Receptors & Clinical Investigation 2014; 1: e144. doi: 10.14800/rci.144; © 2014 by Ru-Jeng Teng.
http://www.smartscitech.com/index.php/rci

REVIEW

Nogo-B Receptor (NgBR): A New Receptor that Modulates Blood Vessel Formation

Ru-Jeng Teng

Department of Pediatrics, Medical College of Wisconsin, United States

Correspondence: Ru-Jeng Teng
E-mail: rteng@mcw.edu
Received: April 08, 2014
Published online: June 13, 2014

Nogo-B is an isoform of reticulon-4 (RTN4) that distributes mainly in the endoplasmic reticulum. Nogo-B binds to its receptor, Nogo-B receptor (NgBR), to modulate blood vessel formation. Animal with Nogo-B knockout is phenotypically normal. NgBR is a type I receptor with a single transmembrane domain that binds Nogo-B and probably other angiogenic factors. NgBR knockout in zebra fish leads to abnormal formation of intersomite vessels suggesting NgBR plays a more important role in the Nogo-B/NgBR signaling pathway. It is reported that NgBR plays a role in dolichol biosynthesis, protein N-glycosylation, stabilizes Niemann-Pick type C2 protein, regulates intracellular cholesterol trafficking, controls blood vessel development, and modulates breast cancer progression. However, the research in NgBR remains in its early stage and needs further exploration.

To cite this article: Ru-Jeng Teng, Nogo-B Receptor (NgBR): A New Receptor that Modulates Blood Vessel Formation. Receptor Clin Invest 2014; 1: e144. doi: 10.14800/rci.144.

Introduction

Nogo system is a relatively newly found signaling pathway that is widely distributed in the biological system. It belongs to the reticulon family and binds to different receptors for specific function. Nogo system contains Nogo-A, -B, and -C isoforms. Nogo-B itself has two isoforms, -B1 and -B2, that bind to same receptor, Nogo-B receptor (NgBR). Nogo-B/NgBR distributes mainly in the vasculature and its role in vascular development was recently been explored. It is considered that NgBR may be more vital during vascular development than Nogo-B judging from the fact that Nogo-A/B knockout animal remains phenotypical normal whereas NgBR knockout leads to impaired vascular formation in zebra fish [1] and is lethal in mouse embryo [*Miao RQ* personal communication]. NgBR also controls other biological functions and awaits further explorations.

Reticulons

Reticulon is a group of proteins and obtain its name due to its distribution mainly in endoplasmic reticulum. Morris *et al* first described reticulon protein, vp20, in rats [2]. Neurite outgrowth inhibitor, also known as Reticulon-4, is a protein that in humans is encoded by the *RTN4* gene that has been identified as an inhibitor of neurite outgrowth specific to the central nervous system and obtained the name as "Nogo" [2, 3]. Using either alternate splicing or different promoter region there are three isoforms of Nogo produced from RTN4 gene (Figure 1) [4-6]. The distribution of the three Nogo isoforms is different. Nogo-A and -C are mainly distributed in the nervous system whereas Nogo-B is distributed mainly in vasculature [6, 7]. There are at least

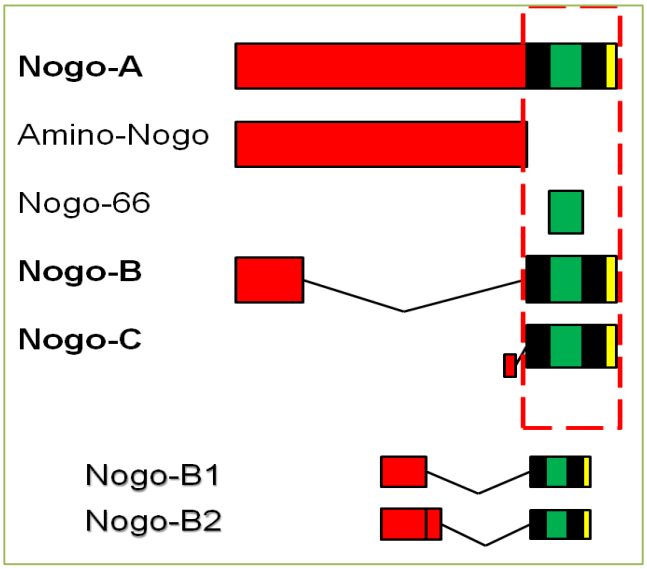

Figure 1. Structure of the different isoforms of Nogo protein. All three major isoforms are encoded by RTN4 gene in human. There are two isoforms of Nogo-B identified.

three receptors have been described to bind Nogo-A that lead to inhibition of neuronal growth [8]. Detailed description of Nogo-A system is beyond the scope of this review and readers are encouraged to refer to several recent review articles about this topic [9, 10].

Nogo-C has been described to play some roles in neurological disease [11, 12], induces apoptosis in vitro via JNK-c-Jun-dependent pathway [13]. Nogo-C expression in brain is increased in schizophrenic patients [14] and mutations in Nogo-C have been linked to hepatic carcinoma in specific Chinese population [15].

