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RhoA/Rho kinase in spinal cord injury

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Abstract

A spinal cord injury refers to an injury to the spinal cord that is caused by a trauma instead of diseases. Spinal cord injury includes a primary mechanical injury and a much more complex secondary injury process involving inflammation, oxidation, excitotoxicity, and cell death. During the secondary injury, many signal pathways are activated and play important roles in mediating the pathogenesis of spinal cord injury. Among them, the RhoA/Rho kinase pathway plays a particular role in mediating spinal degeneration and regeneration. In this review, we will discuss the role and mechanism of RhoA/Rho kinase-mediated spinal cord pathogenesis, as well as the potential of targeting RhoA/Rho kinase as a strategy for promoting both neuroprotection and axonal regeneration.

Key Words: *RhoA; Rho kinase; inflammation; cell death; degeneration; regeneration; spinal cord injury*

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Introduction

It is estimated that 1.5–5.2 million people suffer from spinal cord injury (SCI) and 130,000 new patients are added around the world each year (Schwab et al., 2006). SCI is caused by trauma, a mechanical injury followed by a secondary injury process with much more complex molecular cascade responses (Borgens and Liu-Snyder, 2012). The outcomes of SCI are pain, paralysis, and incontinence. To date, limited effective treatments are available.

RhoA is a small GTPase protein and belongs to Rho GTPase family which contains seven subfamilies including Rho, Rac, Cdc42, Rnd, RhoD, RhoBTB, and RhoH. Among them, RhoA, Rac1, and Cdc42 are the most studied members. RhoA mediates the formation of focal adhesion and stress fibers, which are contractile acting bundles in non-muscle cells that regulate cell contractility, providing force for cell adhesion, migration, and morphogenesis (Stankiewicz and Linseman, 2014). RhoA and its downstream effector Rho kinase (ROCK) control and regulate cytoskeleton dynamic. Rho kinase has two isoforms, ROCK1 and ROCK2, and belongs to the AGC (PKA/PKG/PKC) family of serine-threonine kinase. ROCK1 and ROCK2 share an overall sequence similarity at the amino-acid level of 65% and in their kinase domains of 92% (Amano et al., 2000). The RhoA/Rho kinase pathway regulates a wide range of fundamental cell functions including contraction, motility, proliferation, gene expression, and apoptosis (Loirand et al., 2006). Studies have shown that the RhoA/Rho kinase signal pathway is involved in many diseases, such as cardiovascular diseases (Loirand et al., 2006), cancer (Sahai and Marshall, 2002), and neurological diseases (Mueller et al., 2005). RhoA/Rho kinase pathway plays a role in stroke, Alzheimer's disease, neuropathic pain, multiple sclerosis, and SCI (Mueller et al, 2005). In this brief review, we will discuss the expression profile of RhoA/Rho kinase after SCI and the roles of activated RhoA/Rho kinase pathway in mediating inflammation, neuropathic pain and cell death in the acute phase and mediating axon degeneration in the chronic phase. We will also discuss the therapeutic strategies targeting these signal proteins.

RhoA/Rho Kinase Expression Profiles in SCI

Many studies have shown that RhoA/Rho kinase signal is activated after SCI (Dubreuil et al., 2003; Erschbamer et al., 2005; Wei et al., 2014). After spinal transection or contusion injury in rats and mice, active RhoA is dramatically increased (>10 fold). RhoA is active as early as 1.5 hours after injury and sustains at a high level at 1, 3, and 7 days. One week after injury, RhoA mRNA expression level is still 5-fold higher than normal animals and remains 3-fold higher up to 3 months. Active RhoA and its mRNA are detected in neurons, oligodendrocytes, and reactive astrocytes around the lesion area (Dubreuil et al., 2003). Four to fourteen days after contusion SCI, the signal intensity of RhoA mRNA is significantly higher in spinal cord segments below the injury center compared to segments above the injury center (Dubreuil et al., 2003; Erschbamer et al., 2005).

