication across segmental regions.

5. Elevated pressure-induced effects in segmental regions of the TM

In the normal physiologic response to elevated pressure the TM undergoes ECM turnover and remodeling to correct the outflow resistance and reduce IOP (Acott et al., 2014). TM

cells under mechanical stretch increased MMP14 and MMP2, while TIMP2 was decreased (Bradley et al., 2001). We previously conducted micro-array gene expression studies after mechanical stretching of TM cells and identified many ECM genes that exhibited increased or decreased mRNA levels in response to stretch at varied times (Keller et al., 2007; Vittal et al., 2005). A number of these same ECM genes also showed differences in splice variants in response to stretch (Keller et al., 2007; Vittal et al., 2005). Presumably, a similar process occurs when IOP is elevated over a sustained period of time in tissue, producing a distortion of the ECM in the JCT and inner wall of SC. This stretching or distortion is thought to be sensed by the JCT and SC inner wall cells, possibly through ECM-integrin interactions and other cell surface ECM receptors such as CD44, VCAM-1, syndecans and others. The JCT and SC inner wall cells then make the necessary corrective adjustments by selective ECM degradation and biosynthesis of new ECM to bring the IOP within acceptable physiological ranges.

In the second part of this study the aim was to identify specific ECM genes that were enriched in response to elevated pressure on a per flow region basis. The genes identified in Table 2A are from high flow regions from the TM of anterior segments perfused at elevated pressure compared with those in high flow regions perfused at normal pressure. We also identified differentially expressed genes in low flow regions from TMs perfused at elevated pressure versus low flow regions from TMs perfused at normal pressure (Table 2B). Genes that were up regulated were considered to be enriched at elevated pressure, and those that were down regulated were considered to be enriched at normal pressure.

In high flow regions more genes are downregulated or enriched at normal pressure than at elevated pressure across all groups of ECM genes. The collagen genes COL8A2 and COL14A1 were both enriched at elevated pressure, whereas COL6A2, COL12A1, COL16A1 and COL5A1 were all enriched at normal pressure in high flow regions suggesting that these specific collagens are actively being synthesized in high flow regions in response to either normal or elevated pressure. In low flow regions COL6A1 and COL5A1 were enriched at elevated (2x) pressure, whereas COL7A1 was enriched at normal (1x) pressure. Of the ECM regulator gene group, MMP9 is enriched at elevated pressure in high flow regions, whereas MMP1 and ADAMTS1 are enriched in low flow regions at elevated pressure implying that ECM degradation in response to elevated pressure varies across regions of the TM. Cadherin 1 (CDH1), involved in mechanisms regulating cell-cell adhesion, is the only gene that is enriched at normal pressures in both high flow and low flow regions suggesting an important role in the ongoing maintenance of outflow resistance. No ECM genes were found to be enriched at elevated pressure in both high and low flow regions implying a complex response to pressure in respective regions of the TM. Finally, the COL5A1 gene appears to be not only synthesized in low flow regions in response to elevated pressure, but also in high flow regions at normal pressure. These differentially expressed ECM genes, which vary between low flow and high flow regions, are presumably part of the normal homeostatic mechanism to adjust the outflow resistance back to normal levels after a period of elevated pressure.

6. Discussion

The observation of aqueous humor outflow segmentation has dramatic implications on the resistance adjustments that occur during IOP homeostasis under normal conditions, as well as during sustained pressure increases in the eye. The ECM of the JCT and SC is intricately involved in this complex process presumably through a series of steps including sensing of distortion or stretch relayed to cells triggering signaling pathways and resulting in ECM degradation and remodeling (Acott and Kelley, 2008; Bradley et al., 1998, 2001; Keller et al., 2009a; Overby et al., 2009; Pattabiraman et al., 2013, 2015; Pattabiraman and Rao, 2010; Sanka et al., 2007; Vittitow and Borras, 2004; WuDunn, 2001; Zhang et al., 2008). How segmental outflow regions contribute to this process is poorly understood. How the TM and SC cells within these regions communicate with each other in order to coordinately modulate outflow resistance to ultimately achieve an acceptable IOP is not known; however the data presented here may help lead to a better understanding of this process. In this study we have divided our observations into 2 broad categories: a) differential gene expression between segmental flow regions at a given perfusion pressure (Table 1), and b) differential gene expression in response to elevated perfusion pressure in either of the two flow regions (Table 2). Here we identify subsets of ECM and adhesion gene families based on function that are differentially expressed in high flow or low flow regions when anterior segments were perfused at normal or elevated pressure for a single time point of 48 h. Interestingly, many of the same ECM genes identified in this study were previously shown to be involved in the stretch/pressure response of TM cells at various time points after stretch. Some of these genes common to both studies are: TNC, CD44 COL12A1, COL14A1, COL5A1, VCAM1, MMP2, MMP16, THBS2, LAMC1, and SPARC (Acott et al., 2014; Keller et al., 2007; Vittal et al., 2005).

