·Review·

Rho/ROCK pathway and neural regeneration: a potential therapeutic target for central nervous system and optic nerve damage

Hai-Bo Tan, Yi-Sheng Zhong, Yu Cheng, Xi Shen

Foundation items: Supported by National Nature Science Foundation of China (No.81070728); Shanghai "Science and Technology Innovation Action Plan" Basic Research Key Project, China (No.11JC1407700 and 11JC1407701); Shanghai Nature Science Foundation, China (No.08ZR1413900); Shanghai Leading Academic Discipline Project, China(No.S30205)

Department of Ophthalmology, Ruijin Hospital Affiliated Medical School, Shanghai Jiaotong University,Shanghai 200025, China
Hai-bo Tan and Yi-Sheng Zhong contributed equally to this work.
Correspondence to: Yi-Sheng Zhong and Xi Shen. Department of Ophthalmology, Ruijin Hospital Affiliated Medical School, Shanghai Jiaotong University, Shanghai 200025,China. yszhong68@yahoo.com.cn; carl_shen2005@yahoo.com.cn

Received: 2011-10-10 Accepted: 2011-11-29

Abstract

• Rho-associated kinase (ROCK) is a serine/threonine kinase and one of the major downstream effectors of the small GTPase RhoA. The Rho/ROCK pathway is closely related to the pathogenesis of several central nervous system (CNS) disorders, and involved in many aspects of neuronal functions including neurite outgrowth and retraction. In the adult CNS, the damaged neuron regeneration is very difficult due to the presence of myelin-associated axon growth inhibitors such as Nogo, myelin-associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (Omgp), etc. The effects of these axon growth inhibitors are reversed by blocking the Rho/ROCK pathway *in vitro*, and the inhibition of Rho/ROCK pathway can promote axon regeneration and functional recovery in the injured CNS *in viva*. In addition, the therapeutic effects of the Rho/ROCK inhibitors have also been demonstrated in some animal models and the Rho/ROCK pathway becomes an attractive target for the development of drugs for treating CNS disorders. In this review, we summarized on the effect of the Rho and the downstream factor ROCK in neural regeneration, and the potential therapeutic effect of Rho/ROCK inhibitors in the survival and axonal regeneration of retinal ganglion cells was also discussed.

• KEYWORDS: Rho/ROCK pathway; neural regeneration; potential therapeutic effect; optic nerve damage

DOI:10.3980/j.issn.2222-3959.2011.06.16

Tan HB, Zhong YS, Cheng Y, Shen X. Rho/ROCK pathway and neural regeneration: a potential therapeutic target for central nervous system and optic nerve damage. *Int J Ophthalmol* 2011;4(6):652-657

INTRODUCTION

An important reason that axon regeneration is very difficult, following the grown-up mammal central nervous system (CNS) damaged, is due to the existence of some growth suppression molecules in the damaged environment. Until now had it mainly been discovered three kinds of molecules derived from myelin which can suppress axon growth: Nogo-A、myelin-associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (Omgp)[1]. The axon growth derived from the growth cone, and the growth cone can decide the direction of axon growth, as well as extend length by feeling information from the external environment[2]. After the growth cone contacts these suppression molecules in myelin, the cell skeleton structure changes, subsequently causes the growth cone to collapse, retract, and stop the axon growth [3]. At present, the functional mechanism of myelin-derived inhibitors is not completely clear. Rho is a member of small GTP enzyme of Rho family. Since the first time in 1985 Rho genes had been found [4], the pathophysiology of Rho and Rho-associated kinase (ROCK) has been conducted extensive research. Rho is a member of Rho subfamily of small molecular GTPases superfamily, and the mammalian Rho gene is homologous with the Ras superfamily. Previous studies have confirmed that Rho and their associated signaling molecules participate in and mediate the biological processes of axon regeneration, extension and fiber projection [5-8]. Rho regulates cell actin cytoskeleton by it's downstream effective factor ROCK, which extensively involve in the biological processes of cell migration, movement, apoptosis, gene transcription, nerve regeneration [9]. Previous researches indicate that Nogo-A, MAG, Omgp may activate Rho by common or different way, subsequently causes the growth

cone collapse [10,11]. This review summaries on the effect of the Rho and the downstream factor ROCK in neural regeneration.

