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Glaucoma-associated myocilin: A better understanding but much more to learn

Zachary T. Resch and Michael P. Fautsch *

Department of Ophthalmology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

Abstract

Over a decade has passed since myocilin was identified as the first gene linked to early and late-onset primary open-angle glaucoma. During this time, considerable effort has been put forth to understand the functional role myocilin has in normal and glaucomatous eyes. Myocilin is expressed in many ocular and non-ocular tissues, is found in both intracellular and extracellular spaces, and has been linked to elevations in intraocular pressure. Mutations in the myocilin gene that have been associated with glaucoma appear to confer a gain-of-functional activity rather than loss of function. Unfortunately, what the normal function of myocilin is and how alterations in the function can confer a glaucoma phenotype have yet to be elucidated. We will review the current understanding of myocilin with special emphasis on the structural makeup of the myocilin gene and protein, its possible physiological roles internal and external to ocular cells, the regulation of intraocular pressure as evidenced through the use of perfusion culture systems and animal models, and as a causative agent in some forms of glaucoma.

Keywords

glaucoma; myocilin; intraocular pressure; POAG; JOAG; TIGR

1. Historical perspective

Glaucoma is a heterogeneous group of disorders that alters visual acuity due to the loss of optic nerve function. As the world's population continues to age, the total number of glaucoma cases will continue to rise. By 2020, it is estimated that 70–80 million people worldwide will suffer from the effects of glaucoma (Quigley, 1996). The most common form of glaucoma is primary open-angle glaucoma (POAG). POAG tends to occur in individuals during the 6th to 8th decades of life and patients present clinically with visual field loss associated with elevations in intraocular pressure (IOP). The exact cause of this elevation in IOP is unknown. However, the site of aqueous resistance that mediates this effect is thought to occur primarily within the trabecular meshwork, a filter-like structure comprised of trabecular beams consisting of extracellular matrix and endothelial-like cells (trabecular meshwork cells) located in the angle of the anterior chamber. Continued research efforts are attempting to identify the site of resistance within the trabecular meshwork and the mechanism that changes the overall resistance resulting in elevated IOP (for reviews, see Johnson and Johnson, 2002; Johnson, 2006; Tan et al., 2006).

Clinically, the administration of glucocorticoids, both topically and systemically, can increase IOP and lead to symptoms similar to POAG (Armaly, 1963; Becker and Hahn, 1964; Skuta

* Corresponding author. Tel.: +1 507 284 2244; fax: +1 507 284 8566. *E-mail addresses:* E-mail: resch.zachary@mayo.edu (Z.T. Resch), E-mail: fautsch@mayo.edu (M.P. Fautsch)..

and Morgan, 1996; Wordinger and Clark, 1999). In the late 1980s, glucocorticoid (dexamethasone) treatment of human monolayer trabecular meshwork cells identified several proteins whose expression was altered following treatment. A 55 kDa and 66 kDa protein were identified by Polansky and colleagues based on expression that increased in a time and dose-dependent manner similar to the elevated IOP observed in patients following steroid treatment (Nguyen et al., 1993; Polansky et al., 1989). These products were eventually determined to arise from the same gene and named the Trabecular Meshwork Inducible Glucocorticoid Responsive gene (TIGR) (Nguyen et al., 1993, 1998; Polansky et al., 1997).

Concurrent to the molecular studies of glucocorticoid gene induction that were being performed, genetic linkage studies in a family with an autosomal dominant pedigree for early onset POAG (also called juvenile open-angle glaucoma; JOAG) mapped a glaucoma-associated region to chromosome 1q21–1q31 (Sheffield et al., 1993). Studies of other families diagnosed with JOAG confirmed 1q21–1q31 as a glaucoma susceptibility locus and narrowed the disease-associated region to 1 cM (Morissette et al., 1995; Richards et al., 1994; Sunden et al., 1996; Wiggs et al., 1994). Stone et al. was able to show that the TIGR gene was located within the chromosome 1q susceptibility locus and that the gene was mutated in about 4% of early onset POAG patients (Stone et al., 1997). The location of TIGR was confirmed in an unrelated study identifying unique retinal genes (Kubota et al., 1997). Kubota et al. named the novel gene myocilin (*MYOC*), based on homology to non-muscle myosin and the identification of the gene product in the connecting cilium of photoreceptor cells (Kubota et al., 1997). Conventional nomenclature set forth by the Human Genome Organization Committee officially designated the *MYOC/TIGR* gene as *MYOC* and the protein product produced from this gene as myocilin.