The genes identified here that were found to be up or down regulated in segmental regions of the TM and in response to elevated pressure have the potential to provide a deeper understanding of the complex molecular mechanisms involved in the maintenance of aqueous humor outflow resistance. For example vascular cell adhesion molecule (VCAM1), a protein that interacts with integrins and is known to be important in cell-cell recognition, was previously shown to be upregulated in stretched TM cells and here found to be enriched in high flow regions compared to low flow regions at both normal and elevated pressures, suggesting an important contribution to cell-cell and cell-matrix interactions in high flow regions. Tenascin C (TNC) is an ECM glycoprotein and matricellular protein that interacts with other extracellular and cell-surface proteins including versican, fibronectin, integrins and syndecan. CD44 is a receptor for hyaluronic acid and mediates cell-cell and cell-matrix interactions. Whereas TNC and CD44 were found previously to be upregulated in stretched TM cells, here they were found to be enriched more specifically in low flow regions compared to high flow regions at elevated pressure, indicating a role for both proteins in mediating cell-matrix interactions in low flow regions in response to a pressure challenge. COL14A1 was previously found to be upregulated in stretched TM cells, and in this study it was found to be enriched in high flow regions at elevated pressure compared with high flow regions at normal pressure. Type XIV collagen is a fibril associated collagen that also interacts with other ECM molecules including CD44 and decorin. COL5A1, a fibrillar

forming collagen that binds to heparin and thrombospondin, was previously found to be up regulated in stretched TM cells, and in this study was found to be enriched in high flow regions at normal pressure and enriched in low flow regions at elevated pressure. These results indicate a role for types V and XIV collagen, as well as other collagen molecules as structural components of the TM, to directly modulate outflow resistance in segmental regions of the TM in a normal response to elevated pressure. MMPs 2, 15, and 16 were all previously shown to be upregulated in stretched TM cells, whereas here we show that MMP9, which degrades types IV and V collagen, was enriched in high flow regions, and MMP1, which degrades types I, II and III collagen, was enriched in low flow regions at elevated pressure compared to normal pressure. Cleary, MMPs are important players in the active remodeling of the TM both under both normal and elevated pressure conditions in order to maintain proper outflow resistance. These data suggest a highly complex process which appears to involve a fairly small subset of ECM molecules whose expression levels are both regulated in response to increasing pressure, and are specific to high or low outflow regions of the TM. It is interesting to note that this subset of molecules is a combination of structural ECM molecules, matricellular proteins, and matrix metalloproteinases, all of which are likely involved in ECM turnover in the TM.

We were surprised to find in a high turnover tissue such as the TM differential expression of some fibrillar collagen genes such as COL1A1, COL5A1, and COL6A1 but no differential expression of their corresponding alpha chain partners, such as COL1A2, COL5A2, and COL6A2, respectively. In tissues these proteins form higher ordered triple helical structures in a heteropolymer, and one might expect all genes to be coordinately regulated, but this appears to not be the case. It is intriguing to note that many of the genes identified in this study have been shown to interact with each other as proteins in the extracellular milieu suggesting a shared or coordinately interactive fate during ECM turnover in the TM. For example, tenascin C, thrombospondin and SPARC are all matricellular proteins and are thought to be important in modulating cell-cell and cell-matrix interactions (Acott and Kelley, 2008) (Acott and Kelley, 2008; Bornstein, 2009; Bornstein et al., 2000; Bornstein and Sage, 2002; Calabro et al., 2014) SPARC binds to fibrillar collagens and thrombospondin and has been shown to have an effect on outflow segmentation (Swaminathan et al., 2013). Thrombospondin 1 and 2 both interact with MMPs and SPARC and thrombospondin null mice all have lower IOPs than wild type mice suggesting they may play a role in regulating aqueous outflow facility (Acott and Kelley, 2008; Bornstein, 2009; Bornstein and Sage, 2002; Chatterjee et al., 2014; Swaminathan et al., 2013). CD44 and thrombospondin 2 can bind to fibronectin, laminin and type V collagen, all genes identified in this study to be differentially expressed. Presumably, some of these matricellular proteins serve to modulate binding and organizational interactions between TM cells and the various ECM structural components while they are turned over or reorganized during outflow resistance adjustments.