Rho Profile Rho is a member of Rho subfamily of small molecular GTPases superfamily related to Ras. Rho-GTP kinase, which includes three members: Rho, Rac and Cdc42, is closely related to the development of nerve [12-14]. They are considered as a molecular switch on the signal pathway of neuronal cell membrane surface receptor to actin skeleton, which has played a vital role in adjusting neuron development and axon construction [3]. Rho plays a key role in mediating the process of axon regeneration inhibitors which cause the growth cone collapse [15-17]. Rho has three kind of isomers: RhoA, RhoB and RhoC, mainly RhoA in neuron [18]. Rho normally exists in two forms: one is the non-activated form combined with GDP (Rho-GDP), and the other is the activated form combined with GTP (Rho-GTP) [19]. Rho realizes its molecular switch function by constantly transformation between two forms. At the same time, the combination state of Rho (Rho-GDP or Rho-GTP) is also regulated by many of regulatory proteins, in which guanine nucleotide exchange factors (GEFs) [20-22,] GTPase-activating proteins (GAPs) [23-25] and GDP dissociation inhibitors (GDIs) [26, 27] play a key role. GEFs can urge Rho to release GDP and to unify GTP [20-22]. GAPs can stimulate the Rho activity of their own GTP molecules, which causes GTP to hydrolyze into GDP [23-25]. GDIs may suppress the transformation between Rho-GDP and Rho-GTP [26, 27]. The three types of protein interact with each other and regulate mutually the conversion of Rho between the two forms. Rho-associated kinase (ROCK) belongs to one of serine/ threonine protein activating enzyme family members[28]. The Rho kinase has 2 kinds of isomers: ROCK-I and ROCK-II, 65% of amino acid sequence is the same, the similarity of activating enzyme area reaches as high as 92%[29]. Although their structure is very similar, the distribution of them in organization is relatively different, and their functions have also some differences. ROCK-I mRNA is ubiquitously expressed except in the brain and muscle, whereas ROCK-II mRNA is expressed abundantly in the brain, muscle, heart, lung and placenta[29]. The main biological effect of ROCK is to inactivate myosin light chain phosphatase (myosin light chain phosphatase, MLCP) [30]. MLCP causes phosphorylated myosin light chain to dephosphorylate, which is the main mechanism of contracted-MLC diastole. In the physiological situation, ROCK exists in the non-active form in the cytoplasm, and it may be activated by Rho and arachidonic acid [9,31]. ROCK-I and ROCK-II are, at present, the most distinctive Rho downstream effect members that have researched ever[32-35]. After activated, Rho kinase leads to the phosphorylation and inactivation of myosin phosphatase, makes the extent of myosin phosphorylation increase, thus effects actin-myosin system which led to axon growth cone collapse and axon growth inhibition[32-37].

Inhibition Effect of Rho in Neuronal Regeneration After central nervous system damaged, due to the lack of stimulating factor and a variety of inhibitory molecules in the environment, the axon regeneration process was blocked [38-41]. Some studies have also shown that many of extracellular information-oriented elements, including Nogo. A, MAG, Omgp can inhibit the growth of axons by activating the Rho-mediated signal transduction pathway[42-44]. Lingor *et al* [35] evaluated three inhibitors of ROCK, including Y-27632, fasudil (HA-1077), and dimethylfasudil (H-1152), in the models of neurite outgrowth *in vitro*. All three ROCK inhibitors partially restored neurite outgrowth of Ntera-2 neurons on the inhibitory chondroitin sulphate proteoglycan substrate. In the rat optic nerve crush model, Y-27632 dose-dependently increased regeneration of retinal ganglion cell axons *in vivo*. Application of dimethylfasudil showed a trend toward increasing axon regeneration in an intermediate concentration [35]. Recently, several studies suggested that oxygen-dependent gene expression was of crucial importance in governing the essential steps of neuronal regeneration, such as cell proliferation, survival and differentiation[45-47]. Pacary *et al*[45] analyzed the effect of the hypoxia inducible factor-1 (HIF-1) activation-mimicking agent $CoCl_2$ on mesenchymal stem cells (MSC). $CoCl_2$ treatment increased the expression of the anti-proliferative gene BTG2/PC3 and decreased cyclin D1 expression. Meanwhile, the expressions of HIF-1 alpha and its target genes erythropoietin (EPO), vascular endothelial growth factor (VEGF) and p21 were also upregulated. These changes were followed by inhibiting cell proliferation and morphological changes. Additionally, by using Y-27632, it demonstrated that Rho/ROCK inhibition potentiated $CoCl_2$-induced MSC differentiation, in particular, into dopaminergic neuron-like cells as attested by its effect on tyrosine hydroxylase expression [45,47]. Lingor *et al*[48] evaluated the role of pharmacological inhibition of Rho/ROCK and ciliary neurotrophic factor (CNTF), a potent neurotrophic factor for retinal ganglion cells, in the models of retinal ganglion cell apoptosis and neurite outgrowth regeneration *in vitro* and *in vivo*. It showed for the first time that the ROCK inhibitor Y-27632 significantly enhanced the survival of retinal ganglion cells *in vitro* and *in vivo*. *In vitro*, the co-application of CNTF and Y-27632 potentiated the effect of either substance alone. ROCK inhibition resulted in the activation of the intrinsic mitogen-activated protein kinases (MAPK) pathway [31,48-50],

