Published in final edited form as:

Exp Eye Res. 2009 April ; 88(4): 671–675. doi:10.1016/j.exer.2008.08.006.

Biological Properties of Trabecular Meshwork Cells

Joshua Z. Gasiorowski and Paul Russell

School of Veterinary Medicine, University of Wisconsin – Madison

Abstract

The molecular and physiological mechanisms that lead to the progression of glaucoma are poorly understood. Despite the fact that glaucoma afflicts millions of people worldwide, research on the disease is limited by the current animal models that do not translate well to human forms of the disease. However, recent advances in culturing and manipulating human trabecular meshwork cells may provide a means to elucidate some of the mechanisms that cause glaucoma. This review focuses on the properties of trabecular meshwork cells, from their characteristic expression profile in vivo to their responsiveness to biochemical and biophysical signals in vitro. Hopefully the study of cultured trabecular meshwork cells will provide a better understanding of glaucoma and lead to new, much needed therapies.

Keywords

trabecular meshwork cells; tissue culture; phagocytosis; glucocorticoids; TGF- β ; oxidation; mechanical stretch; biophysical properties

Introduction

The trabecular meshwork (TM) of the eye, composed of cells and matrix, is thought to regulate aqueous humor outflow to control intraocular pressure (IOP). The TM and aqueous humor outflow pathway have a wide anatomical variation from one species to another (Chen et al., 2008; Samuelson, Gelatt, 1984; Smelser, Ozanics, 1971); however, the trabecular meshwork of human, rat and mouse all contain extracellular meshwork organized into a network of beams covered with endothelial cells. There are extensive numbers of elastic-like fibers in the juxtacanalicular (JCT) or cribriform region of the human trabecular meshwork (HTM) that form connections to the inner wall of Schlemm's canal. These fibers appear to function as mechanical tethers to maintain contact of the JCT to the inner wall, resulting in the opposition of hydrodynamic forces generated during perfusion. For this reason, the human meshwork appears unique in that it does not have the perfusion-volume-dependent increase in aqueous outflow facility known as “washout” (Scott et al., 2007). Species variations in the meshwork make the study of glaucoma difficult since no good animal model for human disease exists.

© 2008 Elsevier Ltd. All rights reserved.

Corresponding author: Paul Russell School of Veterinary Medicine 2015 Linden Drive Madison, WI 53706
russellp@svm.vetmed.wisc.edu Ph: 608-262-5652 Fax: 608-263-9713.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Due to the differences in species, this review article will primarily focus on the human trabecular meshwork. However, there is still complexity and variation to consider within the same individual. Tshumper and Johnson found notable differences between fellow eyes(Tschumper, Johnson, 1990) in terms of absolute cell numbers as well as cellularity (nuclei of HTM cells/solid tissue area). The average variation in cell numbers was 15% with deviations up to a maximum of 37%. Interestingly, data also indicate that the cellularity difference may be higher in patients with primary open angle glaucoma (Alvarado et al., 1984). Despite these variables, studying the properties of HTM cells remains a valuable tool to better understand glaucoma.

Culture Systems

Due to the lack of model systems to study glaucoma, the use of cultured TM cells provides a means to evaluate their biological properties when challenged with conditions linked to glaucoma(Polansky et al., 1979; Polansky et al., 1984; Tripathi, Tripathi, 1982; Weinreb et al., 1983). HTM cells are usually obtained from whole donor eyes or from corneal buttons and ideally, one would like to compare HTM cells from glaucomatous tissue to normal HTM cells. (Rhee et al., 2003) However, the culturing of cells from donor glaucomatous tissue has presented a challenge because of limited cellular replication in vitro. Certain groups have had some success using different methods to isolate the HTM cells from glaucomatous meshwork(Stamer et al., 2000; Wordinger et al., 1998), but in general, culturing of HTM cells from glaucomatous meshwork is much more demanding than obtaining and maintaining cells from normal donors. Therefore, normal HTM cells are often stimulated with different techniques to induce glaucoma-like phenotypes in vitro. These techniques will be reviewed later.

