Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Genetics and Genomics 38 (2011) 515−523

JOURNAL OF GENETICS AND GENOMICS

www.jgenetgenomics.org

Nogo receptor 3, a paralog of Nogo-66 receptor 1 (NgR1), may function as a NgR1 co-receptor for Nogo-66

Lei Zhang [a,b,*], Xia Kuang [a], Jian Zhang [a,*]

[a] *State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

[b] *State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China*

Received 15 August 2011; revised 8 October 2011; accepted 16 October 2011

Abstract

Nogo-A is a major myelin associated inhibitor that blocks regeneration of injured axons in the central nervous system (CNS). Nogo-66 (a 66-residue domain of Nogo-A) expressed on the surface of oligodendrocytes has been shown to directly interact with Nogo-66 receptor 1 (NgR1). A number of additional components of NgR1 receptor complex essential for its signaling have been uncovered. However, detailed composition of the complex and its signaling mechanisms remain to be fully elucidated. In this study, we show that Nogo receptor 3 (NgR3), a paralog of NgR1, is a binding protein for NgR1. The interaction is highly specific because other members of the reticulin family, to which Nogo-A belongs, do not bind to NgR3. Neither does NgR3 show any binding activity with Nogo receptor 2 (NgR2), another NgR1 paralog. Majority of NgR3 domains are required for its binding to NgR1. Moreover, a truncated NgR3 with the membrane anchoring domain deleted can function as a decoy receptor to reverse neurite outgrowth inhibition caused by Nogo-66 in culture. These *in vitro* results, together with previously reported overlapping expression profile between NgR1 and NgR3, suggest that NgR3 may be associated with NgR1 *in vivo* and that their binding interface may be targeted for treating neuronal injuries.

Keywords: Nogo-66 receptor 1; NgR3; Neurite outgrowth; Axonal regeneration

1. Introduction

Axonal regeneration in mature or injury within the central nervous system (CNS) of mammalian adults is restricted because of a number of factors. In addition to their limited intrinsic outgrowth capability, injured axons face unfavorable CNS environments, such as inhibitory myelin associated inhibitors (MAIs), including Nogo-A, oligodendrocyte-myelin glycoprotein (OMgP) and myelin associated glycoprotein (MAG) (Schwab, 2010). The most well studied MAI is Nogo-A, which belongs to reticulin protein family. Thus, it is also named as reticulon 4 (RTN4). There are three isoforms for Nogo proteins, Nogo-A, B and C, all of which contain two long hydrophobic stretches linked by a 66 amino acid residue fragment named Nogo-66. Two receptors for Nogo-66 have been identified to mediate its inhibitory activity for axonal sprouting. One is the paired immunoglobulin-like receptor B (PirB), which is an immunoreceptor (Atwal et al., 2008). The other is NgR1 (Fournier et al., 2001). NgR1 is a glycosylphosphatidylinositol (GPI)-linked protein, as a member of leucine rich repeat (LRR) protein family. Other MAIs, such as MAG and OMgP, have also been found to interact with NgR1. Moreover, additional proteins such as amyloid protein fragment amyloid-β (Park et al., 2006), B-lymphocyte stimulator (BLyS) (Zhang et al., 2009) and leucine-rich glioma-inactivated protein 1 (LGI1) (Thomas et al., 2010), also bind to NgR1 and negatively regulate axonal sprouting and neurite outgrowth. Since there are no intracellular domains within NgR1, other transmembrane co-receptors are required to input

* Corresponding authors. Tel/fax: +86 10 6488 8540 (L. Zhang); +86 10 6484 7018 (J. Zhang).

E-mail addresses: leizhang@moon.ibp.ac.cn (L. Zhang); jianzhang84@genetics.ac.cn (J. Zhang).

doi:10.1016/j.jgg.2011.10.001

extracellular signals. Several NgR1 co-receptors have been identified, including p75 (Wang et al., 2002), Lingo1 (Mi et al., 2004) and TROY (Park et al., 2005). Acute blockade of NgR1 functions promotes some axonal regeneration *in vivo* (Giger et al., 2010). However, results from a study using *NgR1* knock-out mice suggested that restricting NgR1 function alone may not be sufficient for corticospinal tract regeneration *in vivo*, indicating the existence of extra negative regulators (Zheng et al., 2005). For example, deficiency of NgR1 may be compensated by other receptors, such as PirB, implicating that blocking all major receptors is essential for significant axonal outgrowth. Indeed, simultaneous disruption of both PirB and NgR1 activities promotes robust neuronal regeneration, suggesting that the interactions between MAIs and their receptors may be essential for future CNS therapy (Atwal et al., 2008). The complexity of MAI receptors and their signaling indicates that some elements involved in the myelin inhibitory pathways remain to be identified.

On receptor level, there are two NgR1 paralogs, NgR2 and NgR3, which share similar protein structures with NgR1 including LRR motif and the GPI-anchored domain. Three NgRs are overlappingly expressed in the developing and adult CNS (Pignot et al., 2003). NgR2 was identified to bind to MAG with a higher affinity and to mediate MAG induced inhibitory effects (Venkatesh et al., 2005). However, biochemical properties and functions of NgR3 remain unclear. Given the structure similarity and expression profiles, we hypothesized that NgR3 may also be involved in negatively regulating neural outgrowth. In this study, we show that NgR3 is a binding protein for NgR1 *in vitro*. Furthermore, we show that disruption of NgR1 and NgR3 interaction reverses neurite inhibition induced by Nogo-66 by using dorsal root ganglion (DRG) neurite outgrowth assay. These results indicate that heterodimerization between NgRs is likely required for transducing MAI signals and that such interactions may be potentially targeted to promote axonal outgrowth.

