Gene Ther Mol Biol Vol 9, 265-268, 2005

Rho/Rho-kinase as potential therapeutic targets for CNS injury

Review Article

Toshihide Yamashita*, Masashi Fujitani, Katsuhiko Hata, Fumiaki Mimura, Junko Taniguchi

Department of Neurobiology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan

---

***Correspondence**: Toshihide Yamashita, Department of Neurobiology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan; Tel: 81-43-2262024; Fax: 81-43-2262025; E-mail: t-yamashita@faculty.chiba-u.jp

Key words: central nervous system, axon, Rho, regeneration

Abbreviations: central nervous system, (CNS); glycosylphosphatidylinositol, (GPI); myelin-associated glycoprotein, (MAG); Nogo-66, (NgR); oligodendrocyte-myelin glycoprotein, (Omgp); Rho guanine nucleotide dissociation inhibitor, (Rho-GDI); spinal cord injury, (SCI)

Received: 1 September 2005; Accepted: 3 October 2005; electronically published: October 2005

Summary

Axons of the adult central nervous system are capable of only a limited amount of regrowth after injury, and that an unfavorable environment plays major roles in the lack of regeneration. Some of the axon growth inhibitory effects are associated with myelin. Three myelin-derived proteins have been identified to inhibit neurite outgrowth in vitro. The p75 receptor, in complex with the Nogo receptor, transduces the signal from all of the myelin-derived inhibitors found to date. The p75 receptor, in response to the myelin-derived proteins, induces activation of Rho, which is one of the key regulators of cytoskeletal organization. Inhibition of Rho or Rho-kinase, downstream effector of Rho, promotes axon regeneration *in vivo*. These findings establish Rho and Rho-kinase as key players in inhibiting the regeneration of the central nervous system, and launched a new wave of studies that aim to promote regeneration of injured axons by modulating this inhibitory pathway.

I. Inability of the adult CNS to regenerate

In 1911, F. Tello described the first successful transplantation of a peripheral nerve into the adult mammalian central nervous system (CNS) (Tello, 1911). Denervated sciatic nerve pieces were implanted into the cortex of rabbits, and he observed fascicles and individual nerve fibers that invaded into these peripheral nerves 2 to 4 weeks after surgery. He and Ramon y Cajal concluded that peripheral nerve Schwann cells reacted to the loss of their axons by the synthesis of attractive and neurite-promoting cues (Ramon y Cajal, 1928). They suggested further that CNS glia would be devoid of such a reaction. Later, Aguayo's group in the early 1980's showed that many neurons can regenerate over long distances if offered a peripheral nerve as a substrate (David and Aguayo, 1981; Richardson et al, 1984; Keirstead et al, 1989).

That CNS myelin is involved in the prevention of axonal regeneration in adult mammals was first suggested by Berry, 1982. He pointed out that non-myelinated axons in the CNS would regenerate after chemical axotomy if damage did not occur to nearby myelinated fibers, but not after mechanical axotomy, which damages myelinated fibers. As damage to the myelinated fibers leads to the release of degeneration products of CNS myelin, it was proposed that this damage would be inhibitory to axonal growth. Subsequently, Schwab's group tested this hypothesis by exposing perinatal DRG or sympathetic neurons to optic and sciatic nerve explants of adult rats in the presence of NGF. However, they observed few or no axons in the optic nerves during 2 weeks in culture, whereas abundant nerve fibers invaded into the sciatic nerves (Schwab and Thoenen, 1985). It was postulated that myelin from the adult CNS is an inhibitory substrate for neurite outgrowth.

II. Myelin derived inhibitors of axon regeneration and their receptors

So far, three major inhibitors expressed by oligodendrocytes and myelinated fiber tracts, Nogo, myelin-associated glycoprotein (MAG) and

oligodendrocyte-myelin glycoprotein (OMgp), have been identified. Nogo was identified as an antigen for the CNS myelin-neutralizing monoclonal antibody IN-1 (Caroni and Schwab, 1988; Chen et al, 2000; GrandPré et al, 2000). MAG, which plays an important role in the formation and maintenance of myelin sheaths (Carenini et al, 1997; Fruttiger et al, 1995; Fujita et al, 1998; Marcus et al, 2002), was found to inhibit neurite outgrowth from some neurons (McKerracher et al, 1994; Mukhopadhyay et al, 1994). OMgp, a major peanut agglutinin-binding polypeptide in the white matter of adult human CNS (Mikol and Stefansson, 1988), was reported to be a third inhibitor of neurite outgrowth (Kottis et al, 2002; Wang et al, 2002b).