During the 12 years since the linkage of myocilin to early and late-onset POAG, considerable effort has been invested to determine what the function of myocilin is in normal eyes and how changes in myocilin expression or presence of myocilin mutations may lead to glaucoma. To date, the physiological function of myocilin has yet to be identified. However, significant progress and new clues to myocilin's pathophysiology have been uncovered.

2. Myocilin gene structure and transcriptional regulation

The gene for human myocilin contains three exons, two introns, spans 17 kb, and maps to chromosome 1q24.3–1q25.2 (Kubota et al., 1997; Nguyen et al., 1998) (Fig. 1). The gene produces several transcripts, ranging in size from 1.8 to 2.3 kb. The key difference in the size of the transcripts appears to be due to differential use of three polyadenylation sites at the 3' end (Adam et al., 1997; Borrás et al., 2002; Ortego et al., 1997). The immediate upstream region of the myocilin gene contains several transcriptional regulatory elements including a TATA-box, NF-κB, AP-1, AP-2, E-box, and glucocorticoid response elements. Kirsten et al. showed that myocilin's basal promoter region was contained within 113 base pairs 5' of the transcription start site (Kirstein et al., 2000). DNase footprinting studies revealed that an upstream stimulatory factor (USF) bound to the E-box was critical for basal promoter activity.

As mentioned previously, myocilin was initially identified by its induction following glucocorticoid treatment. The presence of glucocorticoid-like response elements suggested a correlation between binding to these sites and an increase in transcription. However, analysis of up to 2700 nucleotides upstream of the myocilin transcription start site did not identify a functional steroid response element (Kirstein et al., 2000; Shepard et al., 2001). Furthermore, glucocorticoid induction of myocilin was dependent on new protein synthesis, suggesting that its transcriptional regulation by glucocorticoids is a secondary response, not a direct stimulation (Shepard et al., 2001).

3. Myocilin protein

The protein product produced from the myocilin transcript is a 504 amino acid glycoprotein with an isoelectric point of 5.2 (Kubota et al., 1997; Nguyen et al., 1998; Ortego et al., 1997). Myocilin has a predicted molecular weight of 55.3 kDa, with a commonly identified doublet that separates on SDS-PAGE gels between 53 and 57 kDa. The larger of the two proteins is thought to be the result of N-glycosylation at amino acids (aa) 57–59 (Asn-Glu-Ser) (Caballero and Borras, 2001). Cleavage of myocilin resulting in a 35 kDa C-terminal fragment has been reported in human aqueous humor and select monolayer cells engineered to overexpress myocilin (Aroca-Aguilar et al., 2005; Goldwich et al., 2003; Russell et al., 2001). A 66 kDa form has also been reported; however, not all antibodies recognize this product suggesting that this form may be a myocilin-related protein (Ezzat et al., 2008; Nguyen et al., 1998; Polansky et al., 1997).

Human myocilin contains two translation initiation methionine amino acids at aa1 and aa15. Phylogenetic comparison of other myocilin proteins shows that rat myocilin also contains two initiation codons similar to human myocilin, while all identified myocilin proteins from other species have only one initiation codon that corresponds to the second initiation codon in human and rat. Using the SignalP (version 3.0) software analysis program (Bendtsen et al., 2004), all products of the myocilin gene from different species contain N-terminal sequence suggestive of a signaling peptide. A functional signal peptide for myocilin was confirmed by N-terminal sequencing of immunoprecipitated myocilin (Nguyen et al., 1998) and in vitro using microsomal membranes (Caballero et al., 2000).

The N-terminal region of myocilin contains leucine zipper motifs within two coil–coil domains (aa74–aa110 and aa118–aa186). Leucine zippers are found in a wide variety of proteins and have the ability to form homo- and heteromeric complexes, making these motifs important in regulating protein function. Mutagenesis of the leucines to glycines within the leucine zipper found in the second coil–coil domain (aa118–aa186) have shown that these leucine residues are responsible for homodimerization (Fautsch and Johnson, 2001). Interaction with itself may partially explain the presence of non-monomeric myocilin in aqueous humor (Fautsch and Johnson, 2001; Russell et al., 2001). Gobeil et al. reported that the coil–coil domains of myocilin may be important for interactions of myocilin with intracellular, extracellular and cell surface proteins (Gobeil et al., 2006; Stamer et al., 2006). Heteromeric binding within the coil–coil domain has also been described for myocilin's interaction with exosome-like vesicles (Perkumas et al., 2007; Stamer et al., 2006). Also present in the second coil–coil domain is an arginine-rich motif containing seven arginines each separated by 9–10 amino acids (aa125–200). Interestingly, the arginine-rich motif is found on the opposite side of the α -helix that contains the leucine zipper. Whether this motif is involved in protein binding is yet to be determined (Coca-Prados et al., 1999).