RhoA/Rho Kinase in Injury-induced Inflammation and Neuropathic Pain

As mentioned above, SCI includes a primary injury followed by a secondary injury. The primary injury triggers

the secondary injury, which involves inflammation, reactive oxidation, and excitotoxicity. The outcomes of SCI are mainly influenced by the secondary injury. Previous studies have shown that the RhoA/Rho kinase pathway regulated inflammatory responses (Bao et al., 2004) and mediated inflammatory cell infiltration and migration, and production of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β), interleukin-2 (IL-2) and CXC chemokines (Angkachatchai and Finkel, 1999; Thorlacius et al., 2006; Impellizzeri et al., 2012). ROCK inhibitors reduced leukocyte infiltration into the injured spinal cord (Hara et al., 2000), decreased cytokine production (Angkachatchai and Finkel, 1999), and impaired lymphocyte (T cell) proliferation (Tharaux et al., 2003).

Rho pathway plays a role in SCI-induced neuropathic pain as well. Inflammatory mediators are potential candidates for the induction of neuropathic pain. Lysophosphatidic acid, present at the lesion sites both in the peripheral nervous system and the central nervous system, has been shown to initiate neuropathic pain (Inoue et al., 2004). The mechanism of lysophosphatidic acid-induced neuropathic pain is through its binding with G-protein-coupled LPA receptors that activate RhoA/Rho kinase signaling. Blocking RhoA (with Clostridium botulinum C3 transferase) or ROCK (with Y27632) prevented the initiation of neuropathic pain after nerve injury or lysophosphatidic acid injection (Inoue et al., 2004). ROCK inhibitors, Y27632 and H-1152, relieved neuropathic pain in mouse dorsal root injury and spinal nerve transection models (Ramer et al., 2004; Tatsumi et al., 2005) (**Figure 1**). A recent study found that RhoA/Rho kinase pathway mediates p38 MAPK activation and morphological changes by ATP receptors, P2Y12/13, in spinal microglia in neuropathic pain (Tatsumi et al., 2015).

RhoA/Rho Kinase and Cell Death

After SCI, both neurons and glial cells in and around the lesion area undergo apoptosis induced by the secondary injury. The cell death leads to the formation of a lesion cavity (Liu et al., 1997; Shuman et al., 1997). Although mice do not develop cavitation, apoptotic neurons, astrocytes, and oligodendrocytes are still detected (Dubreuil et al., 2003). Many studies show that RhoA/Rho kinase pathway is highly related to cell death. Inhibition of RhoA, both in mice and rats, can significantly reduce the number of apoptotic cell deaths after SCI. The cells that contain RhoA inhibitor are not apoptotic (Dubreuil et al., 2003). This study also shows that activated RhoA promotes the synthesis of proapoptotic protein such as $p75^{NTR}$, which contributes to the initiation of apoptotic cascades. Reducing $p75^{NTR}$ decreases apoptosis in a contused spinal cord (Brandoli et al., 2001) and protects neurons and glia cells (Dubreuil et al., 2003). Other studies have shown that active RhoA activates p38α and triggers p38α-dependent excitotoxic neuronal death. RhoA is sufficient to induce excitotoxic cell death (Semenova et al., 2007).

Additionally, a number of studies have shown that Rho kinase is very important in the regulation of cell death (Shi and Wei, 2007). Rho kinase regulates myosin light chain phosphorylation and stimulates actomyosin contractility, which induces apoptotic cell membrane blebbing, nuclear disintegration, and cellular fragmentation (Coleman et al., 2001; Croft et al., 2005). ROCK2 can promote apoptosis by increasing erin phosphorylation, which increases Fas, the death receptor, clustering, and expression (Piazzolla et al., 2005). In addition, Rho kinase stimulates phosphatase and tensin homologue (PTEN) and inhibits insulin receptor substrate 1(IRS1) signaling to inactivate Akt, which plays an important role in cell survival (Begum et al., 2002; Li et al., 2005) (**Figure 1**). Lastly, Rho kinase mediates inflammation and reactive oxygen species production to induce cell death (Higashi et al., 2003).