HTM Cell Characteristics

Perhaps the most characteristic marker for HTM cells is the increased expression of myocilin after the cells have been treated with dexamethasone(Polansky et al., 2000b). The induction appears to be specific in HTM cells compared to other ocular cells. This protein has characteristics of a matricellular protein(Peters et al., 2005; Shen et al., 2008; Wentz-Hunter et al., 2004). Other proteins that have been identified in HTM cells are: the alpha-2 adrenergic receptor(Stamer et al., 1996), aquaporin-1(Stamer et al., 1995) and acetylated and acetoacetylated low-density lipoproteins(Chang et al., 1991), but these proteins are also present in a wide variety of cells and are not specific markers for HTM cells. One protein, α B-crystallin, has been reported to be present in cells of the JCT, but not in other cells of the HTM(Welge-Lussen et al., 1999). This heat shock protein can be induced with transforming growth factor- β (TGF- β) as well as heat shock and oxidative stress(Tamm et al., 1996). Because this protein is expressed solely in the JCT region, there is a possibility that there are different sub-types of cells within the meshwork. However, no data exist at this time that differentiates distinct cell types within the HTM. Although it is difficult to use a single marker protein to identify a HTM cell, they do have a pronounced phagocytosis rate that can be used as a behavioral characteristic.

HTM Cell Phagocytosis

The elevated intraocular pressure that often accompanies glucocorticoid treatment has been linked to increased deposition of extracellular matrix material in the outflow pathway(Wordinger, Clark, 1999). This deposition is in part due to decreased HTM cell phagocytosis after steroid treatment(Matsumoto, Johnson, 1997; Zhang et al., 2007). These data highlight phagocytosis as an important function for HTM cells. A variety of particles such as latex beads, zymosan particles, or *Staphylococcus aureus* bioparticles have been used in animal studies in vivo, perfused organ culture of donor human eyes, and HTM cells

in vitro.(Buller et al., 1990; Johnson et al., 1989; Sherwood, Richardson, 1988; Zhang et al., 2007) (Figure 1). In a study using cats, one eye received phagocytic challenge in organ culture while the fellow eye was challenged in vivo(Buller et al., 1990). There were more cells involved with phagocytosis in vivo suggesting an activation of cells possibly by an inflammatory pathway that could only be possible in vivo. There also appeared to be a loss of fibronectin and laminin, indicative of phagocytosis mediated ECM remodeling (Zhou et al., 1995).

Gamma-interferon has also been shown to block phagocytosis(Park, Latina, 1993). This inhibition is apparently related to the alterations of actin filaments in the cell that form radial spoke-like arrangements. Conversely, platelet-derived growth factor enhanced phagocytosis by limiting stress fiber formation in TM cells(Tamura et al., 1989). Altogether, these data provide a correlation between the regulation of intraocular pressure to phagocytosis and the remodeling of the extracellular matrix in HTM cells.

Two of the common ways to study the HTM and progression of glaucoma are tissue culture of HTM cells and the TM organ culture of human, primate and other species within the anterior segment of the eye(Bahler et al., 2008; Comes, Borrás, 2007; Johnson, Tschumper, 1987; Keller et al., 2008; Pang et al., 2000; Wang et al., 2008). In general, hypothesis testing related to glaucoma is first explored with cultured cells and then confirmation of the data is performed with the more difficult organ culture system. This review focuses on the former.

Cell-based Glaucoma Models

HTM cells grown in vitro do not always behave similar to their in vivo counterparts. The changes in gene expression and protein production in tissue culture systems can make it particularly challenging to use HTM cells for glaucoma models. As a result, four different models employing external stimuli have been developed that allow researchers to use normal HTM cells to study glaucoma progression using an in vitro environment. Even though these models recreate some glaucoma-like properties in culture, it is important to note that all of these models still have limitations.