2. Materials and methods

2.1. Plasmids

Human *NgR1* (GenBank: NM_023004.5), *NgR3* (GenBank: NP_848663) and *Lingo1* (GenBank: NP_116197) full length cDNAs were amplified from a human fetal brain cDNA library. Human *DAF* cDNA (GenBank: M31516) was amplified from human spleen cDNA library and then cloned into pRK5-tkneo vector. pBluescriptKS-p75 plasmid containing human *p75* full length cDNA is a generous gift from Dr. Moses V. Chao (New York University, USA). Sequence encoding extracellular domain of p75 (31-249 aa) was cloned and ligated into pFLAG-AP-CMV1 vector to express AP-p75. FLAG-AP-Nogo66 is a fusion protein of human Nogo-66 (amino acids 1055−1120 in Nogo-A) and alkaline phosphatase (AP) at NH_2-terminus and was produced from pFLAG-AP-Nogo66-CMV1. AP-TROY plasmid is a generous gift from Dr. Zhigang He (Children's Hospital, Harvard Medical School, USA).

2.2. Protein expression and purification

All of the proteins were transiently expressed in HEK 293T cells using calcium phosphate transfection method. Conditioned medium was concentrated using Centricon tubes Plus-20 (Millipore, Beijing, China). Human NgR3 (amino acids 1−419) fused with human Fc at C-terminus was purified by immobilized Protein A agarose (RepliGen, MA, USA). FLAG-AP-Nogo66 was purified using anti-FLAG M2 affinity gel (Sigma, St. Louis, MO, USA). AP activities of AP fusion proteins were calculated using standard curves of EIA grade alkaline phosphatase (Roche Applied Science, Mannheim, Germany).

2.3. Expression cloning

The expression cloning procedure was adapted from previous protocols (Zhang et al., 2009). Briefly, 36−48 h after plasmid transfection into COS-7 cells, AP-NgR3 protein was added and further incubated for 2 h at 23°C. After incubation, cells were washed 6 times before fixation with 1.8% formaldehyde. After washing several times, endogenous AP was inactivated by heating at 65°C for 2 h. Cells were then rinsed with AP substrate buffer. Bound AP fusion proteins were visualized by adding 5-bromo-4-chloro-3-indolylophosphate toluidinium-nitroblue tetrazolium (NBT/BCIP) (Roche Applied Science). In competition experiments, COS-7 cells were incubated with NgR3-Fc or anti-human IgG (Ding Guo Corp., Beijing, China) for 1 h before recombinant AP proteins were added. For quantification of NgR3 and NgR1 binding, NgR1 expressing COS-7 cells were lysed in 20 mmol/L Tris, pH 8.0, 0.1% Triton after incubation with AP-NgR3. The cell lysates were incubated at 65°C and the amount of bound AP was revealed by *p*-Nitrophenyl Phosphate Disodium (Sigma, Beijing, China) and then measured at OD_{405nm}. For binding mapping experiment, different AP-NgR3 mutants were as follows: NgR3ΔLRRNF, 57−414 aa; NgR3ΔLRR, (10−32) + (248−414) aa; NgR3ΔLRRCF, (25−247) + (311−414) aa; NgR3ΔBD, 25−310 aa. For NgR3ΔLRR and NgR3ΔLRRCF construction, two fragments were fused by *Kpn* I, equal to introducing Gly and Thy. NgR1 expression was revealed by staining cells with monoclonal antibody against NgR1 (α-NgR1) generously provided by Biogene Inc. (Li et al., 2004).

2.4. Immunoprecipitation

HEK 293T cells were transfected with NgR1-myc and NgR3-FLAG plasmids. Cells were collected and lysed in 1 mL lysis buffer (20 mmol/L Hepes, pH 7.4, 1 mmol/L EGTA, 10% glycerol, 150 mmol/L NaCl, 1% NP-40) for 20 min at 4°C. After centrifugation at 14,000 × *g*, 10 min at 4°C, the supernatant was incubated with 2 μg antibodies against FLAG for 1 h on ice. Ten microliters of Protein A agorose was added to the mixture for another incubation for 1 h on ice. The samples were analyzed by Western blotting.

FLAG-NgR3 was detected with anti-FLAG. NgR1-myc was detected with anti-Myc.

2.5. Modeling of NgR1 and NgR3 binding

Discovery Studio 2.0 software was used in this study. NgR3 protein structure was predicted referring to NgR1 protein crystal structure (PDB: 1OZN) by running modeler program. The highest scored NgR3 structure was locked and optimized according to profile-3D criteria. NgR1 and NgR3 protein docking was then calculated by ZDOCK program. Various docking poses were filtered by interface cutoff and receptor binding site residues.

2.6. Primary cell culture, neurite outgrowth and growth cone collapse assays

Chicken E12-12.5 dorsal root ganglions (DRGs) were dissected in cold HBSS buffer (Hyclone, Beijing, China). For neurite outgrowth assay, glass coverslips in 24-well plates were coated by poly-L-lysine (Sigma). One microgram FLAG-AP-Nogo66 or 100 ng NgR3-Fc in PBS was spotted on the coverslips and air dried in tissue culture hood. DRGs were trypsinized and titrated in DMEM containing 10% fetal calf serum medium (Hyclone, Beijing, China). Three hours after seeding on the plate, half of the medium was replaced with fresh Neurobasal complete medium supplemented with B27 and 30 ng/mL nerve growth factor (NGF) (Invitrogen, Beijing, China).

After culturing for 14−18 h, neurons were rinsed with HBSS twice and fixed with 4% paraformaldehyde (PFA) containing 25% sucrose for 2 h at room temperature. The cells were blocked and then incubated with monoclonal antibody Tuj 1 against class III β-tubulin (Millipore, Beijing, China) for 50 min at 26−28°C. Cells were washed with PBS 3 times, 8 min each. Then cells were incubated with a secondary antibody against mouse conjugated with Rhodamine (ZSGB-Bio, Beijing, China) followed by washing with PBS at least 4 times, 8 min each. After mounting, the DRG neurites were observed under a Nikon ECLIPSE TE2000-U fluorescence microscope. Only well separated neurites were counted. Neurite length was measured from the central cell body to the end of neurite using RS image software (Roper Scientific, Tucson, AZ, USA).