In conclusion, our data show that high and low flow regions of the TM are not only structurally different from each other based on the variety of ECM genes that are enriched in both regions, but that they respond differently in response to elevated pressure. Interestingly, we've observed that the opposite process can also occur whereby when pressure is decreased, there is a corresponding increase in outflow resistance over a series of days

(unpublished observation.) Therefore stretch, or physical distortion as a result of elevated pressure, may be considered as a signal that starts a series of coordinated processes that serve either of two purposes: one is solely to turn over ECM to maintain outflow resistance, the other is to turn over and remodel the existing ECM into a different formation to adjust resistance accordingly. Our data here along with that published previously suggest that a relatively small subset of ECM molecules is working in a complex and coordinated fashion to maintain and adjust outflow resistance. We hypothesize that the high flow regions of the TM are actively undergoing ECM turnover under normal pressures and in response to elevated pressures, however, thus far this has primarily been observed within limited time frames. Our data here show that the low flow regions are also able to sense pressure differences and respond by modifying ECM gene expression levels again within limited time frames. Studies are under way to perform more time points of elevated pressure (for example, 24 and 72 h), as well as analysis of segmental flow regions of the TM by both proteomics and microarrays to get a more complete picture of the molecular mechanisms involved in outflow resistance. Molecules that are differentially expressed or distributed in segmental regions of the TM and in response to pressure challenge could be potential targets for therapies to affect outflow resistance, particularly in specific flow regions of the TM. Thus, design and delivery of more targeted, and thereby more effective, glaucoma treatments will be the outcome of these ongoing studies.

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References

Acott TS, Kelley MJ. Extracellular matrix in the trabecular meshwork. Exp Eye Res. 2008; 86:543–561. [PubMed: 18313051]

Acott TS, Kelley MJ, Keller KE, Vranka JA, Abu-Hassan DW, Li X, Aga M, Bradley JM. Intraocular pressure homeostasis: maintaining balance in a high-pressure environment. J Ocul Pharmacol Ther. 2014; 30:94–101. [PubMed: 24401029]

Battista SA, Lu Z, Hofmann S, Freddo T, Overby DR, Gong H. Reduction of the available area for aqueous humor outflow and increase in meshwork herniations into collector channels following acute IOP elevation in bovine eyes. Invest Ophthalmol Vis Sci. 2008; 49:5346–5352. [PubMed: 18515571]

Bill A, Svedbergh B. Scanning electron microscopic studies of the trabecular meshwork and the canal of Schlemm–an attempt to localize the main resistance to outflow of aqueous humor in man. Acta Ophthalmol. 1972; 50:295–320. [PubMed: 4678226]

Bornstein P. Matricellular proteins: an overview. J Cell Commun Signal. 2009; 3:163–165. [PubMed: 19779848]

Bornstein P, Armstrong LC, Hankenson KD, Kyriakides TR, Yang Z. Thrombospondin 2, a matricellular protein with diverse functions. Matrix biology J Int Soc Matrix Biol. 2000; 19:557–568.

Bornstein P, Sage EH. Matricellular proteins: extracellular modulators of cell function. Curr Opin Cell Biol. 2002; 14:608–616. [PubMed: 12231357]

Braakman ST, Read AT, Chan DW, Ethier CR, Overby DR. Colocalization of outflow segmentation and pores along the inner wall of Schlemm's canal. Exp Eye Res. 2015; 130:87–96. [PubMed: 25450060]

Bradley JM, Kelley MJ, Zhu X, Anderssohn AM, Alexander JP, Acott TS. Effects of mechanical stretching on trabecular matrix metalloproteinases. Invest Ophthalmol Vis Sci. 2001; 42:1505–1513. [PubMed: 11381054]

Bradley JM, Vranka J, Colvis CM, Conger DM, Alexander JP, Fisk AS, Samples JR, Acott TS. Effect of matrix metalloproteinases activity on outflow in perfused human organ culture. Invest Ophthalmol Vis Sci. 1998; 39:2649–2658. [PubMed: 9856774]

Brubaker RF. Flow of aqueous humor in humans [The Friedenwald Lecture]. Invest Ophthalmol Vis Sci. 1991; 32:3145–3166. [PubMed: 1748546]

Buller C, Johnson D. Segmental variability of the trabecular meshwork in normal and glaucomatous eyes. Invest Ophthalmol Vis Sci. 1994; 35:3841–3851. [PubMed: 7928181]

Calabro NE, Kristofik NJ, Kyriakides TR. Thrombospondin-2 and extracellular matrix assembly. Biochim Biophysica acta. 2014; 1840:2396–2402.

