Rho/Rho-associated kinase pathway in glaucoma (Review)

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Abstract. The Rho/ROCK pathway plays important roles in the modulation of the cytoskeletal integrity of cells, the synthesis of extracellular matrix components in the aqueous humor outflow tissue and the permeability of Schlemm's canal endothelial cells. The activation of the Rho/ROCK pathway results in trabecular meshwork (TM) contraction, and the inhibition of this pathway would provoke relaxation of TM with subsequent increase in outflow facility and, thereby, decrease intraocular pressure (IOP). ROCK inhibitors also serve as potent anti-scarring agents via inhibition of transdifferentiation of tenon fibroblasts into myofibroblasts. Furthermore, the RhoA/ROCK pathway is involved in optic nerve neuroprotection. Inactivation of Rho/ROCK signaling increase ocular blood flow, improve retinal ganglion cell (RGC) survival and promote RGC axon regeneration. Considering the IOP modulation, potent bleb anti-scarring effect and neuroprotective properties of ROCK inhibitors, the Rho/ROCK pathway is an attractive target for anti-glaucoma therapy, and it may be used for human therapy in the near future.

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1. Introduction

Glaucoma is a leading cause of permanent blindness and is characterized by progressive retinal ganglion cell (RGC) death that produces characteristic optic nerve head damage and visual field loss (1,2). Some risk factors are related with glaucoma pathogenesis. These include intraocular pressure (IOP), age, family history, clinical appearance of the optic nerve, race and potential vascular disease (3-6). Of these, elevated IOP is considered as a major risk factor for glaucoma, and lowering IOP is the most effective treatment method available for glaucoma (1,2).

Several prospective randomized multi-center studies have identified that IOP reduction with either medicines or surgery can reduce the development and progression of vision loss in glaucoma patients (7-13). The precise mechanisms that lead to the death of RGCs in glaucoma have not been identified conclusively, but might involve the blockade of both anterograde and retrograde axonal transport leading to the deprivation of neurotrophic signals (2). If IOP is beyond the tolerable range of the optic nerve, RGCs axons degenerate at the optic nerve head in the region of the lamina cribrosa, a process that occurs in parallel to the apoptotic death of RGCs. The glaucomatous neuropathy might occur in parallel to a remodeling of the extracellular matrix (ECM) of the optic nerve head (2,14,15).

IOP is determined by the equilibrium between the secretion of aqueous humor by the ciliary body and the drainage of aqueous humor from the eye. There are two main aqueous humor outflow pathways, trabecular (conventional) and uveoscleral (unconventional). The unconventional route of this drainage is through the interstitial spaces of the ciliary muscle and the supraciliary space, whose physiological role is not fully understood. The conventional outflow pathway is composed of the trabecular meshwork (TM), juxtacanalicular tissue (JCT), Schlemm's canal (SC), and the episcleral veins on a continuous basis, and in humans, this pathway represents a predominant route of aqueous humor drainage (16,17). The ciliary secretion of aqueous humor usually remains normal in glaucoma (18), therefore, it is thought that impaired drainage through the trabecular pathway caused by increased resistance is the primary cause for increased IOP in primary open-angle glaucoma (POAG) (18).

The normal aqueous humor outflow resistance resides in the inner wall region of the trabecular meshwork outflow pathways (19,20). The site of highest resistance remains uncertain (21,22), but likely resides at the confluence of the TM, JCT and SC inner wall (21,23). It has been proposed that abnormal accumulation of extracellular material/ECM (ECM hypothesis), and changes in contractile activity and cell adhesive interactions of the cells of aqueous outflow pathway (contractility hypothesis) are contributed to increases resistance to drainage of aqueous humor through the conventional pathway (16-18,24-26). The ECM hypothesis is supported by the observation that perfusion of anterior eye segments in organ cultures with metalloproteinases that digest ECM components leads to a reversible increase in outflow facility (27). The contractility hypothesis is supported by the observation that experimental disruption of the actin cytoskeleton of the trabecular meshwork decreases outflow resistance (28,29) and by recent findings which provide evidence that the trabecular meshwork of patients with primary open angle glaucoma is stiffer than that of age-matched controls (30). The two hypotheses can exist simultaneously, since it is possible that trabecular meshwork cells that increase their contractile capabilities simultaneously synthesize more fibrillar matrix to transmit more force.