Each quantification experiment was repeated at least 3 times. Photos were taken randomly but not repeatedly. Each group was counted double-blindedly. Data was analyzed by Student's *t* test using Graphpad Prisim 5.0 software. All error bars represent SEM.

3. Results

3.1. NgR1 binds to NgR3 in vitro

Although NgR3 shows similar structures and overlapping expression profiles with NgR1 and NgR2, functions of NgR3 have not been reported. As the first step to explore NgR3 activities, we set to identify interacting proteins of NgR3 using an expression cloning approach. To this end, we used a probe composed of soluble NgR3 fused with AP (AP-NgR3) at NH_2-terminus to screen a human fetal brain cDNA library. A cDNA clone was recovered from the screen and was shown to encode an open reading frame of *NgR1* (Fig. 1A). The binding of AP-NgR3 to COS-7 cells transfected with *NgR1* cDNA could be blocked by soluble NgR3, suggesting that NgR3 and NgR1 specifically interact (Fig. 1B and C). To rule out possible nonspecific bindings, we showed that AP-NgR3 did not bind to cells expressing decay-accelerating factor (DAF), another GPI-anchored protein (a negative control) (Fig. 1D). As previously reported (Wang et al., 2002), AP-p75 was shown to bind to cells expressing NgR1 (a positive control, Fig. 1E). In addition, AP protein alone did not bind to NgR1 expressing COS-7 cells (Fig. 1F). The NgR3−NgR1 binding affinity is high as the half-maximal saturation of the binding between the two purified molecules was measured at 4.57 ± 1.71 nmol/L (Fig. 1G). Moreover, we provided further biochemical evidence of NgR1 and NgR3 interaction by a co-immunoprecipitation assay (Fig. 1H). Collectively, these results suggest that NgR3 is a new NgR1 interaction protein *in vitro* and may function in the NgR1 receptor complex.

3.2. NgR3 does not bind to RTN proteins or NgR1 related co-receptors in COS-7 cells

The similarity among NgRs suggests that NgR3 may also function as a ligand binding receptor for MAIs. A previous study demonstrated that Nogo-66 did not bind to NgR3 (Venkatesh et al., 2005), which is consistent with our observation in AP-NgR3 binding assays (data not shown). Since Nogo-A shares similar RTN domain at C-terminus with the other three RTN proteins RTN1, RTN2 and RTN3, we reasoned that these RTNs could be candidate ligands for NgR3. To test this possibility, we used AP-NgR3 to probe COS-7 cells transfected with RTN1, RTN2 or RTN3, respectively. The results showed that none of these transmembrane proteins could bind to AP-NgR3 (Fig. 2), demonstrating that RTN1, RTN2 or RTN3 may not be ligands to NgR3.

On the other hand, the similarity among NgRs prompted us to explore whether NgR3 could bind to NgR2 or to NgR3 itself. In a similar setting, we tested whether NgR1 co-receptors p75, Lingo1 or TROY could interact with AP-NgR3. Our results showed that AP-NgR3 did not bind to COS-7 cells expressing NgR2, NgR3 or any of the known NgR1 co-receptors (Fig. 3). Thus NgR3 may not be a receptor partner for NgR2 or NgR1, further supporting the notion that binding between NgR1 and NgR3 is specific.

3.3. Majority of NgR3 domains are required for binding to NgR1

NgR3 and NgR1 share 45% identity and 56% similarity at amino acid level. Like NgR1, NgR3 has a signal peptide, LRR amino-terminal fragment sequence (LRRNF), LRR (1−8) domain, LRR carboxyl fragment sequence (LRRCF)