Nogo-B is widely expressed in the central nervous system and peripheral tissues, and is mainly located in endoplasmic reticulum and cell membrane. Previous studies have revealed that Nogo-B plays a key role in vascular injury [16, 17], tissue repair and inflammation process [18-20]. However, Nogo-A/B knockout is non-lethal but results in an exaggerated neointimal proliferation, abnormal remodeling and a deficit in ischemia mediated arteriogenesis and angiogenesis. Nogo-B may be critical for apoptosis of tumor cells [21, 22] and central nervous system diseases. Recent studies suggest plasma Nogo-B level may be an indicator for liver cirrhosis [23, 24] and idiopathic pulmonary arterial hypertension [25]. Evidence suggests increased Nogo-B level disrupts the ER-mitochondron unit and suppresses mitochondron-mediated apoptosis especially in the pulmonary artery smooth muscle cells (PASMC) and leads to the thickening of the PASMC layer under pulmonary artery hypertension inducing environment. Two isoforms of Nogo-B are reported in the literature [16, 26, 27] but difference in their biological role remains unknown.

Nogo-B Receptor (NgBR or NUS1)

As the receptor for Nogo-B, it is also known as nuclear undecaprenyl pyrophosphate synthase 1 Homolog (NUS1). Miao *et al* first described this protein and its role in human umbilical vein endothelial cells as a receptor necessary for Nogo-B stimulated chemotaxis and morphogenesis of endothelial cells [28]. His work also indicated NgBR inhibits PDGF-mediated vascular smooth muscle cell migration. There is evidence suggests that VEGF also binds to NgBR offering ~40% of the effect on angiogenesis and may explain why Nogo-A/B knockout mouse is non-lethal and phenotypically normal.

Structure of NgBR

Li *et al* used bioinformatic methodology to study CD and NMR characterization of NgBR and its two dissected domains. They described that the NgBR ectodomain is intrinsically unstructured without both secondary and tertiary structures while the cytoplasmic domain is partially folded with secondary structures but without a tight tertiary packing [29]. Therefore, NgBR is a rare example with the entire ectodomain as a transmembrane receptor.

Function of NgBR

Other than originally described function in vascular endothelial cell migration [28], inhibiting PDGF-mediated PASMC migration, and vascular development [1], it is shown to stabilize Niemann-Pick type C2 protein and regulates intracellular cholesterol trafficking using yeast two-hybrid screen for interacting proteins [30]. Part of its function is believed to be through the binding with vascular endothelial cell growth factor. It is also needed for cellular dolichol biosynthesis, protein N-glycosylation [30]. Miao's group identified NgBR binds farnesylated Ras and recruits Ras to the plasma membrane, which is a critical step required for the activation of Ras signaling in human breast cancer cells and tumorigenesis. Recently his group reported that expression modulates survivin level in estrogen-receptor positive breast cancer tumor cells which may contribute to the progression of breast cancer [31].

Using pulmonary artery endothelial cells (PAEC) and lung tissue obtained from fetal sheep after intrauterine ductus arteriosus constriction induced pulmonary hypertension model, intrauterine pulmonary hypertension (IPH), our group observed a decreased NgBR level in IPH PAEC and lungs [27]. The decreased NgBR level is associated with increased reactive oxygen species formation and decreased nitric oxide availability in PAEC (Figure 2). The changes in ROS and NO formation in

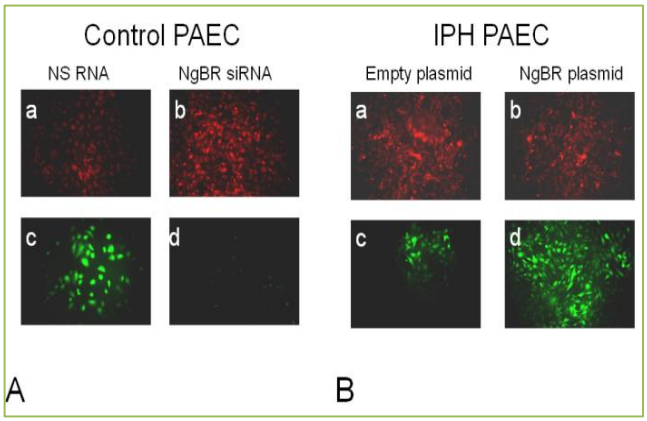

Figure 2. **Nogo-B receptor (NgBR) level modulates reactive oxygen species (ROS) and nitric oxide (NO) formations in pulmonary artery endothelial cells (PAEC)**. (**A**) Control PAEC treated with NgBR silencing RNA increases ROS formation (dihydroethidium stain, a and b) and decreases NO availability (diaminofluoresceine stain, c and d). (**B**) PAEC from intrauterine pulmonary hypertension (IPH) shows increased ROS (a) and decreased NO formation (c). Increasing NgBR level by NgBR containing plasmid decreases ROS formation (b) and increases NO availability (d). NS RNA: Non-silencing RNA; siRNA: silencing RNA