Over the past few years, many studies have shed light on the important role of Rho/Rho-associated kinase (ROCK) pathway in the pathogenesis and treatment of glaucoma. The purpose of this review is to summarize the role of Rho/ROCK pathway in the IOP modulation, subconjunctival scarring of the filtering bleb and neuroprotection of glaucoma.

2. Rho/ROCK pathway

Rho is a member of Rho family of small molecular guanosine triphosphatase (GTPase) superfamily related to Ras. Rho has three isomer types: RhoA, RhoB and RhoC (31). ROCK is a serine/threonine kinase and one of the major downstream effectors of Rho GTPases (32). ROCK has two isomer types: ROCK1 and ROCK2. The structures of ROCK1 and ROCK2 are conserved with 64% overall amino acid identity (33,34). The kinase domain containing both extension segments is more highly conserved between these two proteins (83% identical), suggesting that they may have similar substrate specificity (33,34). Both Rho-kinase proteins are ubiquitously expressed in most tissues; however, higher levels of ROCK2 are found in brain and muscles whereas higher levels of ROCK1 are found in non-neuronal tissues including liver, lung and testis (33,35). ROCK1 is specifically cleaved by caspase-3, whereas ROCK2 is cleaved by granzyme B (36-38). The Rho GTPases act as molecular switches by cycling between an active GTP-bound and an inactive GDP-bound form. In the GTP-bound form, the Rho GTPase interact with specific downstream effector proteins - ROCK, which include Rho kinase, regulators of actin polymerization and adaptor proteins (32). The activity of Rho GTPase is regulated by signaling input originating from different classes of cell surface receptors, including the heterotrimeric G protein-coupled receptors tyrosine kinase receptors, cytokine receptors, frizzled receptors, and adhesion receptors (32,39). Rho/ROCK pathway has critical functions in the formation of actin stress fibers and focal adhesions (40-43), and the regulation of actomyosin cytoskeletal organization, cell adhesion, cell morphology, cell motility, smooth muscle contraction, neurite elongation and neuronal architecture and cytokinesis (44-55).

Rho/ROCK pathway is involved in various cellular functions through phosphorylation of their specific substrates. The main substrates of Rho/ROCK pathway is the myosin light chain (MLC), LIM kinase 1 (LIMK1), LIMK2 and myosin phosphatase target subunit 1 (MYPT1) (56-58). Gene silencing experiments suggest ROCK1 appears to be essential for the formation of stress fibers, whereas ROCK2 appears to be necessary for cytoskeletal rearrangements, cell motility and cell contraction, both of which are dependent on MLC phosphorylation (59,60). The phosphorylation status of MLC is controlled not only by myosin light chain kinase (MLCK), but also by myosin light chain phosphatase (MLCP). MLC is phosphorylated by Ca^{2+}/calmodulin-dependent MLCK and dephosphorylated by Ca^{2+}-independent MLCP, and the balance between these two enzyme activities is a critical determinant of MLC phosphorylation (61-63). Phosphorylation of MLC subsequently results in stimulation of the myosin-actin interactions. Increased and decreased MLC phosphorylation induces contraction and relaxation responses of the cell and influences the formation of actin stress fibers and smooth muscle contraction. ROCK is implicated in the RhoA-mediated inhibition of MLCP (64). Inhibition of ROCK results in an increased activity of MLCP and dephosphorylation of MLC. Thus, Rho/ROCK pathway is a master regulator of the actin cytoskeleton and cell contractility (32,51,65,66). Growth factors, mechanical stretch, cytokines and ECM can activate Rho GTPase through guanine nucleotide exchange factors. This subsequently activates ROCK, which then leads to MLC phosphorylation that enhances actomyosin cross-bridging and contractility, thereby regulating many cell processes including contraction, cytoskeleton organization, adhesive interactions, trafficking and permeability (48,51,65,67-70).