Chang JY, Folz SJ, Laryea SN, Overby DR. Multi-scale analysis of segmental outflow patterns in human trabecular meshwork with changing intraocular pressure. J Ocul Pharmacol Ther. 2014; 30:213–223. [PubMed: 24456518]

Chatterjee A, Villarreal G Jr, Rhee DJ. Matricellular proteins in the trabecular meshwork: review and update. J Ocul Pharmacol Ther. 2014; 30:447–463. [PubMed: 24901502]

de Kater AW, Melamed S, Epstein DL. Patterns of aqueous humor outflow in glaucomatous and nonglaucomatous human eyes. A tracer study using cationized ferritin. Arch Ophthalmol. 1989; 107:572–576. [PubMed: 2705927]

Ethier CR. The inner wall of Schlemm's canal. Exp Eye Res. 2002; 74:161–172. [PubMed: 11950226]

Ethier CR, Chan DW. Cationic ferritin changes outflow facility in human eyes whereas anionic ferritin does not. Invest Ophthalmol Vis Sci. 2001; 42:1795–1802. [PubMed: 11431444]

Ethier CR, Coloma FM, Sit AJ, Johnson M. Two pore types in the inner-wall endothelium of Schlemm's canal. Invest Ophthalmol Vis Sci. 1998; 39:2041–2048. [PubMed: 9761282]

Hann CR, Bahler CK, Johnson DH. Cationic ferritin and segmental flow through the trabecular meshwork. Invest Ophthalmol Vis Sci. 2005; 46:1–7. [PubMed: 15623746]

Inomata H, Bill A, Smelser GK. Aqueous humor pathways through the trabecular meshwork and into Schlemm's canal in the cynomolgus monkey (Macaca irus). An electron microscopic study. Am J Ophthalmol. 1972; 73:760–789. [PubMed: 4623937]

Johnson M. What controls aqueous humour outflow resistance? Exp Eye Res. 2006; 82:545–557. [PubMed: 16386733]

Johnson M, Chan D, Read AT, Christensen C, Sit A, Ethier CR. The pore density in the inner wall endothelium of Schlemm's canal of glaucomatous eyes. Invest Ophthalmol Vis Sci. 2002; 43:2950–2955. [PubMed: 12202514]

Johnson M, Johnson DH, Kamm RD, DeKater AW, Epstein DL. The filtration characteristics of the aqueous outflow system. Exp Eye Res. 1990; 50:407–418. [PubMed: 2338123]

Keller KE, Aga M, Bradley JM, Kelley MJ, Acott TS. Extracellular matrix turnover and outflow resistance. Exp Eye Res. 2009a; 88:676–682. [PubMed: 19087875]

Keller KE, Bradley JM, Acott TS. Differential effects of ADAMTS-1, -4, and -5 in the trabecular meshwork. Invest Ophthalmol Vis Sci. 2009b; 50:5769–5777. [PubMed: 19553617]

Keller KE, Bradley JM, Kelley MJ, Acott TS. Effects of modifiers of glycosaminoglycan biosynthesis on outflow facility in perfusion culture. Invest Ophthalmol Vis Sci. 2008; 49:2495–2505. [PubMed: 18515587]

Keller KE, Bradley JM, Vranka JA, Acott TS. Segmental versican expression in the trabecular meshwork and involvement in outflow facility. Invest Ophthalmol Vis Sci. 2011; 52:5049–5057. [PubMed: 21596823]

Keller KE, Kelley MJ, Acott TS. Extracellular matrix gene alternative splicing by trabecular meshwork cells in response to mechanical stretching. Invest Ophthalmol Vis Sci. 2007; 48:1164–1172. [PubMed: 17325160]

Lu Z, Zhang Y, Freddo TF, Gong H. Similar hydrodynamic and morphological changes in the aqueous humor outflow pathway after washout and Y27632 treatment in monkey eyes. Exp Eye Res. 2011; 93:397–404. [PubMed: 21669200]

Okada Y, Matsuo T, Ohtsuki H. Bovine trabecular cells produce TIMP-1 and MMP-2 in response to mechanical stretching. Jpn J Ophthalmol. 1998; 42:90–94. [PubMed: 9587839]