A protein that binds Nogo-66, one of the inhibitory domains of Nogo, was identified with high affinity (Fournier et al, 2001). Transfection of the cDNA encoding this putative receptor into retinal ganglion cells at a developmental stage when they otherwise are unresponsive to Nogo-66 promotes growth cone collapse by GST-Nogo-66. Mutated forms of the receptor eliminate growth inhibition by Nogo-66. Therefore, this protein is suggested to be a receptor for Nogo-66 (NgR). NgR is a glycosylphosphatidylinositol (GPI) anchor protein that attaches to the outer leaflet of the plasma membrane, and is expressed in the CNS neurons as well as their axons (Josephson et al, 2002; Wang et al, 2002). As release of GPI-anchored proteins by phosphatidylinositol-specific phospholipase C from embryonic DRG results in the abolishment of growth cone collapse in response to Nogo-66, NgR mediates the signal from Nogo-66 in at least these neurons. Interestingly, MAG and OMgp, also bind to NgR. In an expression screening for NgR-interecting proteins, MAG was isolated as a binding partner for NgR (Liu et al, 2002). Another group identified it by direct binding studies based on the similarity in molecular weight to candidates revealed in a previous characterization of MAG binding proteins (Domeniconi et al, 2002). NgR was also obtained by screening for proteins that bind to OMgp (Wang et al, 2002). Thus, it was demonstrated that NgR is necessary for inhibition of axon growth by MAG, Nogo-66 and OMgp *in vitro*. These findings bring three molecules to an intersection at the level of NgR.

From the perspective of trying to develop a therapeutic approach, it is important to note that a fragment of Nogo-66 binds to NgR as a high affinity antagonist (GrandPre et al, 2002). The antagonist peptide, NEP1-40, reduces endogenous inhibitory activity, to promote sprouting of corticospinal tract axons, long distance growth and functional recovery.

III. p75 transduces the signal from MAG, Nogo and OMgp

Although NgR is a binding partner for MAG, Nogo-66 and OMgp, the GPI-linked nature of NgR suggests that there may be a second receptor subunit that spans the plasma membrane and mediates signal transduction. This second subunit was found to be a receptor called the p75 receptor (p75) (Yamashita et al, 2002; Wang et al, 2002). P75 transduces the signal from these proteins. In addition, it was reported recently that Lingo-1 is involved in the signal transduction of myelin-derived inhibitors (Mi et al, 2004), although the precise mechanism of the action remains to be determined. The receptor complex of the three myelin-derived inhibitors therefore consists of at least three elements, Lingo-1, NgR and p75 (**Figure 1**).

One potential clue to understanding the signal transduction mechanism downstream of p75 is found through observations demonstrating that the small GTPase RhoA is a key intracellular effector for growth inhibitory signaling by myelin. In its active GTP-bound form, RhoA rigidifies the actin cytoskeleton, thereby inhibiting axon elongation and mediating growth cone collapse. RhoA is activated by MAG, Nogo-66 and OMgp through a p75-dependent mechanism, thus inhibiting neurite outgrowth from postnatal sensory neurons and cerebellar neurons (Yamashita et al, 2002; Wang et al, 2002; Wong et al, 2002). In neurons, myelin and MAG inhibit growth, that is abolished by the botulinus toxin C3 which inactivates Rho (Lehmann et al, 1999). More specifically, it is directly shown by the affinity precipitation of GTP-bound form of Rho that Rho is activated by MAG-Fc in the cerebellar granule neurons (Yamashita et al, 2002).