RhoA/Rho Kinase and Axon Degeneration

During SCI secondary injury, growth inhibitory proteins such as myelin-associated molecules and glial scar-associated extracellular matrix molecules converge at the RhoA/ROCK pathway to prevent axon regeneration (Forgione and Fehlings, 2014; Fujita and Yamashita, 2014). To date, three myelin-associated growth inhibitors, *i.e.*, Nogo, myelin-associated glycoprotein, and oligodendrocytemyelin glycoprotein (OMgp) (Mckerracher et al., 1994; Chen et al., 2000; Wang et al., 2002), have been reported to block axonal regeneration. For Nogo, there are at least three isoforms: NogoA, NogoB, and NogoC. NogoA is mainly expressed in the nervous system. Two transmembrane domains of Nogo are separated by a 66 amino acid loop, Nogo-66. Nogo-66 is the inhibitory domain that causes growth cone collapse (Fournier et al., 2001). Myelin-associated glycoprotein is required for the formation and maintenance of myelinin normal condition and is identified as a potent inhibitor of neurite outgrowth (Mckerracher et al., 1994). Myelin-associated glycoprotein inhibits axonal growth in older neurons but promotes axonal growth in young neurons depending on the intracellular level of cyclic AMP (cAMP) (Cai et al., 2001). OMgp is a glycosylphophatidylinositol-anchored glycoprotein. It is expressed in both oligodendrocytes and neurons. Notably, all three myelin-associated inhibitory proteins bind to the same receptor, the Nogo receptor. Nogo receptor associates with neurotrophin receptor $p75^{NTR}$ to form a receptor complex. This complex activates RhoA/Rho kinase pathway. Activation of the RhoA/Rho kinase pathway phosphorylates the myosin light chain, LIM kinase, and collapsing response mediator protein-2 to regulate the cytoskeleton dynamics and growth cone collapse, and to inhibit neurite outgrowth (Ohashi et al., 2000; Fukata et al., 2002; Hsieh et al., 2006) (**Figure 1**).

Additionally, repulsive guidance molecule (RGM) also acts as an inhibitor of axon growth. Three homologs of RGM, *i.e.*, RGMa, RGMb, and RGMc, have been identified. Among these molecules, RGMa plays a role in inhibiting axon regeneration (Mueller et al., 2006) and its expression is enhanced around the lesion site after SCI. Treatment with neutralizing anti-RGMa antibodies after SCI in rats promotes axonal regeneration and functional recovery (Hata et