Overby DR, Stamer WD, Johnson M. The changing paradigm of outflow resistance generation: towards synergistic models of the JCT and inner wall endothelium. Exp Eye Res. 2009; 88:656–670. [PubMed: 19103197]

Pattabiraman PP, Inoue T, Rao PV. Elevated intraocular pressure induces Rho GTPase mediated contractile signaling in the trabecular meshwork. Exp Eye Res. 2015; 136:29–33. [PubMed: 25956210]

Pattabiraman PP, Pecen PE, Rao PV. MRP4-mediated regulation of intracellular cAMP and cGMP levels in trabecular meshwork cells and homeostasis of intraocular pressure. Invest Ophthalmol Vis Sci. 2013; 54:1636–1649. [PubMed: 23385799]

Pattabiraman PP, Rao PV. Mechanistic basis of Rho GTPase-induced extracellular matrix synthesis in trabecular meshwork cells. Am J Physiol Cell Physiol. 2010; 298:C749–C763. [PubMed: 19940066]

Quigley HA. Number of people with glaucoma worldwide. Br J Ophthalmol. 1996; 80:389–393. [PubMed: 8695555]

Quigley HA. Glaucoma. Lancet. 2011; 377:1367–1377. [PubMed: 21453963]

Sanka K, Maddala R, Epstein DL, Rao PV. Influence of actin cytoskeletal integrity on matrix metalloproteinase-2 activation in cultured human trabecular meshwork cells. Invest Ophthalmol Vis Sci. 2007; 48:2105–2114. [PubMed: 17460268]

Stamer WD, Acott TS. Current understanding of conventional outflow dysfunction in glaucoma. Curr Opin Ophthalmol. 2012; 23:135–143. [PubMed: 22262082]

Swaminathan SS, Oh DJ, Kang MH, Ren R, Jin R, Gong H, Rhee DJ. Secreted protein acidic and rich in cysteine (SPARC)-null mice exhibit more uniform outflow. Invest Ophthalmol Vis Sci. 2013; 54:2035–2047. [PubMed: 23422826]

Tamm ER. The trabecular meshwork outflow pathways: structural and functional aspects. Exp Eye Res. 2009; 88:648–655. [PubMed: 19239914]

Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A. 2001; 98:5116–5121. [PubMed: 11309499]

Vittal V, Rose A, Gregory KE, Kelley MJ, Acott TS. Changes in gene expression by trabecular meshwork cells in response to mechanical stretching. Invest Ophthalmol Vis Sci. 2005; 46:2857–2868. [PubMed: 16043860]

Vittitow J, Borras T. Genes expressed in the human trabecular meshwork during pressure-induced homeostatic response. J Cell Physiol. 2004; 201:126–137. [PubMed: 15281095]

Vranka J, Keller KE, Acott TS. Extracellular matrix gene expression profiling of high and low flow areas of human trabecular meshwork. Invest Ophthalmol Vis Sci. 2013; 54:3566.

Vranka JA, Bradley JM, Yang YF, Keller KE, Acott TS. Mapping molecular differences and extracellular matrix gene expression in segmental outflow pathways of the human ocular trabecular meshwork. PLoS One. 2015; 10:e0122483. [PubMed: 25826404]

WuDunn D. The effect of mechanical strain on matrix metalloproteinase production by bovine trabecular meshwork cells. Curr Eye Res. 2001; 22:394–397. [PubMed: 11600941]

Zhang M, Maddala R, Rao PV. Novel molecular insights into RhoA GTPase-induced resistance to aqueous humor outflow through the trabecular meshwork. Am J Physiol Cell Physiol. 2008; 295:C1057–C1070. [PubMed: 18799648]

Zhu JY, Ye W, Gong HY. Development of a novel two color tracer perfusion technique for the hydrodynamic study of aqueous outflow in bovine eyes. Chin Med J Engl. 2010; 123:599–605. [PubMed: 20367989]

Zhu JY, Ye W, Wang T, Gong HY. Reversible changes in aqueous outflow facility, hydrodynamics, and morphology following acute intraocular pressure variation in bovine eyes. Chin Med J Engl. 2013; 126:1451–1457. [PubMed: 23595376]

Table 1

Fold change gene expression for high flow (HF) regions in comparison with low flow (LF) regions of the TM from anterior segments perfused at normal (1x) pressure (A) or at elevated (2x) pressure (B).