The C-terminal globular region of myocilin (aa230–504) contains homology to olfactomedins, a protein family with over 100 members involved in many diverse functions (reviewed in Zeng et al., 2005). Across species, the olfactomedin domain of myocilin is highly conserved, with greater than 80% amino acid sequence identity in many mammals, and a >60% sequence identity in diverse species such as zebrafish and pufferfish, suggesting this region is important for the structure–function properties of the protein. The importance of the olfactomedin domain to myocilin function is highlighted by the presence of >90% of the identified glaucoma-associated mutations being found within this domain (Fig. 2).

4. Expression

Myocilin mRNA is expressed in a number of tissues within and outside the eye (for review see Tamm, 2002). Myocilin gene expression has been identified in the ocular tissues of the

trabecular meshwork, sclera, ciliary body, choroid, cornea, iris, lamina cribosa, retina, and optic nerve (Adam et al., 1997; Kubota et al., 1997; Ortego et al., 1997; Ricard et al., 2001; Tamm et al., 1999). Within the eye, the trabecular meshwork is reported to have the highest level of expression (Adam et al., 1997; Tamm et al., 1999) with equal distribution in the uveal, corneoscleral and juxtacanalicular regions (Huang et al., 2000; Swiderski et al., 2000; Takahashi et al., 2000; Wang and Johnson, 2000). Myocilin protein is found in the trabecular meshwork, cornea, lamina cribosa, ciliary body, iris, retina, post laminar optic nerve head, vitreous and aqueous humor (Fautsch and Johnson, 2001; Karali et al., 2000; Rao et al., 2000; Russell et al., 2001).

Outside the eye, expression of myocilin is found in highest levels in the skeletal muscle and heart (Fingert et al., 1998; Nguyen et al., 1998; Ortego et al., 1997). Other tissues in humans, rats and mice that express myocilin include the mammary gland, small intestine, thymus, prostate, testis, colon, stomach, thyroid, trachea, bone marrow, and brain. (Abderrahim et al., 1998; Adam et al., 1997; Ahmed et al., 2001; Fingert et al., 1998; Swiderski et al., 1999; Taguchi et al., 2000; Tomarev et al., 1998). Myocilin has also been detected in Schwann cells and the myelin sheath of peripheral nerves (Ohlmann et al., 2003), renal podocytes and mesangial cells (Goldwich et al., 2005), and intervertebral discs (Gruber et al., 2006). Myocilin protein has been reported in plasma (Muthusamy et al., 2005), but has not been detected in urine (Goldwich et al., 2005).

The expression of myocilin has been shown to be regulated by several molecules. In addition to steroids, transforming growth factor- β 1 (TGF- β 1) (Tamm et al., 1999) and optineurin (Park et al., 2007) have been reported to stimulate the transcription of myocilin in cultured trabecular meshwork cells. Interestingly, addition of human aqueous humor to primary human trabecular meshwork cells also induces myocilin expression (Fautsch et al., 2005). Whether this is due to the ability of aqueous humor to enhance the secretion of myocilin or whether a factor(s) in aqueous humor induces *MYOC* expression remains to be determined. Both steroids and TGF- β 1 molecules have a delayed stimulatory response (days), suggesting that these molecules are inducing myocilin not as a primary target, but as a secondary effect in trabecular meshwork cells (Shepard et al., 2001). Myocilin can also be induced under stress conditions, particularly mechanical and oxidative (Polansky et al., 1997; Tamm et al., 1999). Conversely, glaucoma medications such as timolol and latanoprost have been shown to decrease expression of myocilin while basic fibroblast growth factor (bFGF) and thyroid hormone T_3 reduce the steroid stimulation of myocilin (Lindsey et al., 2001; Polansky et al., 2000, 2003; Rozsa et al., 2008; Zhao et al., 2003).