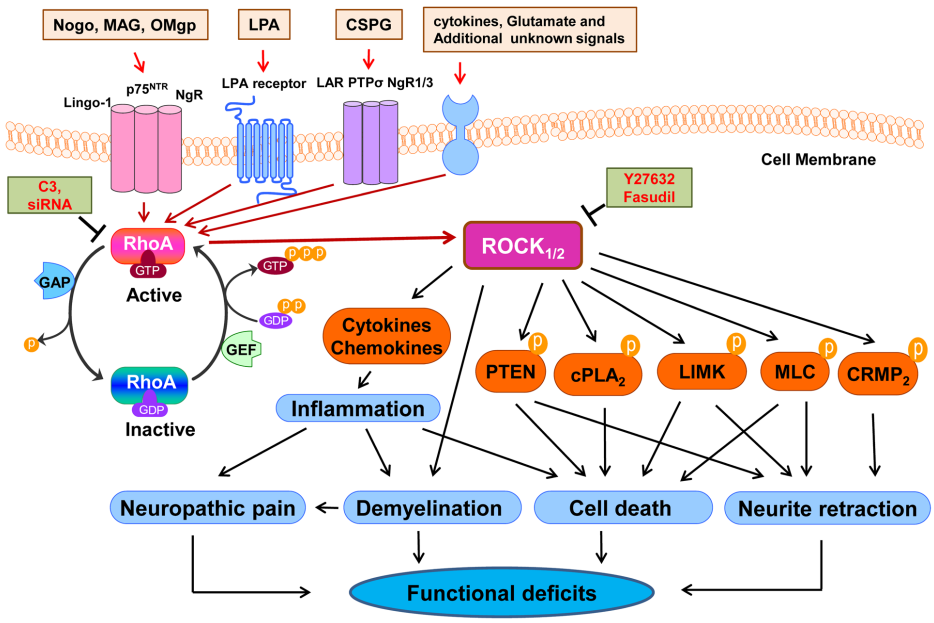

Figure 1 Schematic representation of RhoA/Rho kinase pathway in the pathogenesis of SCI.
The RhoA/Rho kinase pathway is activated by multiple signals and triggers a series of downstream events including inflammation, neuropathic pain, demyelination, cell death, and axon degeneration, all of which contribute to functional deficits. SCI: Spinal cord injury; MAG: myelin-associated glycoprotein; OMgp: oligodendrocytemyelin glycoprotein; LPA: lysophosphatidic acid; CSPG: chondroitin sulfate proteoglycans; LAR: leukocyte common antigen-related phosphatase; ROCK: Rho Kinase; GTP: guanosine triphosphate; GDP: guanosine diphosphate; GEF: guanine nucleotide exchange factor; GAP: GTPase-activating protein; PTPσ: protein tyrosine phosphatase σ; NgR: Nogo receptor; NgR1/3: Nogo receptor 1 and 3; PTEN: phosphatase and tensin homologue; $cPLA_2$: cytosolic phospholipase A2; LIMK: LIM kinase; MLC: myosin light chain; $CRMP_2$: collapsing response mediator protein-2.

al., 2006). RGMa binds with receptor neogenin and activates the RhoA/Rho kinase pathway, leading to neurite outgrowth inhibition (Kubo et al., 2008; Hata et al., 2009).

SCI also triggers a cascade of reactive astrogliosis, which leads to the formation of a glial scar. Reactive astrocytes produce inhibitory extracellular matrix molecule proteoglycans. Chondroitin sulfate proteoglycans are the key component of the glial scar and play important roles in inhibiting axonal regeneration (Yiu and He, 2006). Chondroitin sulfate proteoglycans (CSPGs) bind to a transmembrane protein tyrosine phosphatase (PTPσ) (Shen et al., 2009), leukocyte common antigen-related phosphatase (Fisher et al., 2011), and Nogo receptor 1 and 3 (Dickendesher et al., 2012). These complexes activate the RhoA/Rho kinase signal and, through this pathway, inhibit neurite outgrowth (Dergham et al., 2002; Monnier et al., 2003).

Inhibiting RhoA/Rho Kinase Pathway as a Novel Strategy for SCI Repair

Since the RhoA/Rho kinase pathway is involved in multiple pathophysiologic processes and is a convergence pathway for many inhibition proteins that prevent axon regeneration after SCI, pharmacologic inhibition of RhoA or Rho kinase could be a promising strategy to prevent cell death and promote axon regeneration.

For RhoA inhibition, *Clostridium botulinum* C3 exoenzyme is the prototype of bacterial ADP-ribosyltransferases. C3 selectively modifies RhoA by covalent attachment of an ADP-ribose moiety, which results in inactivation of cellular functions of RhoA (Just et al., 2010). In mouse models of SCI, C3 treatment promoted axonal sprouting, locomotor function recovery and prevented $p75^{NTR}$ dependent cell death after hemisection of the thoracic spinal cord (Dergham et al., 2002; Dubreuil et al., 2003). The next generation of C3 is a cell permeable version which was commercially developed into a clinical grade Rho inhibitor known as BA210 (Trademarked as Cethrin). In rat models of SCI, BA-210 has been shown to penetrate the dura of the spinal cord and cell membrane in a nonspecific, receptor independent manner (Lord-Fontaine et al., 2008).

Based on the promised studies in animal models, BA-210 has been evaluated in a phase I/II clinical trial (Fehlings et al., 2011; McKerracher and Guertin, 2013; Nagoshi et al 2015). In this study, Cethrin was applied to the injury site intraoperatively with a noninvasive, fibrin-mediated delivery system. Forty-eight patients with cervical or thoracic injury were enrolled in this study. Five different doses of Cethrin (0.3–9 mg) were tested. The results showed that the largest neurological recovery occurred in cervical injury patients, whereas patients with thoracic injuries received modest benefits. In the 3-mg dose of Cethrin, 66% of the cervical injured patients changed their ASIA grade from A to C or D (Fehlings et al., 2011).