3. The expression of Rho/ROCK in aqueous humor outflow pathways

In vitro and *in vivo* studies have shown that Rho and ROCKs are expressed in the cells of outflow pathway (53,71-74). Immunoblot analyses have shown that RhoA and ROCKs are present in cultured human TM cells (71-73), SC cells (73) and bovine ciliary muscle (CM) tissues (72). Using RT-PCR analysis, Nakajima and collaborators found ROCK1 and ROCK2 in TM and CM (53). Both in humans and in monkeys ROCKs were expressed in TM more abundantly than in CM (53). Goldhagen *et al* using immunohistochemical analysis found RhoA, ROCK1 and ROCK2 were all distributed in the human aqueous outflow pathway including TM, JCT and SC, and observed no significant difference in Rho/ROCK pathway expression in the outflow tissue between normal eyes and those with glaucoma (74). It is hypothesized that there would be increased expression of the Rho/ROCK pathway in the outflow tissue in glaucomatous eyes. However, the results of Goldhagen *et al* showed no significant expression difference of Rho/ Rho kinase between normal eyes and glaucomatous eyes (74), it can be explained that many medications used for the management of glaucoma may be affecting the expression of Rho/Rho kinase within the outflow pathway.

4. Rho/ROCK pathway and the cytoskeletal integrity of cells in the outflow tissue

The TM beams have been characterized as connective tissue containing elastic and collagen fibers surrounded by endothelial-like trabecular cells resting on a basement membrane (16). The outermost JCT or cribriform region has no collagenous beams, but rather several cell layers immersed in a loose web of ECM fibrils. The adjacent SC is a continuous endothelium-lined channel that drains aqueous humor to the general venous circulation (29). The TM cells exhibit a smooth muscle-like phenotype, based on their expression of various smooth muscle-specific proteins, including α-smooth muscle actin (α-SMA) and CPI-17 (the 17 kDa protein kinase C-potentiated protein phosphatase 1 inhibitor protein) (24,28,29,75,76). The actomyosin system, composed of actin microfilaments and associated proteins, is present in essentially all cells, and is highly organized in TM and SC cells. There are numerous microfilament-based structures in cells along the trabecular outflow pathway. These structures primarily include focal contacts, adherens cell-cell junctions and bundles of microfilaments (77). A physiologically contracted state of the JCT-SC region is required to maintain the microfilament-related structures in the outflow pathway (29). Microfilaments are involved in a variety of cellular processes from cell adhesion and motility to organelle traffic to adhesion-mediated signal transduction. As discussed above, ROCK mainly promotes myosin II activity by inhibiting MLCP as well as by phosphorylating the myosin regulatory light chain. This, in turn, induces the assembly of contractile actomyosin bundles that generate strong tensile forces (65). A specific Rho kinase inhibitor, Y-27632, induces reversible changes in cell shape and decreases in actin stress fibers, focal adhesions, and protein phosphotyrosine staining in human TM cells and SC cells (72,73). In isolated bovine TM strips, Y-27632 completely blocks Ca^{2+}-independent phorbol myristate acetate or endothelin-1-induced contraction (71,78,79). A morphological study in bovine eyes indicates that, with Y-27632, the inner wall of SC and the JCT are significantly distended compared to control eyes, with discernible separation between the inner wall of SC and JCT, which suggests that the structural correlate to the increase in outflow facility of non-human eyes after Y-27632 is physical separation between the JCT and inner wall of SC (80).