and the combination of CNTF and Y-27632 resulted in even more pronounced MAPK activation [48]. *In vivo*, ROCK activity was also decreased in an additive manner by both substances. Both CNTF and Y-27632 enhanced the regeneration of RGC into the non-permissive optic nerve crush model and additive effects were observed after combination treatment [48]. Subsequent studies found that the promoting effect of Y-27632 and fasudil to axon regeneration had the time and dose-dependent [51,52]. Histological analysis showed that, following the spinal cord injury for 4 weeks, local application of fasudil was invalid to axon regeneration and the restoration of motor function, which indicated that the activation of Rho/ROCK pathway mediated the irreversible inhibition effect of axon regeneration in the chronic phase of the central nervous system damaged [53]. These studies have shown that Rho/ROCK pathway plays an important role in mediating inhibitory signals to block the inhibition process of axon regeneration in neuron.

The Role of Rho in Actin Cytoskeleton and Neuronal Regeneration Inhibition Rho has regulated many activities of cell functions which the actin involved in [54]. The studies in the neural cells have discovered that Rho may cause the growth cone to collapse by affecting the actin skeleton system[15, 55-57]. The growth cone is an active vehicle and locates in the expansion part of the terminal of neurite, its surface has the filiform pseudopod and the laminated pseudopod. The filiform pseudopod is the finger-liked protuberance stretched out from the growth cone, which can feel the information from the environment to guide forward the movement of the growth cone[58]. The actin micro silk is the main constitution part of filiform pseudopod, and it is also the main driving force for impelling its advance [58]. Prior to the significant morphogenesis changes of the apical cone, the cell skeleton system, including it's internal actin structures, has changed constantly through the gathering and the depolymerization of actin as well as the unceasing myosin participation, the retraction movements of the filiform pseudopod and the laminated pseudopod allow the apical cone to extend along the specific direction, by which Rho precisely affects the axon growth [58,59]. Alabed *et al*[60] have identified the cytosolic phosphoprotein in collapsin-response mediator protein 4b (CRMP4b) as a protein that physically and functionally interacts with RhoA to mediate neurite outgrowth inhibition. Disruption of CRMP4b-RhoA binding with a competitive inhibitor attenuates neurite outgrowth inhibition on myelin and aggrecan substrate [60]. Stimulation of neuronal growth cones with Nogo leads to colocalization of CRMP4b and RhoA at discrete regions within the actin-rich central and peripheral domains of the growth cone, indicative of a potential function in cytoskeletal rearrangements during neurite outgrowth inhibition[60].

Sarasa-Renedo *et al* [61] found that the actin contractility controlled by RhoA/ROCK has a mechanosensory function in fibroblasts that correlates directly with tenascin-C gene expression. Previous RhoA/ROCK activation, either by chemical or mechanical signals, might render fibroblasts more sensitive to external tensile stress, on the contrary, RhoA/ROCK inhibitors can inhibit tenascin-C gene expression [61,62].

Upstream and Downstream Signal Relation of Rho As a molecular switch that connections molecular surface recceptor and the actin cell skeleton, Rho is playing the vital role in the adjustment of cell skeleton dynamics and the cell movement. Some studies have discovered that the activation of Rho plays an essential function in the mechanism of axon regeneration suppression members that cause the growth cone to collapse [43,44], simultaneously blocking Rho or its downstream signal pathway can reverse the suppression effect of these suppression members against the axon regeneration [35], which shows that these suppression molecules possibly cause the apical cone collapse through Rho-mediated signal pathway [63]. To a great extent, this discoveries have promoted the research for the action mechanism of axon regeneration suppression molecules. Eickholt *et al* [64] have demonstrated that inactivation of p110delta in mice does not affect gross neuronal development, but leads to an increased vulnerability of dorsal root ganglia neurons to exhibit growth cone collapse and results in axon extension decrease. Loss of p110delta activity also dampened axon regeneration following peripheral nerve injury in adult mice and impaired functional recovery of locomotion. The inactivation of p110delta resulted in reducing neuronal signaling by the Akt protein kinase, and increasing the activity of the small GTPase RhoA [64,65]. Pharmacological inhibition of ROCK, a downstream effector of RhoA, can restore axon extension defects in neurons with the inactive of p110delta, suggesting a key role of RhoA in p110delta signaling in neurons [64]. Kubo *et al* [66] have shown that blocking of the Rho/ROCK pathway can reverse the inhibitory effects of these inhibitors *in vitro*, and promote axon regeneration *in vivo*. Therefore, the inhibition of Rho/ROCK has a therapeutic potential against injuries to the human CNS, such as spinal cord injuries [66]. Fu *et al* [67] have reported that the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen and indomethacin, can surmount axon growth restrictions from myelin and proteoglycans by potently inhibiting their downstream pathway ROCK. Systemic administration of