Myocilin expression is also dependent on its external environment. In comparison to trabecular meshworks isolated from fresh human eyes or eyes that have been placed in perfusion organ culture of anterior segments [model for studying the human aqueous humor outflow pathway (Johnson and Tschumper, 1987, 1989)], primary monolayer trabecular meshwork cells have reduced myocilin expression (Tamm et al., 1999). This phenomenon can be attributed, at least in part, to the affect of substrate and culturing conditions. We have shown that trabecular meshwork cells cultured in fetal bovine serum (standard growth supplement for trabecular meshwork cells) have lower levels of myocilin when compared to those cultured in 50% human aqueous humor (Fautsch et al., 2005). Furthermore, myocilin expression and secretion is enhanced when cultured on soft extracellular matrices (Schlunck et al., 2008) or on anisotropically ordered nanopatterned substrates (Russell et al., 2008). Additionally, topographic cues including shear forces present *in situ* may also play an important role in maintaining “normal” myocilin production from the trabecular meshwork (Tamm et al., 1999).

5. Localization

As mentioned previously, myocilin contains a signal peptide sequence indicative of a protein that is secreted from the cell. Proteins with signal peptides are generally synthesized, folded, and processed through the endoplasmic reticulum and golgi apparatus prior to exposure to the extracellular milieu (Hebert and Molinari, 2007; Mellman and Warren, 2000; Nickel, 2003; Ponnambalam and Baldwin, 2003). Therefore, it is not surprising that myocilin has been found in a variety of organelles associated with the secretory pathway including the endoplasmic reticulum and golgi apparatus (Clark et al., 2001a,b; Lutjen-Drecoll et al., 1998; Merts et al., 1999; O'Brien et al., 2000; Polansky et al., 2000; Stamer et al., 1998). However, myocilin also associates with mitochondria (Sakai et al., 2007; Ueda et al., 2000; Wentz-Hunter et al., 2002a, 2003) indicating that myocilin has functions within and outside the cell. The association between myocilin and mitochondria appears to be cell specific, since interactions are seen in trabecular meshwork and astrocyte cells, but not in corneal fibroblasts (Wentz-Hunter et al., 2002a, 2003). Sakai et al. showed that myocilin is imported into the mitochondria and associates with inner and outer membranes and in the intramembrane space, but is not associated with mitochondrial matrix (Sakai et al., 2007). Additionally, myocilin has been shown to associate with microtubules (Merts et al., 1999), myosin regulatory light chain (Wentz-Hunter et al., 2002b), flotillin (Joe et al., 2005; Tawara et al., 2000; Ueda et al., 2000, 2002; Ueda and Yue, 2003), gamma-synuclein (Surgucheva et al., 2005), actin stress fibers, and other proteins involved in signaling and metabolism (Fautsch et al., 2006b). The reproducibility of these interactions has been inconsistent, partially due to limitations with the specificity of available antibodies.

Myocilin also associates with exosome-like vesicles (Hardy et al., 2005; Stamer et al., 2006). Exosomes are small microvesicles that are transported to the plasma membrane via microvesicular bodies, where they are released into the extracellular compartment (Keller et al., 2006). This suggests that in addition to the traditional secretory pathway, myocilin may also use an alternative mechanism to be released from the cell. When secreted, myocilin associates with long spacing collagens, elastic like fibers, and sheath derived plaque material within the trabecular meshwork (Ueda et al., 2000). Structural components of the extracellular matrix including fibronectin, laminin, decorin, and collagen types I, III, V and VI have all been associated with myocilin binding as evidenced by dot-blot analysis and yeast two-hybrid plasmid systems (Joe et al., 2005; Tawara et al., 2000; Ueda et al., 2000, 2002; Ueda and Yue, 2003). The interaction of myocilin with fibronectin has been shown to occur within the Hep II domain of fibronectin, a region critical in regulating adhesion, cytoskeletal organization, signal transduction and phagocytosis (Filla et al., 2002; Peters et al., 2005; Wentz-Hunter et al., 2002b). Hevin, a member of the BM-40/SPARC/osteonectin family has also been identified as a myocilin-binding protein (Li et al., 2006).

6. Function

The normal, physiologic function of myocilin is currently unknown. However, some evidence supports a role in cell-matrix interactions. Overexpression of myocilin from trabecular meshwork cells results in a loss of cellular adhesiveness resulting in increased cAMP/PKA activation and inhibition of Rho kinase (Shen et al., 2008). Loss of actin stress fibers and focal adhesions appears to compromise the cellular attachment to extracellular matrix. When exogenous myocilin is added to a fibronectin matrix, human skin fibroblast cells exhibit decreased adhesion and a decreased rate of cellular spreading suggesting myocilin interferes with cell-matrix communication (Peters et al., 2005). Similarly, in glial scars, neurite outgrowth was inhibited in the presence of myocilin suggesting a possible function of myocilin in inhibiting neural regeneration (Jurynek et al., 2003). Additionally, trabecular meshwork cells that have been “wounded” by physical disruption in culture exhibit decreased migratory ability

and altered morphology, becoming more broadened and stellate with the formation of cellular microspikes in the presence of exogenous myocilin treatment. (Park et al., 2006; Wentz-Hunter et al., 2004a,b).