Dexamethasone

One of the earliest HTM-based glaucoma models, and perhaps the most widely studied to date, involves the use of glucocorticoids. Glucocorticoids are a family of signaling molecules that produce a wide range of effects depending on the receptors they bind and transduction cascades they induce. Glucocorticoids, like dexamethasone, can increase intraocular pressure, and this finding was then translated to cell culture to model glaucoma-like changes in HTM cells(Polansky et al., 2000a).

Dexamethasone has been shown to cause a decrease in the amount of endothelin-1 receptor B (ET-1rB) present in HTM cells grown in culture(Zhang et al., 2003). If ET-1rB is allowed to bind endothelin-1 under normal conditions, it drives nitric oxide release and subsequent vasodilation(Hirata et al., 1993). Thus, dexamethasone treatment decreases the level of ET-1rB available resulting in a drop of nitric oxide levels. Without ET-1rB present, only endothelin-1 receptor A remains available for the ligand. The binding of endothelin-1 to receptor A can start a vasoconstriction signaling cascade, which would presumably promote less outflow in the TM and raise IOP.

Dexamethasone has been reported to induce other hallmarks of glaucoma in HTM cells. Glucocorticoids cause the formation of cross-linked actin networks in cultured HTM cells(Clark et al., 1994). More rigid HTM cells with dense actin networks are less pliable and more likely to inhibit outflow. Dexamethasone downregulates matrix-metalloproteases

(MMP)-2, -3, and -14 when the cells are subjected to high pressures (50 mm Hg)(Ehrich et al., 2005). This results less ECM turnover and less outflow. Untreated HTM cells subjected to 50 mm Hg pressure had a strong upregulation of MMP-3(Ehrich et al., 2005). It appears the normal response of HTM cells to elevated pressure is to increase the amount of MMP-3 to help degrade and remodel ECM, but the presence of dexamethasone blocks this effect.

TGF- β

The second glaucoma model for HTM cells is dependent on treatment with TGF- β . TGF- β is a super-family of signaling proteins that have also been found at high levels in the aqueous humor of glaucomatous eyes(Tripathi et al., 1994). It is believed that high levels of TGF- β 2 contribute to the progression of POAG. TGF- β can exist in active and latent forms, but it has been shown that thrombospondin-1, a TGF- β activator, is upregulated in glaucomatous tissue and may initiate the signaling cascade(Flugel-Koch et al., 2004). In cultured HTM cells, 1 ng/ml of either TGF- β 1 or β 2 will increase tissue transglutaminase, which can cross-link ECM proteins(Welge-Lussen et al., 2000). TGF- β will also promote fibronectin production. Both outcomes can modify the ECM and decrease outflow. However, some of the bone morphogenic proteins (BMPs), which are part of the TGF- β super family, can balance TGF- β effects in the TM. BMPs and BMP receptors have been found in the TM and TM cells(Wordinger et al., 2002). There are some specific differences, though, notably BMP-2 was present at a much higher level in native tissue than in cell culture(Wordinger et al., 2002). While TGF- β induced fibronectin expression in HTM cells, co-treatment with BMP4 inhibited fibronectin expression. TGF- β 2 induced collagen IV and VI, but that expression was blocked by co-treatment with BMP-7(Fuchshofer et al., 2007). The delicate balance of TGF- β and BMP proteins may play a large role in properties of the ECM and ultimately, the pathogenesis of glaucoma.

Oxidative Stress

The third model employs oxidative stress to mediate a glaucoma-like phenotype in HTM cells. Chronic oxidative stress can contribute to reduced outflow by inhibiting the intracellular proteasome system that works to degrade intra- and intercellular debris. After exposure to oxidative stress (40% oxygen) for 10 days, HTM cells lose proteasome activity and have increased cell death(Caballero et al., 2003). Much like the balance between TGF- β and BMPs, there is a balance within the oxidative stress system. Continuous exposure of HTM cells to oxidative stress via H₂O₂ results in ROS generation. This, in turn, stimulated NF- κ B activation and subsequent production of interleukins (ILs), namely IL-1(Li et al., 2007). The positive feedback loop between NF- κ B activation and IL-1 expression may be a response to lower IOP; however, when IL-1 was administered exogenously, aqueous outflow in rat eyes decreased(Kee, Seo, 1997). Interestingly, normal HTM cells had activated NF- κ B and increased levels of IL-1 after treatment with phacoemulsification and this procedure often lowers IOP. This supports the hypothesis that this inflammatory pathway is a cellular attempt to decrease IOP(Wang et al., 2001). The complex and broad influence of proteasome stimulation and inflammation response means more research needs to be performed to understand the role oxidative stress may play in glaucoma.