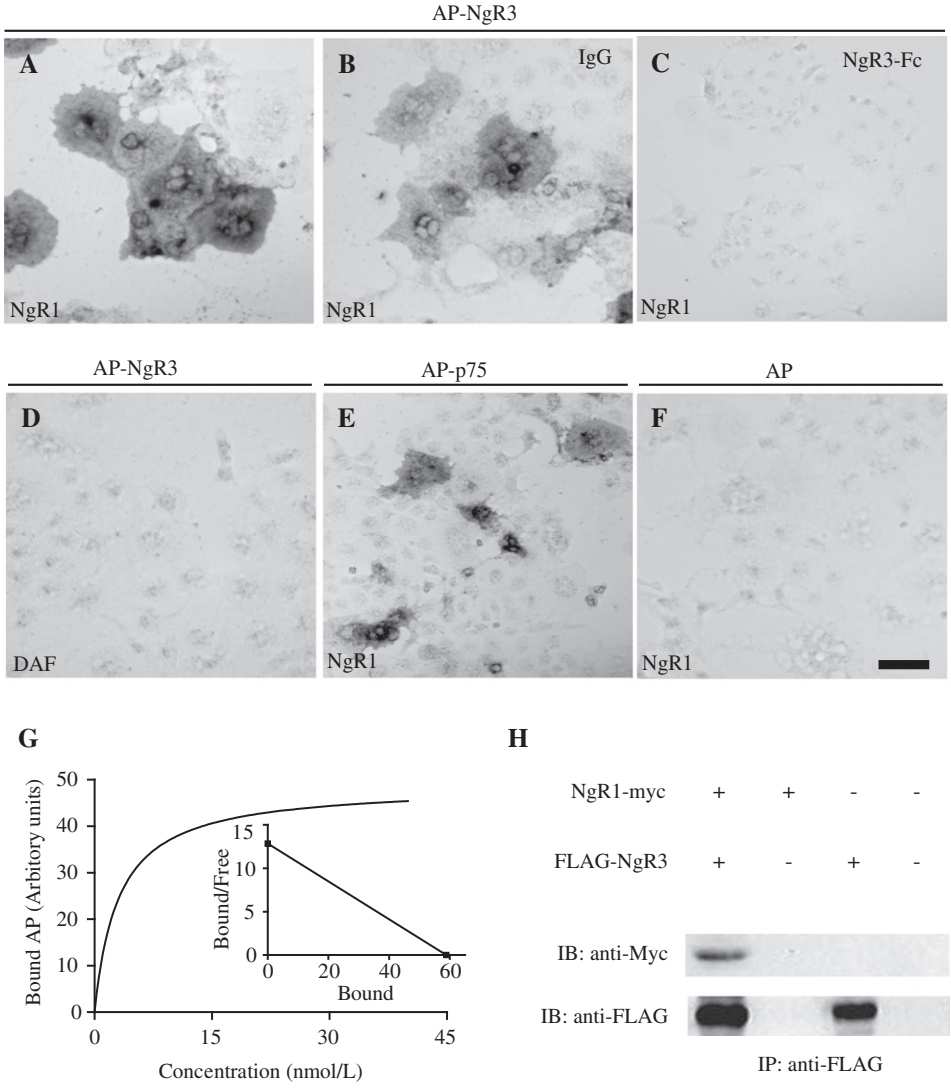

Fig. 1. NgR3 interacts with NgR1 *in vitro*. COS-7 cells transfected with human *NgR1* cDNA are incubated with AP-NgR3 (**A**), with AP-NgR3 but preincubated with IgG (**B**) or NgR3-Fc (**C**), or AP-p75 (**E**), or AP (**F**). Bound AP was visualized using NBT/BCIP as a substrate. DAF (**D**) was transfected into COS-7 cells and then incubated with AP-NgR3. The resulting cells are incubated with AP-NgR3 and stained for AP activity visualization. DAF, decay-accelerating factor, is a GPI linked protein. Scale bar, 100 μm. **G:** quantification of AP-NgR3 binding to NgR1 expressing COS-7 cells. Binding affinity (Kd) is calculated as 4.57 ± 1.71 nmol/L using the binding saturation curve. Inbox is the Scatchard plot. **H:** interaction of NgR1 and NgR3 in HEK 293T cells evaluated by co-immunoprecipitation. NgR1-myc and FLAG-NgR3 were co-transfected into HEK 293T cells. The cell lysates were immunoprecipitated with anti-FLAG. Antibody against FLAG was used to detect FLAG-NgR3. Antibody against Myc is used to detect NgR1-myc.

and a GPI domain. To investigate the structural basis of the interaction between NgR1 and NgR3, we first used a homology modeling method to obtain a putative structure of NgR3 using NgR1 protein structure as a template (Fig. 4A). To predict possible interactions between NgR1 and NgR3, we further generated models for NgR1 and NgR3 interaction using ZDOCK program. Most models suggest that majority domains of both proteins are involved in mutual interactions. Several models also suggest a possible Cys−Cys interaction at C-terminus of proteins (Fig. 4B and C). The Cys residue predicated for disulfide linkage formation is located in the LRRCF region.

To experimentally map domains required for the interaction between NgR1 and NgR3, various domains of NgR3 were fused to the C-terminus of AP (Fig. 5A). The probes were used to stain NgR1 expressing COS-7 cells. Results indicated that mutant AP-NgR3 without LRRNF did not affect its binding to NgR1, while deletion of any other domains except for GPI resulted in negative binding (Fig. 5B). These results demonstrate that most domains in NgR3 are required for NgR1 and NgR3 interaction in COS-7 cells.

3.4. A truncated NgR3 antagonizes Nogo-66 mediated neurite outgrowth inhibition in vitro

Decoy receptors have been extensively used to neutralize functions of their respective receptors both *in vitro* and *in vivo*. NgR1-Fc has been used in some studies to block Nogo-66