Intracellularly, myocilin may influence mitochondria function. Overexpression of myocilin in trabecular meshwork cells reduced mitochondrial respiration, potentially sensitizing cells and triggering apoptotic events (Sakai et al., 2007). This may lead to loss of trabecular meshwork cells, altering trabecular meshwork function and IOP maintenance. Interestingly, the loss of trabecular meshwork cells has been reported in POAG (Alvarado et al., 1984).

Many of myocilin's activities share similarities to matricellular proteins (for reviews on matricellular proteins, see Alford and Hankenson, 2006; Schellings et al., 2004). Matricellular proteins can be found both intracellularly and extracellularly, have diverse functions, bind to extracellular matrix, influence cell-matrix interactions, and are stress and injury response proteins. Whether myocilin is a matricellular protein remains to be determined. However, identification of myocilin's function is essential to determine if myocilin is important in the regulation of intraocular pressure and normal trabecular meshwork function.

7. Myocilin and glaucoma

One of the first hypotheses regarding the association of myocilin and POAG revolved around the idea that elevated IOP may be caused by increased levels of myocilin, thus resulting in a reduction of outflow facility (increase in outflow resistance). Nyguen et al. hypothesized that increased levels of myocilin may accumulate within the trabecular meshwork and interfere with normal flow patterns, thus resulting in elevated IOP (Nguyen et al., 1998). This was supported by immunohistochemical studies indicating that some POAG eyes had elevated levels of myocilin (Lutjen-Drecoll et al., 1998) and that steroid treatment of trabecular meshwork monolayer cells or cultures of anterior segments increased myocilin in a time and dose-dependent manner that correlated with the timing and increase of IOP (Clark et al., 2001b; Nguyen et al., 1993; Polansky et al., 1989). Similar results were observed in primates where treatment with steroids for one-year resulted in elevated IOP and a 3-fold increase in trabecular meshwork myocilin levels (Clark et al., 2001b). We have found that myocilin levels are statistically elevated in human aqueous humor per volume and as a percent of total protein in POAG when compared to normal controls (patients undergoing cataract surgery) suggesting a correlation between increased levels of myocilin and POAG (manuscript submitted). Additional studies by our lab perfusing purified recombinant myocilin overexpressed and isolated from either bacterial lysate or conditioned media from a human transformed trabecular meshwork cell line into human anterior segments, increased outflow resistance by >90% (Fautsch et al., 2000, 2006a). Purified recombinant myocilin from trabecular meshwork cells overexpressing myocilin did not elevate pressure when used in anterior segments cultured in Dulbecco's Modified Eagle's Medium (standard culture medium), but did increase IOP in the presence of porcine aqueous humor, suggesting the formation of an unidentified protein complex with myocilin that was responsible for the elevation in IOP. Goldwich et al. has also shown that perfusion of the C-terminal domain of myocilin did not result in elevated IOP, suggesting the N-terminal region of myocilin may be important for IOP changes (Goldwich et al., 2003).

Additional evidence in animal models suggests an association of increased myocilin levels and IOP. Rats that spontaneously develop increased IOP had a 4-fold increase in myocilin transcription (Naskar and Thanos, 2006). Several canine breeds that have primary glaucoma showed elevated levels of myocilin in aqueous humor, trabecular meshwork and non-pigmented ciliary epithelium within regions of the ciliary processes (Hart et al., 2007; Mackay et al., 2008a,b). Similarly, beagles genetically susceptible to the formation of glaucoma had

increased levels of myocilin in the aqueous humor in a direct relation to the severity of the disease (Mackay et al., 2008b).

Although evidence in human eyes and some animal models seemed to validate the hypothesis that overexpression of myocilin may contribute to elevated IOP, not all studies confirm this opinion. Borrás et al., using adenovirus to express myocilin, showed that overexpression of myocilin in trabecular meshwork cells decreased IOP in perfusion organ culture of human anterior segments (Caballero et al., 2000). Transgenic mice overexpressing either mouse or human myocilin showed no change in IOP (Gould et al., 2004; Zillig et al., 2005). Steroid-treated rats with elevated IOP found normal levels of myocilin in the trabecular meshwork (Sawaguchi et al., 2005) and a feline model overexpressing myocilin for >1 year did not show any change in IOP (Khare et al., 2008). Further complicating the story, deletion of one or both copies of myocilin in mice did not have any effect on IOP or ocular morphologies (Kim et al., 2001). Whether these differences can be attributed to species variations or the mode of delivery of the overexpressed myocilin is unknown.