Mechanical Stretch

Elevated IOP exerts physical forces on HTM cells causing mechanical stretch. As the fourth model for glaucoma, stretching HTM cells can have a profound effect on their expression profile. A gene array study that examined 8,000 possible genes found that mechanical stretch applied over 12, 24, or 48 hours results in a downregulation of 29 and an upregulation of over 100 genes in HTM cells(Vittal et al., 2005). Tenascin C, fibronectin, TGF- β receptor complex protein, secreted protein, acidic, cysteine-rich (SPARC) and some

collagens were upregulated 1.5 - 3.7 fold in the array. Cytoskeleton proteins vimentin, alpha 1 tubulin, and SH2-B as well as inflammatory protein NF- κ B (p105) were also upregulated after stretch. There are also reports that mechanical stretch increases the expression of MMP-2 and MMP-14 through the intracellular signaling molecule, mTOR(Bradley et al., 2001; Bradley et al., 2003). The MMP upregulation, in turn, modulates remodeling of the ECM.

Besides controlling transcription levels of many genes, mechanical stretch can mediate the alternative splicing of several mRNAs as well. Tenascin C, collagen XII and CD44 are alternatively spliced in response to stretch and change the trabecular meshwork ECM composition(Keller et al., 2007). Stretch exerts great control over fibronectin splicing(Vittal et al., 2005). Different fibronectin domains reversibly alter outflow in TM cultures through the available binding domains in the molecule(Santas et al., 2003).

Biophysical Cues

While biochemical signals are commonly used in laboratory experiments to control cell behaviors, the mechanical stretch model demonstrates the importance of also studying the effects of biophysical signals. In addition to fluctuations in IOP, HTM cells are provided with thousands of biophysical cues through cell-cell interactions and cell-ECM interactions. ECM has a rich, 3-dimensional topography through which the cells grow and function(Brody et al., 2006; Diehl et al., 2005; Liliensiek et al., 2006). HTM cells in vivo never see the flat surfaces on which they are typically cultured in vitro. When HTM cells were seeded onto nano and micro scale substrates that biomimetically share topographic features with native ECMs and basement membranes, cellular behaviors and gene expression were altered(Russell et al., 2008). The actin cytoskeleton, and overall HTM cell orientation aligned with the artificial substrata provided in the form of repeating nano and microscale grooves and ridges. In addition, HTM cells dramatically elongated parallel to the anisotropic biomimetic substrates (Figure 2). Finally, myocilin expression was upregulated on topography independent of dexamethasone stimulation(Russell et al., 2008).

The topography of the underlying substrata is not the only physical cue that HTM cells sense. The compliance (rigidity) of the substrate can also modulate HTM cell behaviors and interactions. HTM cells seeded on polyacrylamide gels spread less distance and at a slower rate than HTM cells plated onto stiff tissue culture plastic(Schlunck et al., 2008). The actin cytoskeleton formed less pronounced stress fibers and focal adhesion kinase is minimally phosphorylated on the soft gels compared to plastic. Similar to the topographic surfaces, myocilin expression was upregulated on the more compliant gels. These data, combined with the experiments using biomimetically topographic surfaces reveal the importance of incorporating biophysical cues into HTM cell culture studies.