The precise mechanism of action of $p75^{NTR}$ is suggested by the finding that $p75^{NTR}$ releases Rho from Rho guanine nucleotide dissociation inhibitor (Rho-GDI) (**Figure 1**) (Yamashitra and Tohyama, 2003), thus eliciting activation of Rho. Rho-GDI is an essential part of the signaling mechanism that suppresses the activity of Rho. Rho proteins are regulated either by enzymes that enhance GTP binding and activity (guanine nucleotide exchange factors) or by proteins that increase the hydrolysis of GTP (GTPase activating proteins) and thus decrease activity. Rho is kept in an inactive state in cells by Rho-GDI (Sasaki et al, 1998). Rho-GDI inhibits the activity of Rho by binding to and sequestering Rho in the cytoplasm, by inhibiting the formation of active RhoGTP, and by blocking the binding of Rho to its effectors.

These findings establish Rho as a key player in inhibiting the regeneration of the CNS, and launched a new wave of studies that aimed to promote regeneration of injured axons by modulating this inhibitory pathway. For example, an inhibitor of Rho kinase, a downstream effector of Rho, called Y-27632 has been used to probe the role of Rho in growth inhibiting signaling (Dergham et al, 2002; Fournier et al, 2003). Treating neurons with C3 transferase, a bacterial endotoxin that inactivates Rho, or with Y-27632, promotes growth on inhibitory substrates.

Intriguingly, a peptide that blocks the pathway elicited by MAG, Nogo and OMgp was found (Yamashita and Tohyama, 2003). The binding region of Rho-GDI on $p75^{NTR}$ was identified as the fifth alpha helix in the $p75^{NTR}$. intracellular domain. The short sequence of the fifth helix is similar to mastoparan, a 14-residue peptide of wasp venom that is known to be capable of activating Rho (Koch et al, 1991). A peptide ligand to this region was previously reported by Ilag's group (Ilag et al, 1999) by screening a combinatorial library using a variation of the selectively-infective phage method. This peptide,

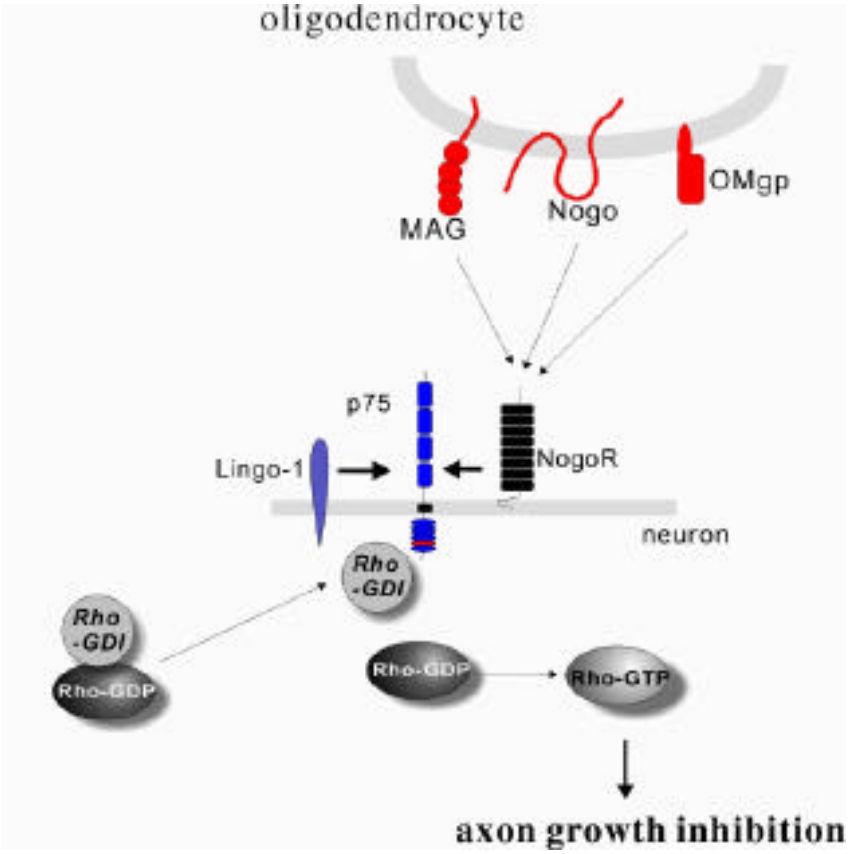

Figure 1. Mechanisms of axon growth inhibition by the Nogo receptor complex. In the absence of myelin-derived inhibitors, growth occurs as a result of Rho-GDI-induced suppression of Rho activity. Rho-GDI maintains Rho in an inactive state by binding, and prevents Rho from interacting with its effectors. Activation of p75 promotes dissociation of Rho-GDI from RhoA, allowing RhoA to become activated through the exchange of GDP for GTP. The activated RhoA then interacts with its signaling molecules to elicit axon growth inhibition in some neurons.