It is important to remember that myocilin is induced by several forms of stress including mechanical and oxidative stress (Polansky et al., 1997; Tamm et al., 1999). Elevated IOP induces myocilin transcription levels, but in a delayed manner, taking up to 24 hours to increase (Borrás et al., 2002). It is feasible to speculate that elevated levels of myocilin, particularly by steroid treatment, may be a secondary effect of elevated IOP, not the primary cause. Therefore, increased levels of myocilin would not cause the elevated IOP, but may be the result of elevated IOP.

8. Myocilin mutants

Myocilin mutations have been found in 2–4% of POAG patients with higher percentages being reported for JOAG (Fingert et al., 1999; Kanagavalli et al., 2003; Sripriya et al., 2004; Stone et al., 1997). In the 12 years since Stone et al. identified myocilin as the gene associated with JOAG and POAG (Stone et al., 1997), over 70 glaucoma-causing mutations have been identified (Fig. 2). Greater than 90% of these mutations are found in the C-terminal globular domain containing homology to olfactomedins. Several of the myocilin mutations are believed to have a common founder (Fingert et al., 1999; Hewitt et al., 2007). For example, the T377M myocilin mutation and its founder effect have been documented in five continents (Hewitt et al., 2007). Myocilin mutations also have a strong genotype–phenotype correlation. Individuals with the T377M mutation are generally diagnosed in their 4th decade, later than the P370L mutation (1st and 2nd decades) and Y437H (2nd decade; 44 mmHg mean maximal IOP), but earlier than the most common disease-associated mutation in Caucasians, Q368X, that has an average onset in the 5th and 6th decades and a mean maximal IOP of 30 mmHg (Fingert et al., 1999; Hewitt et al., 2007; Kanagavalli et al., 2003; Melki et al., 2003; Puska et al., 2005; Shimizu et al., 2000).

Less than 10% of the glaucoma-causing mutations in myocilin have been identified in the N-terminus. Two involve cysteine residues (potential for disulfide bond formation or disruption). One of the mutations (C25R) is in the purported signal peptide sequence, and may interfere with secretion. The other mutation involves the cysteine at aa47. Three other mutations involve arginines at aa82, aa91, and aa126. Whether or not these mutations disrupt structure in the N-terminus (arginine-like zipper) or if they interfere with protein–protein interactions remains to be determined.

The majority of myocilin proteins that contain an amino acid alteration are not secreted, but rather accumulate within the endoplasmic reticulum as homo- or heterodimers (Caballero and Borrás, 2001; Caballero et al., 2000; Gobeil et al., 2004; Joe et al., 2003; Kanagavalli et al., 2007; Oezbey et al., 2007; Wang et al., 2007; Yam et al., 2007a; Yuan et al., 2007). The protein

conformational changes caused by many mutants can be relaxed when cells overexpressing individual myocilin mutants are grown at 30 °C (Liu and Vollrath, 2004). Interestingly, the more virulent glaucoma phenotypes (P370L, I477N, Y437H) were less sensitive to secretion at 30 °C compared to glaucoma phenotypes that are less virulent (T377M, G364 V, I499F, D380A). Additionally, treatment of cells with sodium 4-phenylbutyrate reduced the amount of insoluble heterogeneous myocilin aggregates that were formed and restored the secretion of myocilin mutants C245Y, P370L, and Y437H (Yam et al., 2007b).

Several sequence variations have been identified in the transcriptional regulatory region (for a review see Hewitt et al., 2008). The most common sequence variation appears to be a cytosine to guanine base pair substitution at position –1000 [mt.1(+)] (Alward et al., 2002; Colomb et al., 2001; Copin et al., 2002; Lopez-Martinez et al., 2007; Ozgul et al., 2005; Polansky et al., 2003). This sequence variation has been reported in up to 20% of POAG patients with a suggestion that individuals with the mt.1(+) variation have a more rapid disease progression (Polansky et al., 2003). Not all studies have confirmed the association of the mt.1(+) variant with POAG (Alward et al., 2002) and the use of OcuGene, a genetic test for the mt.1(+) variant to determine potential risk, has been met with limited enthusiasm.