Another study showed a C3 protein-derived 29 animo-acid (154–182) peptide also significantly improved locomotor functional recovery, enhanced regeneration of corticospinal tract fibers and raphespinal fibers, and improved serotonergic input to lumbar alpha-motoneurons (Boato et al., 2010). A recent study has shown that RhoA siRNA was delivered through intraspinal and lumbar intrathecal approaches (Otsuka et al., 2011). The intraspinal delivery improved hindlimb walking over 6 weeks. Although the lumbar intrathecal

delivery did not promote locomotion recovery, it decreased tactile hypersensitivity significantly and improved the white matter sparing. The siRNA approach also decreased the accumulation of $ED1^+$ macrophages, increased PKC-γ immunoreactivity in the corticospinal tract rostral to the injury and, increased serotonergic fiber innervation in the caudal site of injury (Otsuka et al., 2011).

Besides RhoA inhibition, Rho kinase inhibition also shows promise in axonal regeneration and functional recovery. Inhibitors of Rho kinase such as Y27632 and Fasudil have been tested on rat or mouse models of SCI. High doses or locally applied Y27632 enhanced the sprouting of corticospinal tract fibers and locomotor function recovery (Fournier et al., 2003; Tanaka et al., 2004; Chan et al., 2005). However, with oral delivery, Y27632 showed no effect (Sung et al., 2003). Immediate treatment with Fasudil resulted in increased sprouting and improved locomotor scores, whereas delayed treatment at 4 weeks post-SCI was not effective (Nishio et al., 2006).

Watzlawick et al. (2014) conducted a systematic review and meta-analysis on RhoA/Rho kinase blocking-related reference to analyze the impact of bias and determine the normalized effect size of functional locomotor recovery after experimental thoracic SCI (Watzlawick et al., 2014). Thirty studies (725 animals) examined the effect of RhoA or Rho kinase inhibition on spinal cord injuries including hemisection, contusion, and transection. Locomotor recovery was measured using the Basso, Beattie, and Bresnahan (BBB) locomotor rating score or the Basso Mouse Scale. According to the published work, RhoA/Rho kinase inhibition improved locomotor outcome by 21%. In this study, eight different strategies were used to target the RhoA/Rho kinase pathway including RhoA-GTPase inhibitors (BA-210), C3-peptides, C3-ADP-ribosyltransferase, siRNA, ibuprofen, and ROCK inhibitors (fasudil, Y27632 and p21). Additionally, different routes of drug administration were employed such as intrathecal injection, and topical, intraperitoneal, and oral application. The time of drug administration ranged from 30 minutes before the injury up to 4 weeks after SCI. All these elements may affect the variation of outcome assessments. Finally, different animal species and injury models may also influence the outcomes.

Summary

The RhoA/Rho kinase pathway has been shown to play a unique role in the pathogenesis of SCI. Numerous studies have shown that blocking RhoA/Rho kinase pathway protects cell survival and enhances axonal regeneration leading to functional recovery after SCI. Thus, this pathway is a promising target for SCI treatment in patients. Continued research should be conducted to determine the delivery methods, the dose, and the treatment time window for reaching optimal outcomes.

References

Amano M, Fukata Y, Kaibuchi K (2000) Regulation and functions of Rho-associated kinase. Exp Cell Res 261:44-51.

Angkachatchai V, Finkel TH (1999) ADP-ribosylation of rho by C3 ribosyltransferase inhibits IL-2 production and sustained calcium influx in activated T cells. J Immunol 163:3819-3825.

Bao W, Hu E, Tao L, Boyce R, Mirabile R, Thudium DT, Ma XL, Willette RN, Yue TL (2004) Inhibition of Rho-kinase protects the heart against ischemia/reperfusion injury. Cardiovasc Res 61:548-558.