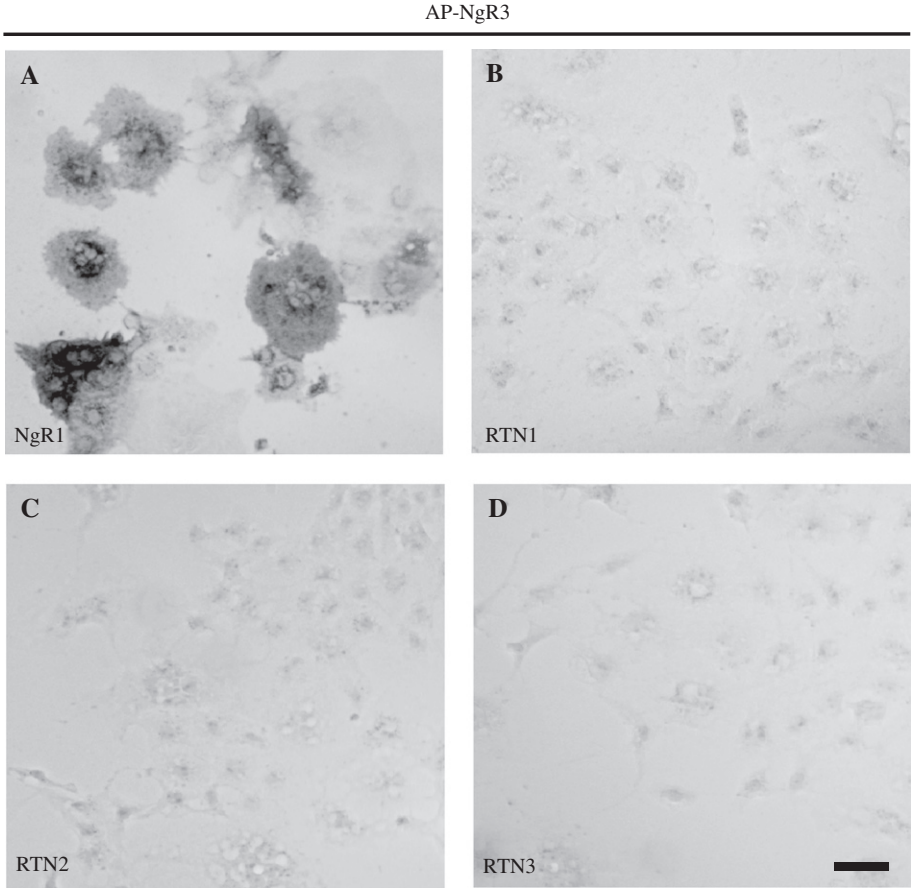

Fig. 2. AP-NgR3 does not bind to COS-7 cells expressing other RTN proteins. NgR1(positive control) (**A**), RTN1 (**B**), RTN2 (**C**) and RTN3 (**D**) were transfected into COS-7 cells, respectively. The transfected cells were incubated with AP-NgR3 and stained for visualization of protein interaction. Scale bar: 100 μm.

inhibitory activities (Fournier et al., 2002). If NgR3 is involved in Nogo-66 signaling through binding to NgR1, it is conceivable that NgR3 may be required for the signaling and that limiting NgR3 activity may also relieve Nogo-66 induced inhibition. To test this hypothesis, we first confirmed that NgR3-Fc (a truncated NgR3 without the GPI domain fused with human Fc fragment at C-terminus) was able to efficiently block binding of AP-Nogo-66 to NgR1 expressing COS-7 cells (Figs. 1C and 6A). Next, we tested whether NgR3-Fc could reverse the inhibitory effects of Nogo-66 on cultured chicken DRG. To do this, we isolated DRG from E12.5 chicken embryos, cultured overnight and used Nogo-66 to suppress their neurite outgrowth. As expected, Nogo-66 suppressed the outgrowth, and addition of NgR3-Fc significantly relieved the inhibition (Fig. 6B and C). Previously, we showed that BLyS is also a potent neurite growth inhibitor (Zhang et al., 2009). We thus tested whether NgR3-Fc could reverse BLyS activity in the same assay. The result showed that NgR3-Fc had no effects on BLyS induced inhibition of neurite sprouting (data not shown), suggesting that NgR3 was not involved in NgR1 mediated BLyS signaling. Together, these results show that truncated NgR3 may be used as an antagonist to alleviate Nogo-66 mediated neural outgrowth inhibition *in vitro*.

4. Discussion

MAIs contribute to the unfavorable environment for neuronal outgrowth. Understanding their signaling pathways is crucial for developing therapeutic strategies for axonal injuries. Multiple CNS growth inhibitors have been isolated and well characterized. Among them Nogo-A has been caught special attention for its possible clinical applications. Currently, Nogo-A neutralizing antibodies have been tested to promote neurite outgrowth after CNS injury in non-human primate (Zorner and Schwab, 2010). In the present study, we identified NgR3 as a novel NgR1 binding partner. This binding was observed both in cell based binding assays and in immunoprecipitation experiments. The functional significance of this interaction was examined in a neurite outgrowth assay. We showed that NgR3-Fc attenuated the inhibitory effects of Nogo-66. Thus we propose that NgR3 may function as a co-receptor for NgR1 to mediate Nogo-66 signaling.

NgR3 has been considered as an orphan receptor. Previous studies revealed that NgR3 showed an overlapping expression profile with that of NgR1 in rodent CNS. More importantly, NgR3 expression is enhanced at rat spinal cord injury (SCI) sites (data not shown), similar to NgR1 expression in SCI we observed before. The expression profiles implicate that NgR3