Summary

The properties of HTM cells are complex in nature and still require additional research. While they provide a useful starting point to understand the different facets of facility and pathogenesis of glaucoma, we must realize that models still have limitations. Aspects of the different HTM in vitro models can be combined and developed to create more accurate representations of TM cell behavior in vivo. The incorporation of biophysical cues will also play an important role in the development of our research systems. Ultimately, a more complete grasp of the cellular properties of HTM cells will provide us with new treatments for glaucoma.

Acknowledgments

Supported by Grants 1R01EY016134-01A1 from the National Eye Institute, 5R01HL079012-02 from the National Heart, Lung, and Blood Institute and DMR-9632527 from the National Science Foundation.

References

- Alvarado J, Murphy C, Juster R. Trabecular meshwork cellularity in primary open-angle glaucoma and nonglaucomatous normals. *Ophthalmology*. 1984; 91:564–579. [PubMed: 6462622]
- Bahler CK, Howell KG, Hann CR, Fautsch MP, Johnson DH. Prostaglandins increase trabecular meshwork outflow facility in cultured human anterior segments. *Am J Ophthalmol*. 2008; 145:114–119. [PubMed: 17988642]
- Bradley JM, Kelley MJ, Rose A, Acott TS. Signaling pathways used in trabecular matrix metalloproteinase response to mechanical stretch. *Invest Ophthalmol Vis Sci*. 2003; 44:5174–5181. [PubMed: 14638714]
- 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]
- Brody S, Anilkumar T, Liliensiek S, Last JA, Murphy CJ, Pandit A. Characterizing nanoscale topography of the aortic heart valve basement membrane for tissue engineering heart valve scaffold design. *Tissue Eng*. 2006; 12:413–421. [PubMed: 16548699]
- Buller C, Johnson DH, Tschumper RC. Human trabecular meshwork phagocytosis. Observations in an organ culture system. *Invest Ophthalmol Vis Sci*. 1990; 31:2156–2163. [PubMed: 2211012]
- Caballero M, Liton PB, Epstein DL, Gonzalez P. Proteasome inhibition by chronic oxidative stress in human trabecular meshwork cells. *Biochem Biophys Res Commun*. 2003; 308:346–352. [PubMed: 12901875]
- Chang IL, Elner G, Yue YJ, Cornicelli A, Kawa JE, Elner VM. Expression of modified low-density lipoprotein receptors by trabecular meshwork cells. *Curr Eye Res*. 1991; 10:1101–1112. [PubMed: 1802612]
- Chen CC, Yeh LK, Liu CY, Kao WW, Samples JR, Lin SJ, Hu FR, Wang JJ. Morphological differences between the trabecular meshworks of zebrafish and mammals. *Curr Eye Res*. 2008; 33:59–72. [PubMed: 18214743]
- Clark AF, Wilson K, McCartney MD, Miggans ST, Kunkle M, Howe W. Glucocorticoid-induced formation of cross-linked actin networks in cultured human trabecular meshwork cells. *Invest Ophthalmol Vis Sci*. 1994; 35:281–294. [PubMed: 8300356]
- Comes N, Borrás T. Functional delivery of synthetic naked siRNA to the human trabecular meshwork in perfused organ cultures. *Mol Vis*. 2007; 13:1363–1374. [PubMed: 17768383]
- Diehl KA, Foley JD, Nealey PF, Murphy CJ. Nanoscale topography modulates corneal epithelial cell migration. *J Biomed Mater Res A*. 2005; 75:603–611. [PubMed: 16106433]
- Ehrlich D, Tripathi B, Tripathi R, Duncker G. Effects of interleukin-1 β and dexamethasone on the expression of matrix metalloproteinase mRNA by trabecular cells exposed to elevated hydrostatic pressure. *Acta Ophthalmol Scand*. 2005; 83:104–108. [PubMed: 15715567]
- Flugel-Koch C, Ohlmann A, Fuchshofer R, Welge-Lüssen U, Tamm ER. Thrombospondin-1 in the trabecular meshwork: localization in normal and glaucomatous eyes, and induction by TGF- β 1 and dexamethasone in vitro. *Exp Eye Res*. 2004; 79:649–663. [PubMed: 15500824]
- Fuchshofer R, Yu AH, Welge-Lüssen U, Tamm ER. Bone morphogenetic protein-7 is an antagonist of transforming growth factor- β 2 in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci*. 2007; 48:715–726. [PubMed: 17251470]
- Hirata Y, Emori T, Eguchi S, Kanno K, Imai T, Ohta K, Marumo F. Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. *J Clin Invest*. 1993; 91:1367–1373. [PubMed: 7682570]
- Johnson DH, Richardson TM, Epstein DL. Trabecular meshwork recovery after phagocytic challenge. *Curr Eye Res*. 1989; 8:1121–1130. [PubMed: 2612200]