ibuprofen to spinal cord lesion rodents reverses the activity of RhoA around injury area measured via Rho-GTP binding assay. Subcutaneous injections of ibuprofen via minipumps to rats with a thoracic spinal cord transection or contusion injury result in substantial corticospinal and serotonergic axon sprouting in the caudal spinal cord and promote locomotor functional recovery, even delaying the treatment 1 week after trauma. In contrast, the non-RhoA-inhibiting NSAID naproxen does not have the axon growth promoting effects on the cultured or lesioned neurons. [67] Gopalakrishnan *et al* [68] have found that in an *in vitro* model of the nerve growth factor-differentiated PC12 cell, the chondroitin sulfate proteoglycans (CSPG) can increase the phosphorylation of myosin phosphatase, which is a substrate immediately downstream of ROCK activation. Fasudil, dimethylfasudil and Y27632 can inhibit the phosphorylation of myosin phosphatase induced by CSGP. In addition, ROCK inhibitors can also reverse cofilin phosphorylation induced by CSPG. These data demonstrate that the interaction between CSPG and the ROCK pathway involves in the downstream effectors of ROCK, such as myosin phosphatase and cofilin [68]. Alabed *et al* [69] have provided the direct evidence that ROCK-II is activated in response to the myelin-associated inhibitor Nogo. Nogo enhances ROCK-II translocation to the cellular membrane of the PC12 cells and enhances ROCK-II kinase activity *in vitro*. In addition, Nogo also enhances the phosphorylation of myosin light chain II, a known ROCK substrate. Furthermore, the primary dorsal root ganglia neurons can be rendered insensitive to the inhibitory effects of myelin via infection with dominant negative ROCK[69].

Recently, Sagawa *et al* [70] investigated the effect of a novel ROCK inhibitor, Y-39983, on neurite regeneration *in vitro* and axon regeneration in the crushed cat optic nerve *in vivo*. Y-39983 was injected into the vitreous and the crushed site. An injection of 10 microM Y-39983 induced the crushed axons to regenerate and pass over the crush site. In contrast, very few axons passed beyond the crush site in the optic nerve with phosphate-buffered saline injection. The second injection of 10 microM Y-39983 on day 7 doubled the number of regenerated axons[70].

CONCLUSION AND PROSPECT

To sum up, some axon growth suppression molecules in the damaged environment, including Nogo.A, MAG, Omgp can inhibit the growth of axons by activating the Rho-mediated signal transduction pathway (Figure 1). Many of these processes demonstrate that the dynamic reorganization of actin cytoskeleton of which Rho signaling has now emerged as a major molecular switch. The involvement of dynamic changes of Rho GTPases in the axon regeneration

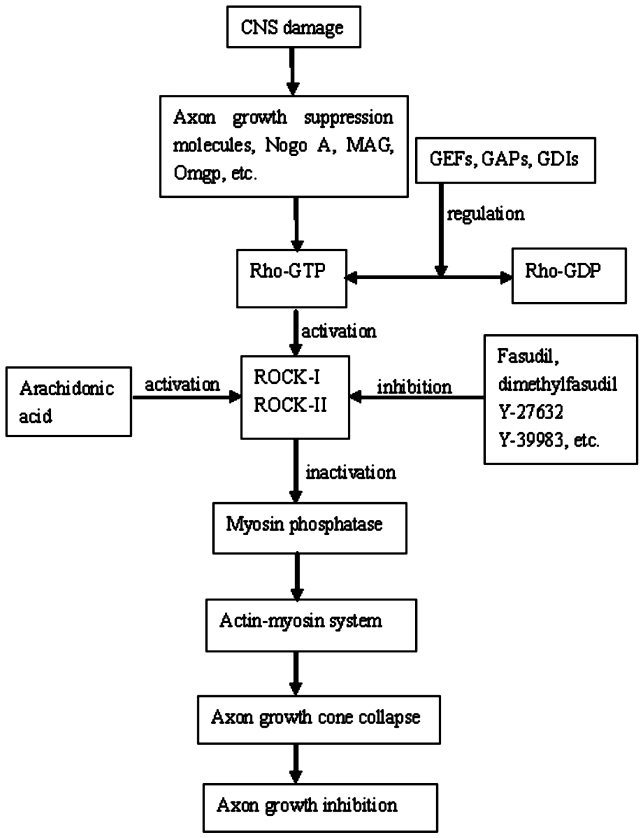

Figure 1 Proposed Rho/ROCK pathway leading to axon growth inhibition in CNS damage