Regulation of mechanical and contractile properties of the pressure-sensitive TM cells is recognized to play a significant role in modulation of aqueous humor outflow and ocular pressure homeostasis (20,24,81-83). There is growing evidence, that contraction of TM reduces aqueous humor outflow and thus enhances intraocular pressure, whereas relaxation exerts the opposite effect (24,29,72,75,76,84). The activation of Rho/ROCK pathway could result in TM contraction, and the inhibition of this pathway would provoke relaxation of TM with subsequent increase in outflow facility (53,72,73,82). As expected, ROCK inhibitors, such as Y-27632, Y-39983, HA-1077, H-1152, increase outflow facility and/or decrease IOP in animals (72,85-88). Conversely, agents that activate Rho GTPase and myosin II activity, including lysophosphatidic acid (LPA), sphingosine-1-phosphate, TGF-β2, and endothelin-1, decrease aqueous humor outflow facility concomitant with increased contractile activity of the TM cells, indicating a potential importance of actomyosin organization and the contractile force generated by the actomyosin system in the regulation of aqueous humor drainage (76,84,89-91). In addition to the effect on the contractility of cells in trabecular (conventional) outflow, Rho/ROCK pathway may also modulate the contractility of tissues in uveoscleral (unconventional) outflow (72,92). CM is one of the main tissues in the uveoscleral outflow pathway, and CM cells morphologically and electrophysiologically express properties that are typical of smooth muscle cells (93). The ROCK inhibitor Y-27632 has been shown to induce inhibition of smooth muscle contraction and alter various cellular behavior (94,95). Moreover, Y-27632 can relax the excised ciliary muscle which is previously constricted by carbachol, suggesting that the inhibitor acts to increase the uveoscleral outflow (92). However, there is also evidence to the contrary. ROCK and its substrates show higher expression in TM compared to CM (53), and ROCK inhibitor Y-39983 leads to relaxation of TM, but Y-39983 is only slightly effective in CM (86). Honjo *et al* also reported that only a modest increase in the uveoscleral outflow was found in rabbit eyes by Y-27632, and its effects were not statistically significant (72). These results suggest that the mechanism for decreased IOP by ROCK inhibitor is largely mediated by enhancement of aqueous outflow facility through relaxation of TM in the conventional outflow pathway (24).

5. Rho/ROCK pathway and ECM in the outflow tissue

Alterations in ECM content and organization have been found to be associated with increased resistance in the outflow pathway of human glaucomatous eyes (96-100). Rho/ROCK pathway has an important role for modulating the synthesis of ECM components in the trabecular pathway. Pattabiraman and Rao found that human TM cells expressing a constitutively activated form of RhoA (RhoAV14) demonstrated increased levels of fibronectin, fibronectin fibril formation, laminin, tenascin C and α-SMA (101). Furthermore, the changes in expression of ECM proteins could be suppressed by the Rho GTPase inhibitor (C3 transferase) and ROCK inhibitor (Y-27632), in association with decreased MLC phosphorylation, actin stress fibers, focal adhesions and fibronectin fibrils (101). Zhang *et al* reported that TM cells expressing a constitutively activated form of RhoA had increased expression of various ECM-related genes and cytokines such as TGF-β, interleukin-1, and connective tissue growth factor (CTGF) in TM cells (91). The stimulation of TM cells with physiological agonists such as LPA and TGF-β2, which are known to induce Rho GTPase activation and MLC phosphorylation in TM (90,102), leads to an increase in levels of fibronectin, fibronectin fibrils, laminin and α-SMA in a RhoA- and Rho kinase-dependent manner. In the case of TGF-β2, increased resistance to aqueous humor outflow is reported to be associated with increased levels of synthesis of ECM components (25,98,103). CTGF has also an important role in ECM synthesis, Iyer *et al* reported that stimulation of human TM cells with CTGF treatment for 24 h led to an increase in the levels of laminin, fibronectin, and in the levels of phosphorylated MLC in human TM cells, and that the expression of CTGF is regulated closely by Rho GTPase (104).

There is a potential interplay among the contractile activity, ECM synthesis and Rho GTPase activation (105-109). As mentioned above, the activation of Rho GTPase and ROCK was able to promote myosin II phosphorylation and contractile activity (53,72,73,82), and to induce ECM synthesis/assembly in TM cells (91,101,104). On the other hand, the actomyosin-derived contractile force induced ECM synthesis/assembly and, conversely, ECM assembly/rigidity could influence actomyosin contraction and induce Rho GTPase activation (91). ECM rigidity has been reported to increase fibronectin fibril formation, Erk activation, focal adhesion kinase activity, α-SMA, and actin stress fibers in TM cells (110). The interplay among contractile activity, ECM synthesis/assembly and Rho GTPase activation in the cells of aqueous humor outflow pathway, including TM, JCT and SC cells, represents a crucial regulatory component in the homeostasis of aqueous humor outflow resistance (101).