- Johnson DH, Tschumper RC. Human trabecular meshwork organ culture. A new method. *Invest Ophthalmol Vis Sci.* 1987; 28:945–953. [PubMed: 3583633]
- Kee C, Seo K. The effect of interleukin-1 α on outflow facility in rat eyes. *J Glaucoma.* 1997; 6:246–249. [PubMed: 9264304]
- 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, 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]
- Li G, Luna C, Liton PB, Navarro I, Epstein DL, Gonzalez P. Sustained stress response after oxidative stress in trabecular meshwork cells. *Mol Vis.* 2007; 13:2282–2288. [PubMed: 18199969]
- Liliensiek SJ, Campbell S, Nealey PF, Murphy CJ. The scale of substratum topographic features modulates proliferation of corneal epithelial cells and corneal fibroblasts. *J Biomed Mater Res A.* 2006; 79:185–192. [PubMed: 16817223]
- Matsumoto Y, Johnson DH. Dexamethasone decreases phagocytosis by human trabecular meshwork cells in situ. *Invest Ophthalmol Vis Sci.* 1997; 38:1902–1907. [PubMed: 9286282]
- Pang IH, McCartney MD, Steely HT, Clark AF. Human ocular perfusion organ culture: a versatile ex vivo model for glaucoma research. *J Glaucoma.* 2000; 9:468–479. [PubMed: 11131754]
- Park CH, Latina MA. Effects of gamma-interferon on human trabecular meshwork cell phagocytosis. *Invest Ophthalmol Vis Sci.* 1993; 34:2228–2236. [PubMed: 8505204]
- Peters DM, Herbert K, Biddick B, Peterson JA. Myocilin binding to Hep II domain of fibronectin inhibits cell spreading and incorporation of paxillin into focal adhesions. *Exp Cell Res.* 2005; 303:218–228. [PubMed: 15652337]
- Polansky JR, Fauss DJ, Zimmerman CC. Regulation of TIGR/MYOC gene expression in human trabecular meshwork cells. *Eye.* 2000a; 14:503–514. [PubMed: 11026980]
- Polansky JR, Fauss DJ, Zimmerman CC. Regulation of TIGR/MYOC gene expression in human trabecular meshwork cells. *Eye.* 2000b; 14(Pt 3B):503–514. [PubMed: 11026980]
- Polansky JR, Weinreb RN, Baxter JD, Alvarado J. Human trabecular cells. I. Establishment in tissue culture and growth characteristics. *Invest Ophthalmol Vis Sci.* 1979; 18:1043–1049. [PubMed: 383640]
- Polansky JR, Wood IS, Maglio MT, Alvarado JA. Trabecular meshwork cell culture in glaucoma research: evaluation of biological activity and structural properties of human trabecular cells in vitro. *Ophthalmology.* 1984; 91:580–595. [PubMed: 6540429]
- Rhee DJ, Tamm ER, Russell P. Donor corneoscleral buttons: a new source of trabecular meshwork for research. *Exp Eye Res.* 2003; 77:749–756. [PubMed: 14609563]
- Russell P, Gasiorowski JZ, Nealy PF, Murphy CJ. Response of human trabecular meshwork cells to topographic cues on the nanoscale level. *Invest Ophthalmol Vis Sci.* 2008; 49:629–635. [PubMed: 18235008]
- Samuelson DA, Gelatt KN. Aqueous outflow in the beagle. I. Postnatal morphologic development of the iridocorneal angle: pectinate ligament and uveal trabecular meshwork. *Curr Eye Res.* 1984; 3:783–794. [PubMed: 6734259]
- Santas AJ, Bahler C, Peterson JA, Filla MS, Kaufman PL, Tamm ER, Johnson DH, Peters DM. Effect of heparin II domain of fibronectin on aqueous outflow in cultured anterior segments of human eyes. *Invest Ophthalmol Vis Sci.* 2003; 44:4796–4804. [PubMed: 14578401]
- Schlunck G, Han H, Wecker T, Kampik D, Meyer-Ter-Vehn T, Grehn F. Substrate rigidity modulates cell matrix interactions and protein expression in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci.* 2008; 49:262–269. [PubMed: 18172101]
- Scott PA, Overby DR, Freddo TF, Gong H. Comparative studies between species that do and do not exhibit the washout effect. *Exp Eye Res.* 2007; 84:435–443. [PubMed: 17173894]
- Shen X, Koga T, Park BC, SundarRaj N, Yue BY. Rho GTPase and cAMP/protein kinase A signaling mediates myocilin-induced alterations in cultured human trabecular meshwork cells. *J Biol Chem.* 2008; 283:603–612. [PubMed: 17984096]