underscores the need to produce effective inhibitors for their therapeutic applications [71]. Fasudil, Y-27632 and Y-39983, with many newer additions, are three classes of widely used chemical compounds that inhibit ROCK, an important downstream effector of RhoA subfamily GTPases. These inhibitors have been successful in some animal models, indicating the potential benefit of clinical Rho pathway inhibition. Because of a rapidly growing number of studies deciphering the role of the Rho proteins in CNS diseases, specific and potent pharmaceutical modulators of various steps of Rho GTPase signaling pathway are critically needed to target for therapeutic intervention in CNS disease.

REFERENCES

1 Woolf CJ, Bloechlinger S. Neuroscience. It takes more than two to Nogo. *Science* 2002;297(5584):1132-1134

2 Laishram J, Avossa D, Shahapure R, Torre V. Mechanical computation in neurons. *Dev Neurobiol*2009;69(11):731-751

3 Hall A, Nobes CD. Rho GTPases: molecular switches that control the organization and dynamics of the actin cytoskeleton. *Philos Trans R Soc Lond B Biol Sci*2000;355(1399):965-970

4 Madaule P, Axel R. A novel ras-related gene family. *Cell*1985;41(1):31-40

5 Sivasankaran R, Pei J, Wang KC, Zhang YP, Shields CB, Xu XM, He Z. PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. *Nat Neurosci*2004;7(3):261-268

6 Zhou FQ, Walzer M, Wu YH, Zhou J, Dedhar S, Snider WD. Neurotrophins support regenerative axon assembly over CSPGs by an ECM-integrin-independent mechanism. *J Cell Sci*2006;119(Pt 13):2787-2796

7 Douglas MR, Morrison KC, Jacques SJ, Leadbeater WE, Gonzalez AM, Berry M, Logan A, Ahmed Z. Off-target effects of epidermal growth factor receptor antagonists mediate retinal ganglion cell disinhibited axon growth. *Brain* 2009;132 (Pt 11):3102-3121

8 Duffy P, Schmandke A, Schmandke A, Sigworth J, Narumiya S, Cafferty WB, Strittmatter SM. Rho-associated kinase II (ROCKII) limits axonal growth after trauma within the adult mouse spinal cord. *J Neurosci* 2009;29(48):15266-15276

9 Bhadriraju K, Yang M, Alom Ruiz S, Pirone D, Tan J, Chen CS. Activation of ROCK by RhoA is regulated by cell adhesion, shape, and cytoskeletal tension. *Exp Cell Res* 2007;313(16):3616-3623

10 Schimchowitsch S, Cassel JC. Polyamine and aminoguanidine treatments to promote structural and functional recovery in the adult mammalian brain after injury: a brief literature review and preliminary data about their combined administration. *J Physiol Paris* 2006;99(2-3):221-231

11 Schweigreiter R, Walmsley AR, Niederöst B, Zimmermann DR, Oertle T, Casademunt E, Frentzel S, Dechant G, Mir A, Bandtlow CE. Versican V2 and the central inhibitory domain of Nogo-A inhibit neurite growth via p75NTR/NgR-independent pathways that converge at RhoA. *Mol Cell Neurosci* 2004;27(2):163-174

12 Negishi M, Katoh H. Rho family GTPases and dendrite plasticity. Neuroscientist. 2005;11(3):187-191

13 Ahmed I, Calle Y, Iwashita S, Nur-E-Kamal A. Role of Cdc42 in neurite outgrowth of PC12 cells and cerebellar granule neurons. *Mol Cell Biochem* 2006; 281(1-2):17-25

14Causeret F, Hidalgo-Sanchez M, Fort P, Backer S, Popoff MR, Gauthier-Rouvi é re C, Bloch-Gallego E. Distinct roles of Rac1/Cdc42 and Rho/Rock for axon outgrowth and nucleokinesis of precerebellar neurons toward netrin 1. *Development* 2004;131(12):2841-2852

15 Hall A. Rho GTPases and the actin cytoskeleton. *Science* 1998;279 (5350): 509-514

16 Jiang P, Enomoto A, Takahashi M. Cell biology of the movement of breast cancer cells: intracellular signalling and the actin cytoskeleton. *Cancer Lett* 2009; 284(2):122-130