6. Rho/ROCK pathway and permeability of the SC endothelial cells

The permeability of SC endothelial cells is suggested to play important roles in the regulation of aqueous outflow (17,111). Breaks have been found in the endothelial lining of the SC and aqueous plexus after perfusion with certain cytoskeletal drugs (112-114). Additionally, SC endothelial cells have transcellular pores accompanied by giant vacuoles (111,115). ROCK inhibitor Y-27632 resulted in Rho/ROCK-dependent filamentous actin reorganization and disruption of proteins associated with tight junction, increased SC endothelial-cell monolayer permeability, which may lead to increased aqueous humor outflow facility (73,111).

7. Rho/ROCK pathway and IOP modulation

Rho/ROCK pathway has a crucial role in IOP modulation. In general, the activation of Rho/ROCK pathway in the outflow tissue results in reduction of aqueous humor outflow, and thereby increase IOP, whereas the inhibition of Rho/ROCK pathway tissue results in increase of aqueous humor outflow, and thereby decrease IOP. Organ-cultured anterior segments from porcine eyes expressing RhoAV14 exhibited significant reduction of aqueous humor outflow (91). However, inhibiting RhoA expression in TM with siRNA is effective in suppressing elevated IOP in mice (116). Furthermore, several ROCK inhibitors, such as Y-27632, Y-39983, HA-1077 and H-1152, increase outflow facility and/or decrease IOP in living rabbits, mouse, rat, monkeys, human and enucleated porcine eyes (72,73,76,85-88,92,117-120). In monkey eyes, 0.05% Y-39983 induces significant IOP reduction almost equal to that obtained with 0.005% latanoprost (86). SNJ-1656, an ophthalmic solution of Y-39983, has been proved as a safe topical agent that is effective in reducing IOP in healthy adult volunteers (87). Thus, ROCK inhibitors might be a candidate for the next generation of glaucoma therapy (53,119).

8. Rho/ROCK pathway and wound healing of filtration canal

Filtration surgery, such as trabeculectomy, is the most widely used anti-glaucoma surgery. The most frequent cause of failure of glaucoma filtration surgery is postoperative scarring in the filtering bleb. Fibroblasts from the subconjunctival space play a key role in the scarring process. Perioperative administration of antimetabolites such as 5-fluorouracil and mitomycin C (MMC) is effective in limiting the scarring process. However, use of these antiproliferative agents is accompanied by severe side-effects (121,122). Therefore, alternative anti-scarring agents that do not cause extensive tissue damage are needed.

Subconjunctival scarring of the filtering bleb site is mainly mediated by tenon fibroblasts (TFs) proliferation, migration, and contraction (123-125). Transdifferentiation of fibroblasts into myofibroblasts is a crucial step in wound healing and scar formation (126), which is associated with expression of α-SMA (127). Enhanced α-SMA expression indicates the presence of activated fibroblasts with increased synthesis of ECM proteins, growth factors and integrins (128,129). Myofibroblasts are responsible for fibrosis via increased ECM synthesis, for granulation tissue formation, wound contraction and scar formation (126,130-132). TFs are stimulated by growth factors to differentiate into myofibroblasts both *in vitro* (133), and *in vivo* (134). LPA and serum, as well as TGF-β, could activate myofibroblast differentiation (135-138), which is supposedly one of the most potent stimulators of TFs (124). After glaucoma filtration surgery, TFs are likely to be exposed to LPA via serum and/or plasma, because the blood-aqueous humor barrier breaks down, and circulating aqueous humor bathes the wound site (139).

ROCK inhibitors can inhibit cell migration, invasion (140) and cytokinesis (141,142), all of which have a role in wound healing and scar formation, therefore, the Rho/ROCK pathway has critical functions in regulation of wound healing of filtration canal. ROCK inhibitors (Y-27632, HA-1077 and H-1152) have been reported to reduce or block LPA-induced and TGF-β-induced α-SMA expression in TFs (133,143-146), which suggests that ROCK inhibitors serve as a potent anti-scarring agent via inhibition of transdifferentiation of TFs into myofibroblasts (146). Meyer-ter-Vehn *et al* found that ROCK inhibitors did not alter the Smad2 phosphorylation pattern, but inhibited TGF-β-induced phosphorylation of p38 in TFs (133). Honjo *et al* (146) reported that Y-27632 induced profound changes in cultured human TFs without significant toxicity or inhibition of human TFs proliferation. In addition, topical instillation of Y-27632 was effective in preventing fibroproliferation and scar formation in a rabbit model of glaucoma surgery (146). Therefore, the ROCK inhibitors have potential to be anti-scarring agents after glaucoma filtering surgery.