Other sequence variations in the transcriptional control region have also been identified. Saura et al. identified five sequence variations associated with potential transcription regulatory sequences in a cohort of 79 patients with POAG (Saura et al., 2005). Three of the sequence changes (–77G>C, –78G>C, –83G>A) were identified in the E-box, a DNA sequence found to be essential for basal transcriptional regulation of myocilin (Kirstein et al., 2000). Whether these sequence variations interfere with transcription factors binding to these DNA motifs and alter myocilin transcription levels remains to be determined.

9. Mutant myocilin and glaucoma

While the mutations within the transcription regulatory region (promoter and enhancer region) may change the level of expression of the myocilin mRNA transcript, the alterations in the protein coding sequence have the ability to alter the three dimensional structure, effectively burying important charges that regulate cleavage sites and presumably normal function (Kanagavalli et al., 2007; Rozsa et al., 1998). The sequestration of myocilin mutants in the endoplasmic reticulum may result in a gain-of-function, unrelated to the normal function of myocilin (Kim et al., 2001). The identification of homozygous and heterozygous family members for myocilin mutant R46X, which deletes all but 13 amino acids of the processed protein, does not result in glaucoma. This phenomenon suggests that myocilin may not be a factor in glaucoma development (Lam et al., 2000). Furthermore, targeted disruption of myocilin in mice did not cause ocular changes or elevated IOP in mice carrying one normal copy or no normal copies of the myocilin gene (Kim et al., 2001). These studies have laid the foundation for the hypothesis that an as yet unidentified function gained through the mutation must influence the physiologic development of glaucoma.

Mutant myocilin forms heterodimers with normal myocilin, effectively reducing the levels of secreted normal myocilin from trabecular meshwork cells (Caballero and Borrás, 2001; Gobeil et al., 2004, 2006; Jacobson et al., 2001; Yam et al., 2007a). Liu and Vollrath speculated that prolonged expression of mutant myocilin may lead to retention of the mutant, causing an endoplasmic reticulum overload response or unfolded protein response (Liu and Vollrath, 2004). Yam et al. have shown that heteromeric myocilin complexes aggregate to form inclusion bodies typical of Russell bodies (Yam et al., 2007a). Like Russell bodies, heteromeric myocilin complexes do not get translocated to the cytosol or extracellularly, leading to the induced expression of immunoglobulin heavy chain binding protein (BiP), endoplasmic reticulum-localized eukaryotic initiation factor (eIF-2 α) kinase, and activation of caspase 3 and 12, all

indicative of an endoplasmic reticulum storage disease leading to apoptotic death (Gobeil et al., 2004; Yam et al., 2007a). Furthermore, if normal myocilin has a de-adhesive effect as postulated (Wentz-Hunter et al., 2004a,b), then retention of normal myocilin in the endoplasmic reticulum by mutant myocilin would reduce the level of extracellular myocilin and potentially cause a stiffer, less-pliable trabecular meshwork, possibly resulting in elevated IOP.

Glaucoma is mainly a human disease, with only sporadic cases found in other animals. Therefore, if myocilin is involved in glaucoma, specific characteristics of human myocilin may give us clues as to how mutated myocilin may function. Shepard et al. reported that the human myocilin sequence contains a peroxisomal targeting signal-1 receptor binding site (PTS1R) that is not found in any other myocilin identified from different species (Shepard et al., 2007). Shepard et al. showed that transduction of mouse eyes with an adenovirus carrying the human Y437H myocilin mutation caused an increase in IOP while mouse eyes transduced with either the mouse Y423H mutation (equivalent mutation in mouse to Y437H in humans), human Q368X (removes PTS1R), or S502P (disrupts PTS1R binding site) failed to show a myocilin mutation-related increase in IOP. Although location and amounts of mutant myocilin following adenovirus transduction were not reported, Shepard et al. hypothesized that the association with the PTS1R leads to the misfolding of myocilin and shuttling to peroxisomes, thus leading to cellular stress and death. Furthering this notion that myocilin mutations only have a phenotype in selected species, Gould and colleagues report that mutated mouse myocilin Y423H (equivalent to human Y437H) is not secreted and does not cause increase in IOP (Gould et al., 2006). Feline Y423H (equivalent to human Y437H) also did not cause a change in baseline IOP (Khare et al., 2008). Recently, Zhou et al. confirmed that human myocilin mutant Y437H when overexpressed in mice resulted in a glaucoma phenotype, supporting the idea that only human myocilin mutations may be able to function in a manner that leads to glaucoma (Zhou et al., 2008). In contrast, work by Senatorov et al. suggests that overexpression of mutant murine myocilin (Y423H) was able to increase IOP marginally in mice (Senatorov et al., 2006). Additional studies aimed at delineating these differences in animal models overexpressing human, murine, or feline myocilin mutants will be important for determining how mutated myocilin alters IOP and displays a glaucoma-like phenotype.