17 Arnsdorf EJ, Tummala P, Kwon RY, Jacobs CR. Mechanically induced osteogenic differentiation--the role of RhoA, ROCKII and cytoskeletal dynamics. *J Cell Sci* 2009;122(Pt 4):546-553

18 Erschbamer MK, Hofstetter CP, Olson L. RhoA, RhoB, RhoC, Rac1, Cdc42, and Tc10 mRNA levels in spinal cord, sensory ganglia, and corticospinal tract neurons and long-lasting specific changes following spinal cord injury. *J Comp Neurol* 2005;484(2):224-233

19 Faur é J, Dagher MC. Interactions between Rho GTPases and Rho GDP dissociation inhibitor (Rho-GDI). *Biochimie* 2001;83(5):409-414

20 Estrach S, Schmidt S, Diriong S, Penna A, Blangy A, Fort P, Debant A. The Human Rho-GEF trio and its target GTPase RhoG are involved in the NGF pathway, leading to neurite outgrowth. *Curr Biol* 2002;12(4):307-312

21 Overbeck AF, Brtva TR, Cox AD, Graham SM, Huff SY, Khosravi-Far R, Quilliam LA, Solski PA, Der CJ. Guanine nucleotide exchange factors: activators of Ras superfamily proteins. *Mol Reprod Dev* 1995;42(4):468-476

22 Blomquist A, Schwörer G, Schablowski H, Psoma A, Lehnen M, Jakobs KH, R ü menapp U. Identification and characterization of a novel Rho-specific guanine nucleotide exchange factor. *Biochem J* 2000;352 Pt 2:319-325

23 Moon SY, Zheng Y. Rho GTPase-activating proteins in cell regulation. Trends Cell Biol. 2003 Jan;13(1):13-22

24 Brinkmann T, Daumke O, Herbrand U, K ü hlmann D, Stege P, Ahmadian MR, Wittinghofer A. Rap-specific GTPase activating protein follows an alternative mechanism. *J Biol Chem* 2002;277(15):12525-12531

25 Kozma R, Ahmed S, Best A, Lim L. The GTPase-activating protein n-chimaerin cooperates with Rac1 and Cdc42Hs to induce the formation of lamellipodia and filopodia. *Mol Cell Biol* 1996;16(9):5069-5080

26 Qiao J, Holian O, Lee BS, Huang F, Zhang J, Lum H. Phosphorylation of GTP dissociation inhibitor by PKA negatively regulates RhoA. *Am J Physiol Cell Physiol* 2008;295(5):C1161-1168

27 DerMardirossian C, Bokoch GM. GDIs: central regulatory molecules in Rho GTPase activation. *Trends Cell Biol* 2005;15(7):356-363

28 Riento K, Totty N, Villalonga P, Garg R, Guasch R, Ridley AJ. RhoE function is regulated by ROCK I-mediated phosphorylation. *EMBO J* 2005;24 (6): 1170-1180

29 Nakagawa O, Fujisawa K, Ishizaki T, Saito Y, Nakao K, Narumiya S. ROCK-I and ROCK-II, two isoforms of Rho-associated coiled-coil forming protein serine/threonine kinase in mice. *FEBS Lett* 1996;392(2):189-193

30 Wang Y, Zheng XR, Riddick N, Bryden M, Baur W, Zhang X, Surks HK. ROCK isoform regulation of myosin phosphatase and contractility in vascular smooth muscle cells. *Circ Res* 2009 ;104(4):531-540

31 Garcia MC, Ray DM, Lackford B, Rubino M, Olden K, Roberts JD. Arachidonic acid stimulates cell adhesion through a novel p38 MAPK-RhoA signaling pathway that involves heat shock protein 27. *J Biol Chem* 2009;284(31):20936-20945

32 Bito H, Furuyashiki T, Ishihara H, Shibasaki Y, Ohashi K, Mizuno K, Maekawa M, Ishizaki T, Narumiya S. A critical role for a Rho-associated kinase, p160ROCK, in determining axon outgrowth in mammalian CNS neurons. *Neuron* 2000;26(2):431-441

33 Nikolic M. The role of Rho GTPases and associated kinases in regulating neurite outgrowth. *Int J Biochem Cell Biol* 2002;34(7):731-745

34 Fournier AE, Takizawa BT, Strittmatter SM. Rho kinase inhibition enhances axonal regeneration in the injured CNS. *J Neurosci* 2003;23(4):1416-1423

35 Lingor P, Teusch N, Schwarz K, Mueller R, Mack H, Bähr M, Mueller BK. Inhibition of Rho kinase (ROCK) increases neurite outgrowth on chondroitin sulphate proteoglycan *in vitro* and axonal regeneration in the adult optic nerve *in vivo*. *J Neurochem* 2007;103(1):181-189