- Sherwood ME, Richardson TM. Phagocytosis by trabecular meshwork cells: sequence of events in cats and monkeys. *Exp Eye Res.* 1988; 46:881–895. [PubMed: 3197758]
- Smelser GK, Ozanics V. The development of the trabecular meshwork in primate eyes. *Am J Ophthalmol.* 1971; 1:366–385. [PubMed: 5542135]
- Stamer DW, Roberts BC, Epstein DL, Allingham RR. Isolation of primary open-angle glaucomatous trabecular meshwork cells from whole eye tissue. *Curr Eye Res.* 2000; 20:347–350. [PubMed: 10855028]
- Stamer WD, Huang Y, Seftor RE, Svensson SS, Snyder RW, Regan JW. Cultured human trabecular meshwork cells express functional alpha 2A adrenergic receptors. *Invest Ophthalmol Vis Sci.* 1996; 37:2426–2433. [PubMed: 8933759]
- Stamer WD, Seftor RE, Snyder RW, Regan JW. Cultured human trabecular meshwork cells express aquaporin-1 water channels. *Curr Eye Res.* 1995; 14:1095–1100. [PubMed: 8974838]
- Tamm ER, Russell P, Johnson DH, Piatigorsky J. Human and monkey trabecular meshwork accumulate alpha B-crystallin in response to heat shock and oxidative stress. *Invest Ophthalmol Vis Sci.* 1996; 37:2402–2413. [PubMed: 8933757]
- Tamura M, Iwamoto Y, Nakatsuka K, Yamanouchi U. Immunofluorescence studies of the cytoskeletal and contractile elements in cultured human trabecular cells. *Jpn J Ophthalmol.* 1989; 33:95–102. [PubMed: 2659860]
- Tripathi RC, Li J, Chan WF, Tripathi BJ. Aqueous humor in glaucomatous eyes contains an increased level of TGF-beta 2. *Exp Eye Res.* 1994; 59:723–727. [PubMed: 7698265]
- Tripathi RC, Tripathi BJ. Human trabecular endothelium, corneal endothelium, keratocytes, and scleral fibroblasts in primary cell culture. A comparative study of growth characteristics, morphology, and phagocytic activity by light and scanning electron microscopy. *Exp Eye Res.* 1982; 35:611–624. [PubMed: 6185354]
- Tschumper RC, Johnson DH. Trabecular meshwork cellularity. Differences between fellow eyes. *Invest Ophthalmol Vis Sci.* 1990; 31:1327–1331. [PubMed: 2365563]
- 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]
- Wang N, Chintala SK, Fini ME, Schuman JS. Activation of a tissue-specific stress response in the aqueous outflow pathway of the eye defines the glaucoma disease phenotype. *Nat Med.* 2001; 7:304–309. [PubMed: 11231628]
- Wang WH, McNatt LG, Pang IH, Hellberg PE, Fingert JH, McCartney MD, Clark AF. Increased expression of serum amyloid A in glaucoma and its effect on intraocular pressure. *Invest Ophthalmol Vis Sci.* 2008; 49:1916–1923. [PubMed: 18223246]
- Weinreb RN, Mitchell MD, Polansky JR. Prostaglandin production by human trabecular cells: in vitro inhibition by dexamethasone. *Invest Ophthalmol Vis Sci.* 1983; 24:1541–1545. [PubMed: 6581147]
- Welge-Lüssen U, May CA, Eichhorn M, Bloemendal H, Lutjen-Drecoll E. AlphaB-crystallin in the trabecular meshwork is inducible by transforming growth factor-beta. *Invest Ophthalmol Vis Sci.* 1999; 40:2235–2241. [PubMed: 10476788]
- Welge-Lüssen U, May CA, Lutjen-Drecoll E. Induction of tissue transglutaminase in the trabecular meshwork by TGF-beta1 and TGF-beta2. *Invest Ophthalmol. Vis. Sci.* 2000; 41:2229–2238. [PubMed: 10892867]
- Wentz-Hunter K, Kubota R, Shen X, Yue BY. Extracellular myocilin affects activity of human trabecular meshwork cells. *J Cell Physiol.* 2004; 200:45–52. [PubMed: 15137056]
- Wordinger RJ, Agarwal R, Talati M, Fuller J, Lambert W, Clark AF. Expression of bone morphogenetic proteins (BMP), BMP receptors, and BMP associated proteins in human trabecular meshwork and optic nerve head cells and tissues. *Mol Vis.* 2002; 8:241–250. [PubMed: 12131877]
- Wordinger RJ, Clark AF. Effects of glucocorticoids on the trabecular meshwork: towards a better understanding of glaucoma. *Prog Retin Eye Res.* 1999; 18:629–667. [PubMed: 10438153]
- Wordinger RJ, Clark AF, Agarwal R, Lambert W, McNatt L, Wilson SE, Qu Z, Fung BK. Cultured human trabecular meshwork cells express functional growth factor receptors. *Invest Ophthalmol Vis Sci.* 1998; 39:1575–1589. [PubMed: 9699547]