9. Rho/ROCK pathway and optic nerve neuroprotection

Normal human optic nerve head (ONH) express RhoA, ROCK1 and ROCK2 (74). The cultured glaucomatous ONH astrocytes exhibit upregulated expression of Rho GTPase and certain ECM proteins (147), and the RhoA expression in the ONH of human glaucoma eyes is increased significantly when compared with age-matched normal subjects, indicating a possible involvement of RhoA/ROCK pathway in the pathophysiology of the optic nerve damage from glaucoma (74).

Increase in ocular blood flow. The Rho/ROCK pathway is expressed in vascular smooth muscles (148-151), and ROCK inhibitors have been known to relax various vascular smooth muscles (152-156), which may enhance ocular and retinal blood flow by inducing vasodilatation and, thus, provide neuroprotective action. Cell culture experiments and studies using isolated vessel preparations demonstrated that the constrictor effects of endothelin and angiotensin II and the generation of myogenic tone are mainly mediated by ROCK activation (157-160), thus the inhibition of ROCK activation could eliminate the vessel contraction induced by endothelin and angiotensin II. The experiment *in vitro* showed that selective ROCK inhibitors (Y-27632 and Y-39983) induced concentration-dependent relaxation of isolated rabbit ciliary arteries (161), and demonstrated that the mechanism of relaxation may decrease the Ca^{2+} sensitivity of key intracellular contractile protein(s) and/or alter regulation of Rho kinases (161). The studies *in vivo* also demonstrated that topical application of ROCK inhibitor (Y-27632, Y-39983 and fasudil) increased ONH blood flow in rabbit (161-163). Okamura *et al* reported that systemic administration of fasudil had vasodilator effects on retinal arterioles in stroke-prone spontaneously hypertensive rats (164). Sugiyama *et al* also found that systemic or topical fasudil suppressed impairment of ONH blood flow, function and morphology induced by NG-nitro-L-arginine methyl ester (L-NAME) or endothelin-1 (163), and the amelioration could be inhibited by a specific inhibitor of Akt/PI-3 kinase, which suggests that these actions of fasudil might be caused by inhibition of ROCK, leading to phosphorylation and activation of Akt via the PI-3 kinase (165). Decreased perfusion is thought to be one of the causative factors in loss of vision in glaucoma (166-169), ROCK inhibitors could increase blood flow velocity in optic nerve head, which suggests that Rho/ROCK signaling may be a promising target for the treatment of glaucoma optic neuropathy.

Improved RGC survival. There is increasing evidence demonstrating the protective effects of RhoA/ROCK-inhibition on adult retinas. The intraocular injection of the RhoA antagonist C3 is reported to increase both axonal regeneration and RGC survival after optic nerve axotomy in rats (170). The inhibition of ROCK (fasudil) was shown to decrease the extent of N-methyl-D-aspartic acid (NMDA)-induced neurotoxicity in rat retinas (171). In an *in vivo* rat model of glaucoma, intraperitoneal injection of the Rho kinase inhibitor fasudil protected against neuronal loss (172,173), which suggested that abnormal activity of Rho/Rho kinase pathway may participate in the pathophysiology of glaucoma (82). Inactivation of Rho/ROCK signaling also contributes to the neuroprotectivity of neuronal cells in the retinal ischemia/reperfusion injury. Retinal ischemia/reperfusion injury leads to a loss of neuronal cells in the inner retinal layers such as RGCs and amacrine cells (174,175), and neuronal cell apoptosis induced by transient retinal ischemia progresses through the reperfusion phase rather than the ischemic phase. Injury during reperfusion is caused by the infiltration of leukocytes into the neural tissue through vascular endothelial cells (176). The Rho/Rho kinase pathway contributes to leukocyte extravasation by regulating the leukocyte cytoskeleton and tight junction of endothelial cells (177,178). ROCK inhibitors attenuate the ischemia/reperfusion induced apoptosis of retinal cells in the inner retinal layers (including RGCs) by decreasing Bax/Bcl-2 mRNA ratio and the expression of caspase-3 and iNOS (179), and by regulating leukocyte infiltration in the neural tissue (180,181). The treatment with the ROCK inhibitor Y-27632 could promote the viability of primary RGCs, the RGCs-5 cell line (182). Moreover, ROCK inhibition also rescues RGCs from axotomy-induced apoptosis *in vivo*, and the neuroprotective effects of the ROCK inhibitor Y-27632 are mediated by the activation of well-established cell survival pathways, such as the Akt and MAPK pathways (182). Furthermore, Tura *et al* found that the neuroprotective effect of H-1152P on retinal cells, particularly in RGCs, was associated with a decrease in the reactivity of astrocytes, Müller cells, and microglia both in retinas cultured under serum deprivation and after optic nerve crush, which suggested the neuroprotective effect of H-1152P-mediated ROCK-inhibition on retinal cells under stress may rely partly on the attenuation of glial cell reactivity (183).