36 Dergham P, Ellezam B, Essagian C, Avedissian H, Lubell WD, McKerracher L. Rho signaling pathway targeted to promote spinal cord repair. *J Neurosci* 2002;22 (15):6570-6577

37 Wettschureck N, Offermanns S. Rho/Rho-kinase mediated signaling in physiology and pathophysiology. *J Mol Med* 2002;80(10):629-638

38 Jones LL, Sajed D, Tuszynski MH. Axonal regeneration through regions of chondroitin sulfate proteoglycan deposition after spinal cord injury: a balance of permissiveness and inhibition. *J Neurosci* 2003;23(28):9276-9288

39 Yiu G, He Z. Glial inhibition of CNS axon regeneration. *Nat Rev Neurosci* 2006;7(8):617-627

40Filbin MT. Recapitulate development to promote axonal regeneration: good or bad approach? *Philos Trans R Soc Lond B Biol Sci* 2006;361(1473):1565-1574

41 Chen ZJ, Ughrin Y, Levine JM. Inhibition of axon growth by oligodendrocyte precursor cells. *Mol Cell Neurosci* 2002;20(1):125-139

42 Bouquier N, Vignal E, Charrasse S, Weill M, Schmidt S, L é onetti JP, Blangy A, Fort P. A cell active chemical GEF inhibitor selectively targets the Trio/RhoG/Rac1 signaling pathway. *Chem Biol* 2009;16(6):657-666

43 Kang MJ, Seo JS, Park WY. Caveolin-1 inhibits neurite growth by blocking Rac1/Cdc42 and p21-activated kinase 1 interactions. *Neuroreport* 2006;17 (8): 823-827

44 Niederöst B, Oertle T, Fritsche J, McKinney RA, Bandtlow CE. Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1. *J Neurosci* 2002;22(23):10368-10376

45 Pacary E, Legros H, Valable S, Duchatelle P, Lecocq M, Petit E, Nicole O, Bernaudin M. Synergistic effects of $CoCl_2$ and ROCK inhibition on mesenchymal stem cell differentiation into neuron-like cells. *J Cell Sci* 2006;119 (Pt 13): 2667-2678

46 Pacary E, Tixier E, Coulet F, Roussel S, Petit E, Bernaudin M. Crosstalk between HIF-1 and ROCK pathways in neuronal differentiation of mesenchymal stem cells, neurospheres and in PC12 neurite outgrowth. *Mol Cell Neurosci* 2007; 35(3):409-423

47 Pacary E, Petit E, Bernaudin M. Concomitant inhibition of prolyl hydroxylases

and ROCK initiates differentiation of mesenchymal stem cells and PC12 towards the neuronal lineage. *Biochem Biophys Res Commun* 2008;377(2):400-406

48 Lingor P, Tönges L, Pieper N, Bermel C, Barski E, Planchamp V, Bähr M. ROCK inhibition and CNTF interact on intrinsic signalling pathways and differentially regulate survival and regeneration in retinal ganglion cells. *Brain* 2008;131(Pt 1):250-263

49 Khatiwala CB, Kim PD, Peyton SR, Putnam AJ. ECM compliance regulates osteogenesis by influencing MAPK signaling downstream of RhoA and ROCK. *J Bone Miner Res* 2009;24(5):886-898

50 Rodrigues-D ì ez R, Carvajal-González G, Sánchez-López E, Rodr ì guez-Vita J, Rodrigues D ì ez R, Selgas R, Ortiz A, Egido J, Mezzano S, Ruiz-Ortega M. Pharmacological modulation of epithelial mesenchymal transition caused by angiotensin II. Role of ROCK and MAPK pathways. *Pharm Res* 2008; 25(10):2447-2461

51 Chan CC, Khodarahmi K, Liu J, Sutherland D, Oschipok LW, Steeves JD, Tetzlaff W. Dose-dependent beneficial and detrimental effects of ROCK inhibitor Y27632 on axonal sprouting and functional recovery after rat spinal cord injury. *Exp Neurol* 2005;196(2):352-364

52 Zhang Z, Ottens AK, Larner SF, Kobeissy FH, Williams ML, Hayes RL, Wang KK. Direct Rho-associated kinase inhibition [correction of inhibiton] induces cofilin dephosphorylation and neurite outgrowth in PC-12 cells. *Cell Mol Biol Lett* 2006;11(1):12-29