A. Genes at 1x Pressure	Fold change (HF/LF)[a]	B. Genes at 2x Pressure	Fold change (HF/LF)[a]
Collagens		*Collagens*	
Collagen 1α1	6.14	Collagen 14α1	0.35
Collagen 6α2	2.09		
Collagen 6α1	1.93		
Collagen 4α2	1.76		
Collagen 16α1	1.58		
Collagen 15α1	0.28		
ECM glycoproteins		*ECM glycoproteins*	
Thrombospondin 2	3.4	Thrombospondin 2	1.85
SPARC	1.97	Tenascin C	0.48
Laminin β1	1.78	Laminin β1	0.42
Laminin γ1	0.62	Laminin α1	0.41
Laminin α3	0.49	Laminin β3	0.39
Osteopontin	0.42	ECM protein 1	0.31
Laminin β3	0.36	Osteopontin	0.20
ECM receptors/adhesion		*ECM receptors/adhesion*	
Vascular cell adhesion molecule 1	5.22	Integrin αV	3.95
		Vascular cell adhesion molecule 1	2.17
Contactin 1	4.65	CD44	0.46
Catenin α1	4.54	Intercellular adhesion molecule 1	0.43
Catenin β1	2.49		
Integrin β1	1.77		
Neural cell adhesion molecule 1	1.59		
Integrin β3	1.54		
Integrin α7	1.50		
Integrin β4	0.48		
ECM regulators		*ECM regulators*	
Matrix metalloproteinase 12	3.53	Matrix metalloproteinase 14	0.45
Matrix metalloproteinase 3	3.09	Tissue inhibitor of metalloproteinases 1	0.39
Matrix metalloproteinase 1	3.07	Matrix metalloproteinase 1	0.38
Tissue inhibitor of metalloproteinases 1	2.73	Matrix metalloproteinase 11	0.37
Matrix metalloproteinase 2	2.39	Tissue inhibitor of metalloproteinases 2	0.35
A disintegrin and metalloproteinase with thrombospondin motifs 8	0.52		
Matrix metalloproteinase 16	0.44		

[a]Threshold fold-change differences semi-arbitrarily determined to be biologically significant were >1.5 and ≤ 0.5 as previously described (Vranka et al., 2015). SAM (significance analysis of microarrays), version 4.01 (Tusher et al., 2001), with 4 biological replicates, was used to determine statistically significant gene changes. The analysis was two class paired, with arrays median centered.

Table 2

Fold change gene expression in either high flow (HF) regions (A), or in low flow (LF) regions (B) from TMs perfused at elevated (2x) pressure compared with the same region from TMs perfused at normal pressure (1x).

A. Genes in HF	**Fold change (2x/1x)[a]**	**B. Genes in LF**	**Fold change (2x/1x)[a]**
Collagens		*Collagens*	
Collagen 8α2	1.75	Collagen 6α1	1.94
Collagen 14α1	1.54	Collagen 5α1	1.60
Collagen 6α2	0.44	Collagen 7α1	0.49
Collagen 12α1	0.42		
Collagen 16α1	0.36		
Collagen 5α1	0.35		
ECM glycoproteins		*ECM glycoproteins*	
Laminin γ1	2.26	ECM protein 1	2.30
Tenascin C	0.49	Neural cell adhesion molecule 1	1.84
Laminin α3	0.34		
Vitronectin	0.34		
Laminin β1	0.27		
Osteopontin	0.26		
ECM receptors/adhesion		*ECM receptors/adhesion*	
Integrin α4	1.66	Catenin β1	1.81
Integrin β1	0.47	Integrin α2	1.74
Catenin α1	0.40	CD44	1.57
Cadherin 1	0.24	Integrin β5	0.37
		Cadherin 1	0.30
ECM regulators		*ECM regulators*	
Matrix metalloproteinase 9	1.65	Matrix metalloproteinase 1	2.48
A disintegrin and metalloproteinase with thrombospondin motifs 1	0.38	Tissue inhibitor of metalloproteinases 1	1.89
Transforming growth factor β1	0.38	A disintegrin and metalloproteinase with thrombospondin motifs 1	1.50
		A disintegrin and metalloproteinase with thrombospondin motifs 13	0.50
		Tissue inhibitor of metalloproteinases 3	0.38
		Matrix metalloproteinase 8	0.36

[a]Threshold fold-change differences semi-arbitrarily determined to be biologically significant were >1.5 and ≤ 0.5 as previously described (Vranka et al., 2015). SAM (significance analysis of microarrays), version 4.01 (Tusher et al., 2001), with 4 biological replicates, was used to determine statistically significant gene changes. The analysis was two class paired, with arrays median centered.