designated Pep5, inhibits the interaction of $p75^{NTR}$ with Rho-GDI *in vitro* and *in vivo*. The inhibitory peptide completely abolishes the effects mediated by MAG or Nogo-66 in adult DRG neurons and postnatal cerebellar granule neurons (Yamashita and Tohyama, 2003), establishing the Rho-GDI- $p75^{NTR}$ association as an important mecahnism of $p75^{NTR}$ -induced suppression of axon growth by myelin proteins. An especially notable aspect is that the peptide does not inhibit other functions of $p75^{NTR}$, such as axon elongation or cell death by neurotrophins.

IV. Axon regeneration by blocking Rho or Rho-kinase

As the signaling pathway of MAG, Nogo and OMgp is considered to contribute to the lack of regeneration of the injured central nervous system, blocking the signal is expected to promote regeneration of the injured axons. Indeed, inhibition of Rho-kinase facilitates axonal regeneration of the injured neurons and improves locomotor activity after spinal cord injury (SCI) (Dergham et al, 2002; Fournier et al, 2003; Tanaka et al., 2004). In these studies, small-molecule Rho-kinase inhibitors (Y-27632 and Fasudil) and the peptide inhibitor derived from p21 (WAF1/Cip1) lacking nuclear localization signal were used. Rho-kinase inhibition not only enhanced nerve-fibre growth beyond the lesion site, but was also neuroprotective and decreased tissue damage and cavity formation. Altogether, Rho-kinase may be a good molecular target against injuries in the CNS and the therapeutic potential of blocking Rho/Rho-kinase activation for the treatment of CNS injury, in fact, has been studied.

References

Berry M (**1982**) Post-injury myelin-breakdown products inhibit axonal growth: An hypothesis to explain the failure of axonal regeneration in the mammalian central nervous system. **Bibliotheca Anatomica** 23, 1-11.

Carenini S, Montag D, Cremer H, Schachner M, and Martini R (**1997**) Absence of the myelin-associated glycoprotein (MAG) and the neural cell adhesion molecule (N-CAM) interferes with the maintenance, but not with the formation of peripheral myelin. **Cell Tissue Res** 287, 3-9.

Caroni P, and Schwab ME (**1988**) Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate properties of CNS white matter. **Neuron** 1, 85-96.

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

David S, and Aguayo AJ (**1981**) Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. **Science** 214, 931-933.

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

Domeniconi M, Cao Z, Spencer T, Sivasankaran R, Wang K, Nikulina E, Kimura N, Cai H, Deng K, Gao Y, He Z, and Filbin M (**2002**) Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. **Neuron** 35, 283-290.

GrandPré T, Nakamura F, Vartanian T, and Strittmatter SM (**2000**) Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. **Nature** 403, 439-444.

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.

Fruttiger M, Montag D, Schachner M, and Martini R (**1995**) Crucial role for the myelin-associated glycoprotein in the maintenance of axon-myelin integrity. **Eur J Neurosci** 7, 511-515.

Fujita N, Kemper A, Dupree J, Nakayasu H, Bartsch U, Schachner M, Maeda N, Suzuki K, and Popko B (**1998**) The cytoplasmic domain of the large myelin-associated glycoprotein isoform is needed for proper CNS but not peripheral nervous system myelination. **J Neurosci** 18, 1970-1978.

GrandPre T, Li S, and Strittmatter SM (**2002**) Nogo-66 receptor antagonist peptide promotes axonal regeneration. **Nature** 417, 547-551.

Ilag LL, Rottenberger C, Liepinsh E, Wellnhofer G, Rudert F, Otting G, and Ilag LL (**1999**) Selection of a peptide ligand to the p75 neurotrophin receptor death domain and determination of its binding sites by NMR. **Biochem Biophys Res Commun** 1999, 255, 104-109.