Promotion of RGC axon regeneration. The failure of axon regeneration after injury to the mammalian CNS is attributable to the limited availability of neurotrophic factors (NTF) which promote neuron survival and axon regeneration, and the presence of myelin- and scar-derived inhibitory molecules such as Nogo-A, myelin associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (Omgp), chondroitin sulphate proteoglycan (CSPG), ephrins and semaphorins (184-191). After binding to their cognate receptors, these inhibitory molecules converge on the Rho/ROCK pathway to change actin dynamics and initiate growth cone collapse (184,186,187,192). The Rho/ROCK pathway has been mainly associated with inhibitory signaling for neurite elongation (193), and inactivation of Rho/ROCK pathway can promote the regeneration (193). As discussed above, ONH expresses RhoA, ROCK1 and ROCK2 (74), and their presence in the optic nerve suggests a potential role for the Rho/ROCK pathway in neurite outgrowth and axon regeneration through actin cytoskeletal reorganization (147). Inhibition of Rho and ROCK has been shown to increase RGC axon regeneration (193,194). It has been shown that intraocular delivery of C3-exoenzyme, an inactivator of Rho GTPase, can promote the regeneration of RGC axons in the optic nerve after a microcrush lesion (193,195). On the other hand, ROCK inhibitors have also been shown to increase regeneration in different optic nerve lesion models (182,195-198). In terms of their intensity, the effect of Y-39983 in promotion of neurite outgrowth was stronger than that of Y-27632, fasudil and dimethylfasudil (194,196). In addition to a ROCK inhibitor used alone, the treatment with ROCK inhibitor (Y-27632) in combination with CNTF and/or raised cAMP levels has additive effects, and promotes robust RGCs axon regeneration (182,197). We recently found that RhoA/ROCK signaling pathway was involved in the erythropoietin (EPO) effect to promote RGC axon regeneration after optic nerve crush, and EPO and Y-27632 had additive effects in promoting RGC axon regeneration (199).