Begum N, Sandu OA, Ito M, Lohmann SM, Smolenski A (2002) Active Rho kinase (ROK-alpha) associates with insulin receptor substrate-1 and inhibits insulin signaling in vascular smooth muscle cells. J Biol Chem 277:6214-6222.

Boato F, Hendrix S, Huelsenbeck SC, Hofmann F, Grosse G, Djalali S, Klimaschewski L, Auer M, Just I, Ahnert-Hilger G, Holtje M (2010) C3 peptide enhances recovery from spinal cord injury by improved regenerative growth of descending fiber tracts. J Cell Sci 123:1652-1662.

Borgens RB, Liu-Snyder P (2012) Understanding secondary injury. Q Rev Biol 87:89-127.

Brandoli C, Shi BT, Pflug B, Andrews P, Wrathall JR, Mocchetti I (2001) Dexamethasone reduces the expression of p75 neurotrophin receptor and apoptosis in contused spinal cord. Mol Brain Res 87:61-70.

Cai D, Qiu J, Cao Z, McAtee M, Bregman BS, Filbin MT (2001) Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate. J Neurosci 21:4731-4739.

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

Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA, Christ F, Schwab ME (2000) Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 403:434-439.

Coleman ML, Sahai EA, Yeo M, Bosch M, Dewar A, Olson MF (2001) Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. Nat Cell Biol 3:339-345.

Croft DR, Coleman ML, Li SX, Robertson D, Sullivan T, Stewart CL, Olson MF (2005) Actin-myosin-based contraction is responsible for apoptotic nuclear disintegration. J Cell Biol 168:245-255.

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

Dickendesher TL, Baldwin KT, Mironova YA, Koriyama Y, Raiker SJ, Askew KL, Wood A, Geoffroy CG, Zheng BH, Liepmann CD, Katagiri Y, Benowitz LI, Geller HM, Giger RJ (2012) NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. Nat Neurosci 15:703-712.

Dubreuil CI, Winton MJ, McKerracher L (2003) Rho activation patterns after spinal cord injury and the role of activated Rho in apoptosis in the central nervous system. J Cell Biol 162:233-243.

Erschbamer MK, Hofstetter CP, Olson L (2005) 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 484:224-233.

Fehlings MG, Theodore N, Harrop J, Maurais G, Kuntz C, Shaffrey CI, Kwon BK, Chapman J, Yee A, Tighe A, McKerracher L (2011) A phase I/IIa clinical trial of a recombinant Rho protein antagonist in acute spinal cord injury. J Neurotrauma 28:787-796.

Fisher D, Xing B, Dill J, Li H, Hoang HH, Zhao ZZ, Yang XL, Bachoo R, Cannon S, Longo FM, Sheng M, Silver J, Li SX (2011) Leukocyte common antigen-related phosphatase is a functional receptor for chondroitin sulfate proteoglycan axon growth inhibitors. J Neurosci 31:14051-14066.

Forgione N, Fehlings MG (2014) Rho-ROCK Inhibition in the treatment of spinal cord injury. World Neurosurg 82:E535-539.

Fournier AE, GrandPre T, Strittmatter SM (2001) Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature 409:341-346.

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

Fujita Y, Yamashita T (2014) Axon growth inhibition by RhoA/ROCK in the central nervous system. Front Neurosci 8:338.

Fukata Y, Itoh TJ, Kimura T, Menager C, Nishimura T, Shiromizu T, Watanabe H, Inagaki N, Iwamatsu A, Hotani H, Kaibuchi K (2002) CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. Nat Cell Biol 4:583-591.

Hara M, Takayasu M, Watanabe K, Noda A, Takagi T, Suzuki Y, Yoshida J (2000) Protein kinase inhibition by fasudil hydrochloride promotes neurological recovery after spinal cord injury in rats. J Neurosurg 93:94-101.

Hata K, Kaibuchi K, Inagaki S, Yamashita T (2009) Unc5B associates with LARG to mediate the action of repulsive guidance molecule. J Cell Biol 184:737-750.