10. Concluding remarks

From the identification in the late 1980s to the association with glaucoma in the late 1990s, the immense research conducted by many scientists has led to a foundational understanding of myocilin as we near the end of the first decade of the 21st century. However, many important questions remain unanswered. What is myocilin's normal physiologic function in the eye? How do changes in myocilin function lead to glaucoma? What is the "gain-of-function" attributed to the mutant forms of the proteins? Is myocilin-associated glaucoma a "protein conformational disease"? What is the function of normal myocilin outside of the eye and how do myocilin mutations alter its function in other tissues? These questions, as well as further studies in animal models with additional myocilin mutant proteins, will help to clarify its functional role. However, while the use of animal models, particularly mice, is attractive due to the relative ease in which investigators can manipulate their genome, the physiological regulation of IOP can be significantly different when compared to primates. Therefore, understanding how results in animal models translate to humans must be considered (Table 1).

Myocilin's expression throughout the eye and its secretion into the outflow pathway as part of aqueous humor suggest possible roles for myocilin in maintaining or influencing outflow that is only partly substantiated by published reports. Changes in stress, aqueous humor protein content, or the effect of potential modifier genes such as CYP1B1 (Vincent et al., 2002) need to be factored into the role they may have on myocilin function. An appreciation of myocilin

in concert with other factors is necessary to help delineate its role in outflow regulation. Future endeavors aimed at myocilin function will be essential to understanding myocilin's role in the eye as well as other tissues of the body.

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Fig. 1.

Structure of human myocilin. Analysis of myocilin's primary sequence identifies several structural motifs: a signal peptide sequence [amino acid (aa)1–32], a helix-turn-helix (HtH) domain (aa18–58), two coil-coil (CC) domains (aa74–110 and aa118–186), and a C-terminal globular domain (aa230–504) that contains homology to olfactomedins (aa326–501), a protein family involved in many diverse functions. Asterisks represent leucine amino acids involved in leucine zipper. Number sign denotes N-glycosylation site. Arrow indicates calpain II cleavage site.

Fig. 2.

Glaucoma-causing mutations in myocilin. Over 70 myocilin glaucoma-causing mutations have been identified in myocilin, with >90% found in exon 3 that codes for the olfactomedin-homology domain of myocilin. Gene structure is shown on right. Numbers under the exons denote codon location in gene sequence.

Table 1**Myocilin animal models**

Species	Myocilin expressed	Effect on IOP	Reference
Mouse	Mutant Y437H ^a	Increased	Zhou et al. (2008)
Mouse	MYOC overexpression ^a	No effect	Shepard et al. (2007)
Mouse	Mutant G364V, Y437H ^a	Increased	Shepard et al. (2007)
Mouse	Mutant Q368X, S502P ^a	No effect	Shepard et al. (2007)
Mouse	Mutant Y423H ^b	Increased	Senatorov et al. (2006)
Mouse	Mutant Y423H ^b	No effect	Gould and Reedy (2006)
Mouse	Mutant Y437H ^a	Increased	Zillig et al. (2005)
Mouse	MYOC overexpression ^a	No effect	Zillig et al. (2005)
Mouse	MYOC overexpression ^b	No effect	Gould et al. (2004)
Mouse	MYOC Knockout	No effect	Kim et al. (2001)
Feline ^d	MYOC overexpression ^c	No effect	Khare et al. (2008)
Feline ^d	Mutant Y423H ^c	No effect	Khare et al. (2008)
<i>Naturally occurring models</i>			
Species	Phenotype	Myocilin levels	Reference
Canine	Glaucomatous	Elevated	Mackay et al. (2008a)
Beagle	Glaucomatous	Elevated	Mackay et al. (2008b)
Rat	Elevated IOP	Elevated	Naskar and Thanos 2006

^a Human mutant.^b Murine mutant.^c Feline myocilin or myocilin mutant.^d Overexpression due to transduction of the anterior chamber with feline-immunodeficiency virus (FIV) containing myocilin constructs.