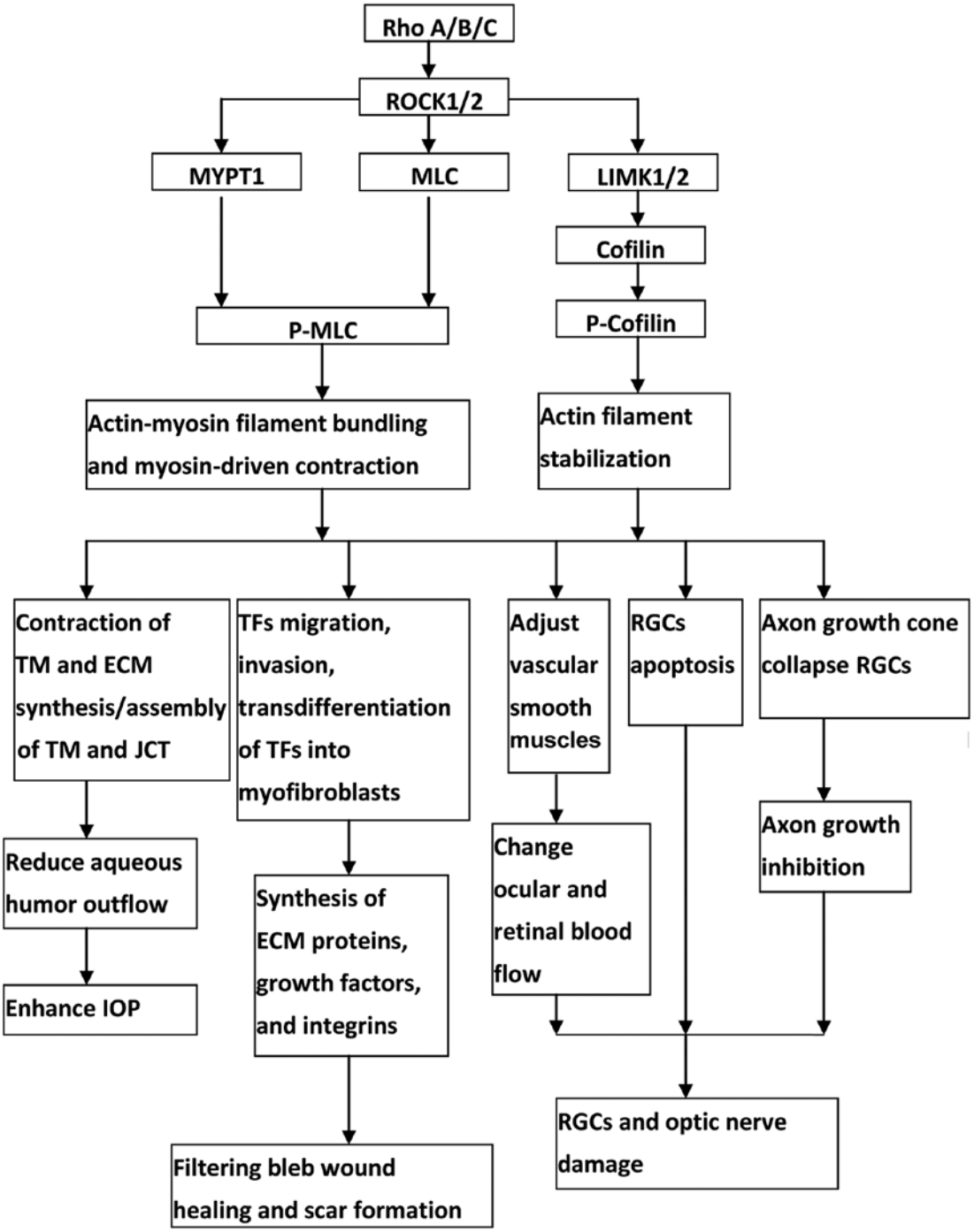

Figure 1. The diagram of Rho/ROCK effect on IOP, filtering bleb and optic nerve. ROCK, Rho/Rho-associated kinase; MYPT1, myosin phosphatase target subunit 1; MLC, myosin light chain; P-MLC, phosphorylation of MLC; LIMK1/2, LIM kinase1/2; P-Cofilin, phosphorylation of cofilin; TM, trabecular meshwork; ECM, extracellular matrix; JCT, juxtacanalicular tissue; TFs, Tenon fibroblasts; IOP, intraocular pressure; RGCs, retinal ganglion cells.

10. Conclusion

Rho/ROCK pathway has important roles for modulating the cytoskeletal integrity of cells, the synthesis of ECM components in the outflow tissue, and the permeability of the SC endothelial cells. The activation of Rho/ROCK pathway in the outflow tissue results in reduction of aqueous humor outflow, and thereby increase IOP, whereas the inhibition of Rho/ROCK pathway in the outflow tissue results in increase of aqueous humor outflow, and thereby decrease IOP. ROCK inhibitors also serve as a potent anti-scarring agent via inhibition of transdifferentiation of TFs into myofibroblasts. Furthermore, RhoA/ROCK pathway is involved in optic nerve neuroprotection. Inactivation of Rho/ROCK signaling could increase ocular blood flow, improve RGCs survival and promote RGCs axon regeneration. Considering the IOP modulation, potent bleb anti-scarring effect and neuroprotective properties of ROCK inhibitors (Fig. 1), Rho/ROCK pathway is an attractive target for anti-glaucoma therapy, and it may be used for human therapy in the near future.

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