Hata K, Fujitani M, Yasuda Y, Doya H, Saito T, Yamagishi S, Mueller BK, Yamashita T (2006) RGMa inhibition promotes axonal growth and recovery after spinal cord injury. J Cell Biol 173:47-58.

Higashi M, Shimokawa H, Hattori T, Hiroki J, Mukai Y, Morikawa K, Ichiki T, Takahashi S, Takeshita A (2003) Long-term inhibition of Rho-kinase suppresses angiotensin II-induced cardiovascular hypertrophy in rats in vivo - Effect on endothelial NAD(P)H oxidase system. Circ Res 93:767-775.

Hsieh SHK, Ferraro GB, Fournier AE (2006) Myelin-associated inhibitors regulate cofilin phosphorylation and neuronal inhibition through LIM kinase and slingshot phosphatase. J Neurosci 26:1006-1015.

Impellizzeri D, Mazzon E, Paterniti I, Esposito E, Cuzzocrea S (2012) Effect of fasudil, a selective inhibitor of Rho kinase activity, in the secondary injury associated with the experimental model of spinal cord trauma. J Pharmacol Exp Ther 343:21-33.

Inoue M, Rashid MH, Fujita R, Contos JJ, Chun J, Ueda H (2004) Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling. Nat Med 10:712-718.

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

Li Z, Dong X, Wang Z, Liu WZ, Deng N, Ding Y, Tang L, Hla T, Zeng R, Li L, Wu D (2005) Regulation of PTEN by Rho small GTPases. Nat Cell Biol 7:399-404.

Liu XZ, Xu XM, Hu R, Du C, Zhang SX, McDonald JW, Dong HX, Wu YJ, Fan GS, Jacquin MF, Hsu CY, Choi DW (1997) Neuronal and glial apoptosis after traumatic spinal cord injury. J Neurosci 17:5395-5406.

Loirand G, Guerin P, Pacaud P (2006) Rho kinases in cardiovascular physiology and pathophysiology. Circ Res 98:322-334.

Lord-Fontaine S, Yang F, Diep Q, Dergham P, Munzer S, Tremblay P, McKerracher L (2008) Local inhibition of Rho signaling by cell-permeable recombinant protein BA-210 prevents secondary damage and promotes functional recovery following acute spinal cord injury. J Neurotrauma 25:1309-1322.

McKerracher L, Guertin P (2013) Rho as a target to promote repair: translation to clinical studies with cethrin. Curr Pharm Design 19:4400-4410.

Mckerracher L, David S, Jackson DL, Kottis V, Dunn RJ, Braun PE (1994) Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron 13:805-811.

Monnier PP, Sierra A, Schwab JM, Henke-Fahle S, Mueller BK (2003) The Rho/ROCK pathway mediates neurite growth-inhibitory activity associated with the chondroitin sulfate proteoglycans of the CNS glial scar. Mol Cell Neurosci 22:319-330.

Mueller BK, Mack H, Teusch N (2005) Rho kinase, a promising drug target for neurological disorders. Nat Rev Drug Discov 4:387-398.

Mueller BK, Yamashita T, Schaffar G, Mueller R (2006) The role of repulsive guidance molecules in the embryonic and adult vertebrate central nervous system. Philos Trans R Soc Lond B Biol Sci 361:1513-1529.

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

Ohashi K, Nagata K, Maekawa M, Ishizaki T, Narumiya S, Mizuno K (2000) Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. J Biol Chem 275:3577-3582.

Otsuka S, Adamson C, Sankar V, Gibbs KM, Kane-Goldsmith N, Ayer J, Babiarz J, Kalinski H, Ashush H, Alpert E, Lahav R, Feinstein E, Grumet M (2011) Delayed intrathecal delivery of RhoA siRNA to the contused spinal cord inhibits allodynia, preserves white matter, and increases serotonergic fiber growth. J Neurotrauma 28:1063-1076.

Piazzolla D, Meissl K, Kucerova L, Rubiolo C, Baccarini M (2005) Raf-1 sets the threshold of Fas sensitivity by modulating Rok-alpha signaling. J Cell biol 171:1013-1022.