Josephson A, Trifunovski A, Widmer HR, Widenfalk J, Olson L, Spenger C (**2002**) Nogo-receptor gene activity: cellular localization and developmental regulation of mRNA in mice and humans. **J Comp Neurol** 453, 292-304.

Keirstead SA, Rasminsky M, Fukuda Y, Carter DA, Aguayo AJ, and Vidal-Sanz M (**1989**) Electrophysiologic responses in hamster superior colliculus evoked by regenerating retinal axons. **Science** 246, 255-257.

Koch G, Haberman B, Mohr C, Just I, and Aktories K (**1991**) Interaction of mastoparan with the low molecular mass GTP-binding proteins rho/rac. **FEBS Lett** 291, 336-340.

Kottis V, Thibault P, Mikol D, Xiao ZC, Zhang R, Dergham P, and Braun PE (**2002**) Oligodendrocyte-myelin glycoprotein (OMgp) is an inhibitor of neurite outgrowth. **J Neurochem** 82, 1566-1569.

Lehmann M, Fournier A, Selles-Navarro I, Dergham P, Sebok A, Leclerc N, Tigyi G, and McKerracher L (**1999**) Inactivation of Rho signaling pathway promotes CNS axon regeneration. **J Neurosci** 19, 7537-7547.

Liu BP, Fournier A, GrandPre T, and Strittmatter SM (**2002**) Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. **Science** 297, 1190-1193.

Marcus J, Dupree JL, and Popko B (**2002**) Myelin-associated glycoprotein and myelin galactolipids stabilize developing axo-glial interactions. **J Cell Biol** 156, 567-577.

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

Mi S, Lee X, Shao Z, Thill G, Ji B, Relton J, Levesque M, Allaire N, Perrin S, Crowell T, Cate RL, McCoy JM, and Pepinsky RB (**2004**) LINGO-1 is a component of the NOGO-66 receptor/p75 signaling complex. **Nat Neurosci** 7, 221-228.

Mikol DD, and Stefansson K (**1988**) A phosphatidylinositol-linked peanut agglutinin-binding glycoprotein in central nervous system myelin and on oligodendrocytes. **J Cell Biol** 106, 1273-1279.

Mukhopadhyay G, Doherty P, Walsh FS, Crocker PR, and Filbin MT (**1994**) A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. **Neuron** 13, 757-767.

Ramon y Cajal S (**1928**) Degeneration and regeneration of the nervous system. London: **Oxford Unversity Press.**

Richardson PM, Issa VM, and Aguayo AJ (**1984**) Regeneration of long spinal axons in the rat. **J Neurocytol** 13, 165-182.

Sasaki T, and Takai Y (**1998**) The Rho small G protein family-Rho GDI system as a temporal and spatial determinant for cytoskeletal control. **Biochem Biophys Res Commun** 245, 641-645.

Schwab ME, and Thoenen H (**1985**) Dissociated neurons regenerate into sciatic but not optic nerve explants in culture irrespective of neurotrophic factors. **J Neurosci** 5, 2415-2423.

Tanaka H, Yamashita T, Yachi K, Fujiwara K, Yoshikawa K, and Tohyama M (**2004**) Cytoplasmic p21Cip1/WAF1 enhances axonal regeneration and functional recovery after spinal cord injury in rats. **Neurosci** 127, 155-164.

Tello F (**1911**) La influencia del neurotropismo en la regeneracion de los centros nerviosos. **Trab Lab Invest Biol** 9, 123-159.

Wang KC, Kim JA, Sivasankaran R, Segal R, and He Z (**2002**) p75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. **Nature** 420, 74-78.

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

Wang X, Chun SJ, Treloar H, Vartanian T, Greer CA, and Strittmatter SM (**2002**) Localization of Nogo-A and Nogo-66 receptor proteins at sites of axon-myelin and synaptic contact. **J Neurosci** 22, 5505-5515.

Wong ST, Henley JR, Kanning KC, Huang KH, Bothwell M, and Poo MM (**2002**) A p75(NTR) and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. **Nat Neurosci** 5, 1302-1308.

Yamashita T. Higuchi H, and Tohyama M (**2002**) The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. **J Cell Biol** 157, 565-570.

Yamashita T, and Tohyama M (**2003**) The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. **Nat Neurosci** 6, 461-467.

Toshihide Yamashita