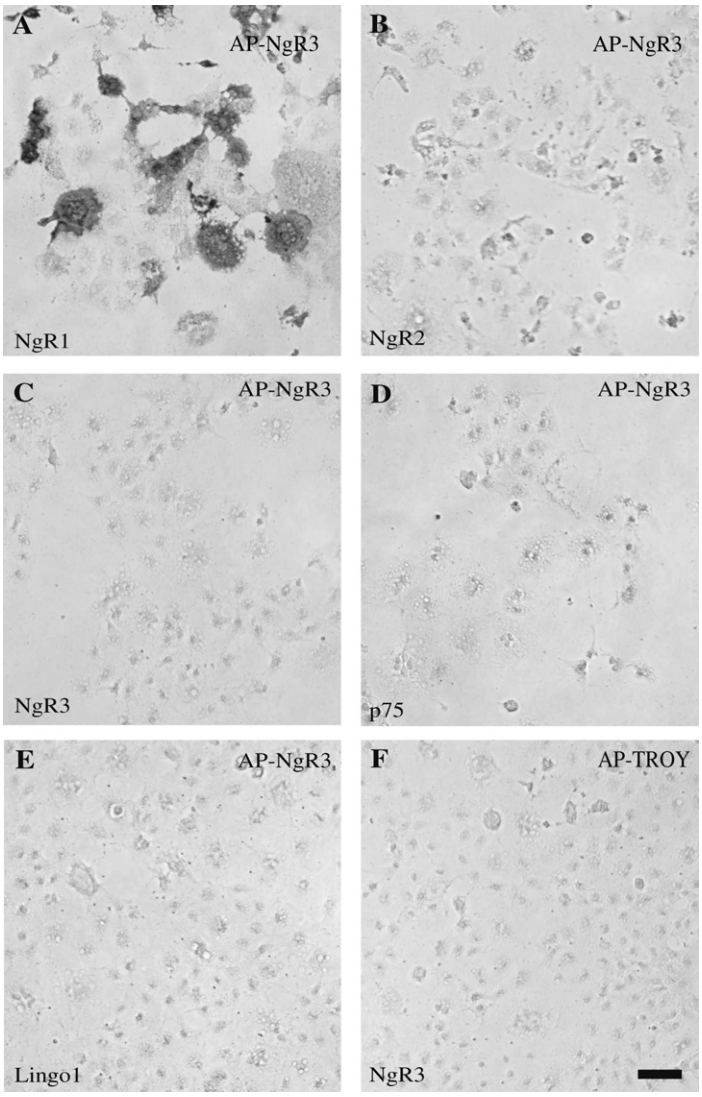

Fig. 3. AP-NgR3 does not bind to COS-7 cells expressing NgR1 related receptors. COS-7 cells were transfected with NgR1 (**A**); NgR paralog molecules, NgR2 and NgR3 (**B** and **C**); and NgR1 co-receptors, p75 and Lingo1 (**D** and **E**). The resulting cells were incubated with AP-NgR3 and stained for AP visualization. AP-TROY (**F**) was used to incubate with NgR3 expressing COS-7 cells and stained by NBT/BCIP. Scale bar, 100 μm.

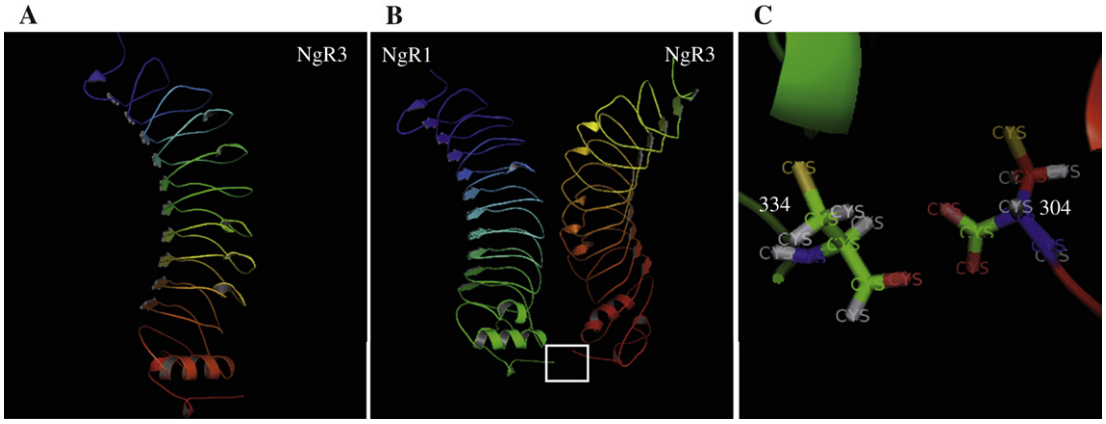

Fig. 4. Modeling NgR1 and NgR3 interaction. Discovery Studio 2.0 software was used to establish NgR3 protein modeling and protein docking. **A:** NgR3 protein structure was predicted referring to NgR1 protein crystal structure by running modeler program. **B:** NgR1 and NgR3 protein docking was calculated by ZDOCK program. Possible interaction between the two proteins are shown. Small rectangle indicates the binding interface by Cys−Cys interaction. **C:** disulfide connection by two Cys residues between NgR1 (334 aa) and NgR3 (304 aa).