Ramer LM, Borisoff JF, Ramer MS (2004) Rho-kinase inhibition enhances axonal plasticity and attenuates cold hyperalgesia after dorsal rhizotomy. J Neurosci 24:10796-10805.

Sahai E, Marshall CJ (2002) RHO-GTPases and cancer. Nat Rev Cancer 2:133-142.

Schwab JM, Brechtel K, Mueller CA, Failli V, Kaps HP, Tuli SK, Schluesener HJ (2006) Experimental strategies to promote spinal cord regeneration--an integrative perspective. Prog Neurobiol 78:91-116.

Semenova MM, Maki-Hokkonen AM, Cao J, Komarovski V, Forsberg KM, Koistinaho M, Coffey ET, Courtney MJ (2007) Rho mediates calcium-dependent activation of p38alpha and subsequent excitotoxic cell death. Nat Neurosci 10:436-443.

Shen YJ, Tenney AP, Busch SA, Horn KP, Cuascut FX, Liu K, He ZG, Silver J, Flanagan JG (2009) PTP sigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. Science 326:592-596.

Shi J, Wei L (2007) Rho kinase in the regulation of cell death and survival. Arch Immunol Ther Exp 55:61-75.

Shuman SL, Bresnahan JC, Beattie MS (1997) Apoptosis of microglia and oligodendrocytes after spinal cord contusion in rats. J Neurosci Res 50:798-808.

Stankiewicz TR, Linseman DA (2014) Rho family GTPases: key players in neuronal development neuronal survival, and neurodegeneration. Front Cell Neurosci 8.

Sung JK, Miao L, Calvert JW, Huang LX, Harkey HL, Zhang JH (2003) A possible role of RhoA/Rho-kinase in experimental spinal cord injury in rat. Brain Res 959:29-38.

Tanaka H, Yamashita T, Yachi K, Fujiwara T, Yoshikawa H, Tohyama M (2004) Cytoplasmic p21(Cip1/WAF1) enhances axonal regeneration and functional recovery after spinal cord injury in rats. Neuroscience 127:155-164.

Tatsumi E, Yamanaka H, Kobayashi K, Yagi H, Sakagami M, Noguchi K (2015) RhoA/ROCK pathway mediates p38 MAPK activation and morphological changes downstream of P2Y12/13 receptors in spinal microglia in neuropathic pain. Glia 63:216-228.

Tatsumi S, Mabuchi T, Katano T, Matsumura S, Abe T, Hidaka H, Suzuki M, Sasaki Y, Minami T, Ito S (2005) Involvement of Rho-kinase in inflammatory and neuropathic pain through phosphorylation of myristoylated alanine-rich C-kinase substrate (MARCKS). Neuroscience 131:491-498.

Tharaux PL, Bukoski RC, Rocha PN, Crowley SD, Ruiz P, Nataraj C, Howell DN, Kaibuchi K, Spurney RF, Coffman TM (2003) Rho kinase promotes alloimmune responses by regulating the proliferation and structure of T cells. J Immunol 171:96-105.

Thorlacius K, Slotta JE, Laschke MW, Wang Y, Menger MD, Jeppsson B, Thorlacius H (2006) Protective effect of fasudil, a Rho-kinase inhibitor, on chemokine expression, leukocyte recruitment, and hepatocellular apoptosis in septic liver injury. J Leukoc Biol 79:923-931.

Wang KC, Koprivica V, Kim JA, Sivasankaran R, Guo Y, Neve RL, He ZG (2002) Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature 417:941-944.

Watzlawick R, Sena ES, Dirnagl U, Brommer B, Kopp MA, Macleod MR, Howells DW, Schwab JM (2014) Effect and reporting bias of RhoA/ROCK-blockade intervention on locomotor recovery after spinal cord injury: a systematic review and meta-analysis. JAMA Neurol 71:91-99.

Wei WJ, Yu ZY, Yang HJ, Xie MJ, Wang W, Luo X (2014) Cellular expression profile of RhoA in rats with spinal cord injury. J Huazhong U Sci-Med 34:657-662.

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