- Zhang X, Clark AF, Yorio T. Interactions of endothelin-1 with dexamethasone in primary cultured human trabecular meshwork cells. *Invest Ophthalmol Vis Sci.* 2003; 44:5301–5308. [PubMed: 14638730]
- Zhang X, Ognibene CM, Clark AF, Yorio T. Dexamethasone inhibition of trabecular meshwork cell phagocytosis and its modulation by glucocorticoid receptor beta. *Exp Eye Res.* 2007; 84:275–284. [PubMed: 17126833]
- Zhou L, Fukuchi T, Kawa JE, Higginbotham EJ, Yue BY. Loss of cell-matrix cohesiveness after phagocytosis by trabecular meshwork cells. *Invest Ophthalmol Vis Sci.* 1995; 36:787–795. [PubMed: 7706026]

Figure 1. Primary HTM cells from non-glaucomatous donors show high rates of phagocytosis in culture. HTM cells were plated and challenged with particles that emit fluorescence (green) when actively internalized. Four hours after challenge, a high percentage of the particles were phagocytosed by the HTM cells.

Figure 2.

HTM cell alignment is modulated by underlying topographic cues. Cells were cultured for 24 hours prior to fixation, phalloidin staining and imaging. Cells oriented between 0-10° to the repeating ridge and groove pattern of the biomimetic substrate were defined as parallel. On the control planar surface, the cells were equally likely to be aligned parallel as perpendicular to an arbitrary, consistent axis.