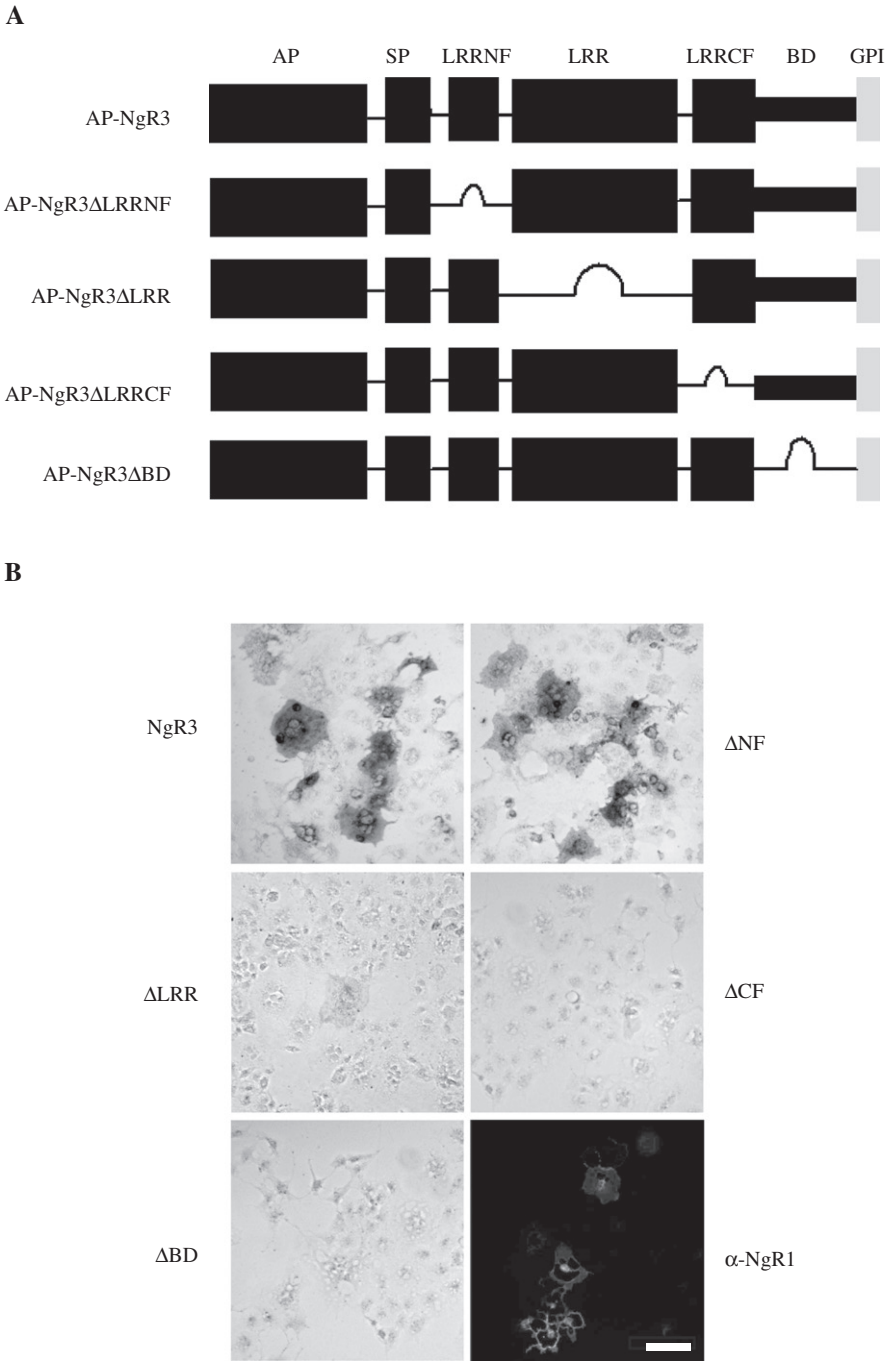

Fig. 5. Different soluble AP-NgR3 mutants bind to NgR1 expressing COS-7 cells. **A:** schematic AP-NgR3 and the deletion mutants used in this study. The mutant AP-NgR3 includes deletion of LRRNF (ΔNF), LRR 1−8 (ΔLRR), LRRCF (ΔCF) and LRRBD (ΔBD). **B:** NgR1 expressing COS-7 cells were incubated with different mutant AP-NgR3 with the same concentration. NgR1 expression was detected by immunostaining with a monoclonal antibody against NgR1. Scale bar, 100 μm.

and NgR1 may be functional coupled *in vivo*. Interestingly, although NgR3 is a homolog of NgR1 with highly similar structures, NgR3 fails to interact with Nogo-66 and other three RTN proteins—RTN1, RTN2 and RTN3. Furthermore, NgR3 fails to directly bind to NgR1 related receptors, including p75, Lingo1 and TROY. Although the structural basis of selective binding remains to be identified, these results re-enforce that NgR1−NgR3 interaction is specific. Based on the binding assays and *in vitro* neurite sprouting assay, we speculate that NgR3 may function as a component of NgR1/Lingo1/p75/TROY receptor complex (Giger et al., 2010). Moreover, we demonstrated that NgR3-Fc was able to block Nogo-66 induced neurite outgrowth, but we have not investigated the exact underlying mechanisms. It is very likely that NgR3 is an integral part of NgR1 receptor complex and functions as an accessory protein to modulate Nogo-66 signaling.

Taken together, our study suggests that NgR3 is a binding partner for NgR1 and may function together with NgR1 to

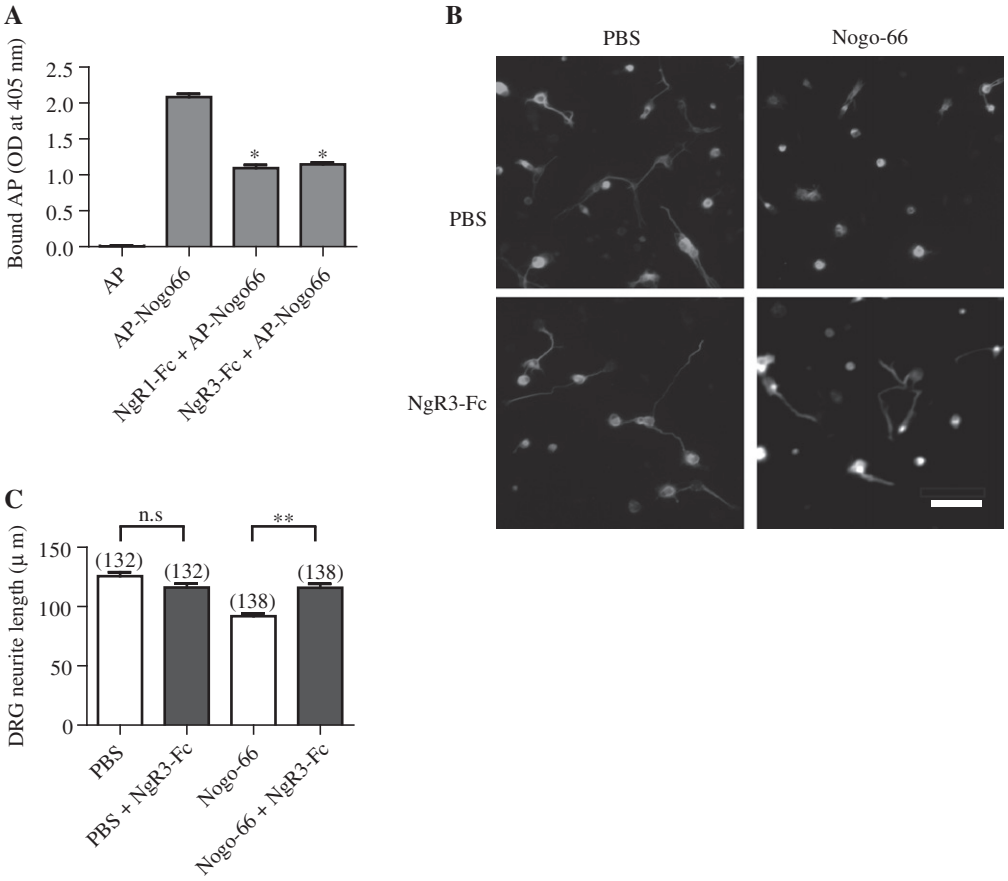

Fig. 6. Truncated NgR3 blocks DRG neurite outgrowth inhibition stimulated by Nogo-66. **A:** different recombinant probes including AP, AP-Nogo66, AP-Nogo66 with NgR1-Fc or NgR3-Fc were incubated with NgR1 expressing COS-7 cells. The resulting cells were lysed and assayed for bound AP at OD_{405nm}. Results are the means of three repeated experiments with Student's *t* test with 95% confidence interval. *: $P < 0.001$ by Student's two tail *t* test. **B:** E12 chicken embryo DRGs were isolated and cultured on different immobilized substrates. Cells were fixed and stained using monoclonal antibody Tuj 1 against class III β-tubulin. Scale bar, 100 μm. **C:** quantification of E12 chicken DRG neurite length on different immobilized substrates. Two tailed **: $P < 0.0001$, by Student's two tail *t* test with 95% confidence interval. Error bars represent SEM. The representative results of three experiments are shown.