53 Nishio Y, Koda M, Kitajo K, Seto M, Hata K, Taniguchi J, Moriya H, Fujitani M, Kubo T, Yamashita T. Delayed treatment with Rho-kinase inhibitor does not enhance axonal regeneration or functional recovery after spinal cord injury in rats. *Exp Neurol* 2006;200(2):392-397

54 Santarius M, Lee CH, Anderson RA. Supervised membrane swimming: small G-protein lifeguards regulate PIPK signalling and monitor intracellular PtdIns(4,5) P2 pools. *Biochem J* 2006;398(1):1-13

55 Kubo T, Endo M, Hata K, Taniguchi J, Kitajo K, Tomura S, Yamaguchi A, Mueller BK, Yamashita T. Myosin IIA is required for neurite outgrowth inhibition produced by repulsive guidance molecule. *J Neurochem* 2008;105(1):113-126

56 Shi L, Fu WY, Hung KW, Porchetta C, Hall C, Fu AK, Ip NY. Alpha2-chimaerin interacts with EphA4 and regulates EphA4-dependent growth cone collapse. *Proc Natl Acad Sci U S A* 2007;104(41):16347163-52

57 Gallo G, Letourneau PC. Regulation of growth cone actin filaments by guidance cues. *J Neurobiol* 2004;58(1):92-102

58 Withers GS, James CD, Kingman CE, Craighead HG, Banker GA. Effects of substrate geometry on growth cone behavior and axon branching. *J Neurobiol* 2006; 66(11):1183-1194

59 Thies E, Davenport RW. Independent roles of Rho-GTPases in growth cone and axonal behavior. *J Neurobiol* 2003;54(2):358-369

60 Alabed YZ, Pool M, Ong Tone S, Fournier AE. Identification of CRMP4 as a convergent regulator of axon outgrowth inhibition. *J Neurosci* 2007;27 (7): 1702-1711

61 Sarasa-Renedo A, Tun ? Civelek V, Chiquet M. Role of RhoA/ROCK-dependent actin contractility in the induction of tenascin-C by cyclic tensile strain. *Exp Cell Res* 2006;312(8):1361-1370

62 Maier S, Lutz R, Gelman L, Sarasa-Renedo A, Schenk S, Grashoff C, Chiquet M. Tenascin-C induction by cyclic strain requires integrin-linked kinase. *Biochim Biophys Acta* 2008;1783(6):1150-1162

63 Borisoff JF, Chan CC, Hiebert GW, Oschipok L, Robertson GS, Zamboni R, Steeves JD, Tetzlaff W. Suppression of Rho-kinase activity promotes axonal growth on inhibitory CNS substrates. *Mol Cell Neurosci* 2003;22(3):405-416

64 Eickholt BJ, Ahmed AI, Davies M, Papakonstanti EA, Pearce W, Starkey ML, Bilancio A, Need AC, Smith AJ, Hall SM, Hamers FP, Giese KP, Bradbury EJ, Vanhaesebroeck B. Control of axonal growth and regeneration of sensory neurons by the p110delta PI 3-kinase. *PLoS One* 2007;2(9):e869

65 Papakonstanti EA, Ridley AJ, Vanhaesebroeck B. The p110delta isoform of PI 3-kinase negatively controls RhoA and PTEN. *EMBO J* 2007;26(13):3050-3061

66 Kubo T, Hata K, Yamaguchi A, Yamashita T. Rho-ROCK inhibitors as emerging strategies to promote nerve regeneration. *Curr Pharm Des* 2007;13(24): 2493-2499

67 Fu Q, Hue J, Li S. Nonsteroidal anti-inflammatory drugs promote axon regeneration via RhoA inhibition. *J Neurosci* 2007;27(15):4154-4164

68 Gopalakrishnan SM, Teusch N, Imhof C, Bakker MH, Schurdak M, Burns DJ, Warrior U. Role of Rho kinase pathway in chondroitin sulfate proteoglycan-mediated inhibition of neurite outgrowth in PC12 cells. *J Neurosci Res* 2008;86(10):2214-2226

69 Alabed YZ, Grados-Munro E, Ferraro GB, Hsieh SH, Fournier AE. Neuronal responses to myelin are mediated by rho kinase. *J Neurochem* 2006;96 (6): 1616-1625

70 Sagawa H, Terasaki H, Nakamura M, Ichikawa M, Yata T, Tokita Y, Watanabe M. A novel ROCK inhibitor, Y-39983, promotes regeneration of crushed axons of retinal ganglion cells into the optic nerve of adult cats. *Exp Neurol* 2007;205(1): 230-240

71 Lu Q, Longo FM, Zhou H, Massa SM, Chen YH. Signaling through Rho GTPase pathway as viable drug target. *Curr Med Chem* 2009;16(11):1355-1365