mediate Nogo-66 induced axonal inhibition. Thus, NgR3 may also play a negative role in neuronal outgrowth during CNS injuries. Finally, the NgR3−NgR1 interaction interface may be a potential target for designing molecules to promote axonal outgrowth.

Acknowledgements

This work was supported by Ministry of Science and Technology (2007CB947201), Chinese Academy of Sciences (XDA01010108) and National Science Foundation of China (30425013) to J.Z. We thank Drs. Moses V. Chao and Zhigang He for sharing plasmids. We appreciate Biogen Idec Inc. for generously providing anti-NgR1 monoclonal antibody. We thank Mr. Xiaoguang Wei for his assistance in expression cloning and neurite outgrowth experiments. Dr. Zhanli Wang kindly helped the modeling of protein interactions.

References

Atwal, J.K., Pinkston-Gosse, J., Syken, J., Stawicki, S., Wu, Y., Shatz, C., Tessier-Lavigne, M., 2008. PirB is a functional receptor for myelin inhibitors of axonal regeneration. Science 322, 967−970.

Fournier, A.E., Gould, G.C., Liu, B.P., Strittmatter, S.M., 2002. Truncated soluble Nogo receptor binds Nogo-66 and blocks inhibition of axon growth by myelin. J. Neurosci. 22, 8876−8883.

Fournier, A.E., GrandPre, T., Strittmatter, S.M., 2001. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature 409, 341−346.

Giger, R.J., Hollis, E.R., Tuszynski, M.H., 2010. Guidance molecules in axon regeneration. Cold Spring Harb. Perspect. Biol. 2, a001867.

Li, W., Walus, L., Rabacchi, S.A., Jirik, A., Chang, E., Schauer, J., Zheng, B.H., Benedetti, N.J., Liu, B.P., Choi, E., Worley, D., Silvian, L., Mo, W., Mullen, C., Yang, W., Strittmatter, S.M., Sah, D.W., Pepinsky, B., Lee, D.H., 2004. A neutralizing anti-Nogo66 receptor monoclonal antibody reverses inhibition of neurite outgrowth by central nervous system myelin. J. Biol. Chem. 279, 43780−43788.

Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., Levesque, M., Allaire, N., Perrin, S., Sands, B., Crowell, T., Cate, R.L., McCoy, J.M., Pepinsky, R.B., 2004. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat. Neurosci. 7, 221−228.

Park, J.B., Yiu, G., Kaneko, S., Wang, J., Chang, J., He, X.L., Garcia, K.C., He, Z., 2005. A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. Neuron 45, 345−351.

Park, J.H., Gimbel, D.A., GrandPre, T., Lee, J.K., Kim, J.E., Li, W., Lee, D.H., Strittmatter, S.M., 2006. Alzheimer precursor protein interaction with the Nogo-66 receptor reduces amyloid-beta plaque deposition. J. Neurosci. 26, 1386−1395.

Pignot, V., Hein, A.E., Barske, C., Wiessner, C., Walmsley, A.R., Kaupmann, K., Mayeur, H., Sommer, B., Mir, A.K., Frentzel, S., 2003.

Characterization of two novel proteins, NgRH1 and NgRH2, structurally and biochemically homologous to the Nogo-66 receptor. J. Neurochem. 85, 717−728.

Schwab, M.E., 2010. Functions of Nogo proteins and their receptors in the nervous system. Nat. Rev. Neurosci. 11, 799−811.

Thomas, R., Favell, K., Morante-Redolat, J., Pool, M., Kent, C., Wright, M., Daignault, K., Ferraro, G.B., Montcalm, S., Durocher, Y., Fournier, A., Perez-Tur, J., Barker, P.A., 2010. LGI1 is a Nogo receptor 1 ligand that antagonizes myelin-based growth inhibition. J. Neurosci. 30, 6607−6612.

Venkatesh, K., Chivatakarn, O., Lee, H., Joshi, P.S., Kantor, D.B., Newman, B.A., Mage, R., Rader, C., Giger, R.J., 2005. The Nogo-66 receptor homolog NgR2 is a sialic acid-dependent receptor selective for myelin-associated glycoprotein. J. Neurosci. 25, 808−822.

Wang, K.C., Kim, J.A., Sivasankaran, R., Segal, R., He, Z., 2002. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. Nature 420, 74−78.

Zhang, L., Zheng, S., Wu, H., Wu, Y., Liu, S., Fan, M., Zhang, J., 2009. Identification of BLyS (B lymphocyte stimulator), a non-myelin-associated protein, as a functional ligand for Nogo-66 receptor. J. Neurosci. 29, 6348−6352.

Zheng, B., Atwal, J., Ho, C., Case, L., He, X.L., Garcia, K.C., Steward, O., Tessier-Lavigne, M., 2005. Genetic deletion of the Nogo receptor does not reduce neurite inhibition *in vitro* or promote corticospinal tract regeneration *in vivo*. Proc. Natl. Acad. Sci. USA 102, 1205−1210.

Zorner, B., Schwab, M.E., 2010. Anti-Nogo on the go: from animal models to a clinical trial. Ann. N.Y. Acad. Sci. 1198 (Suppl. 1), E22−E34.