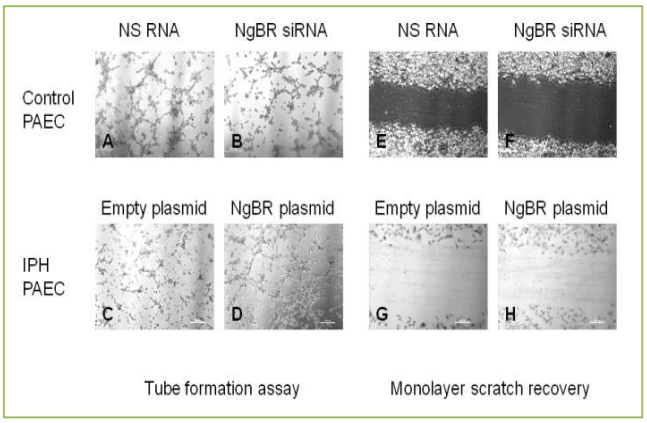

Figure 3. NgBR level in pulmonary artery endothelial cell affects the in vitro angiogenesis. Tube formation assay and monolayer scratch recovery assay are used. Tube formation for control PAEC (A) decreases after NgBR knockdown (B) whereas monolayer scratch recovery (C) is impaired after NgBR knockdown (D). In IPH PAEC tube formation (E) improves after NgBR overexpression (F) whereas monolayer scratch recovery (G) is enhanced after NgBR overexpression (H).

PAEC parallel to the in vitro angiogenesis assays (Figure 3) as we previously described [32]. We believe that increased NgBR level in PAEC helps eNOS activity through activation of Akt/PKB pathway. In the same report we also observed an increased GTP cyclohydrolase-1 level and MnSOD level/activity. Judging from the decreased GTP cyclohydrolase-1 level [33] and MnSOD level/activity in IPH PAEC [34] impair angiogenesis in IPH we believe NgBR provides several levels of benefit to vascular development in the developing lungs.

Conclusions

NgBR is a newly described protein with limited information available in literature to help us understanding its role in biologic system. Knockout animal models suggest NgBR is vital to normal embryo development. In human cancer tissue NgBR may modulate the disease progression. Manipulating NgBR level in PAEC demonstrates its function in angiogenesis is through eNOS coupling. Research in this protein remains in its early stage but its involvement in cancer progression and lung development deserve our endeavor for further explorations.

Conflicting interests

The authors have declared that no competing interests exist.

References

1. Zhao B, Chun C, Liu Z, Horswill MA, Pramanik K, Wilkinson GA, *et al*. Nogo-B receptor is essential for angiogenesis in zebrafish via Akt pathway. Blood 2010;116:5423-5433.
2. Morris NJ, Ross SA, Neveu JM, Lane WS, Lienhard GE. Cloning and characterization of a 22 kDa protein from rat adipocytes: a new member of the reticulon family. Biochim Biophys Acta 1999;1450:68-76.
3. GrandPre T, Nakamura F, Vartanian T, Strittmatter SM. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 2000;403:439-444.
4. Yang J, Yu L, Bi AD, Zhao SY. Assignment of the human reticulon 4 gene (RTN4) to chromosome 2p14-->2p13 by radiation hybrid mapping". Cytogenet Cell Genet 2000;88:101-102.
5. Bandtlow CE1, Schwab ME. NI-35/250/nogo-a: a neurite growth inhibitor restricting structural plasticity and regeneration of nerve fibers in the adult vertebrate CNS. Glia 2000;29:175-181.
6. Dodd DA, Niederoest B, Bloechlinger S, Dupuis L, Loeffler J-P, Schwab ME. Nogo-A, -B, and -C are found on the cell surface and interact together in many different cell types. J Biol Chem 2005; 280:12494-12502.
7. Josephson A, Trifunovski A, Widmer HR, Widenfalk J, Olson L, Spenger C. Nogo-receptor gene activity: cellular localization and developmental regulation of mRNA in mice and humans. J Comp Neurol 2002;453:292-304.
8. Martin E, Schwab. Functions of Nogo proteins and their receptors in the nervous system. Nat Rev Neurosci 2010;11:799-811.
9. Kempf A, Schwab ME. Nogo-A represses anatomical and synaptic plasticity in the central nervous system. Physiology 2013;28:151-163.

10. 10. Schwab ME, Strittmatter SM. Nogo limits neural plasticity and recovery from injury. Curr Opin Neurobiol. 2014;27C:53-60.
11. Dupuis L, Gonzalez de Aguilar JL, di Scala F, Rene F, de Tapia M, Pradat PF, *et al*. Nogo provides a molecular marker for diagnosis of amyotrophic lateral sclerosis. Neurobiol Dis 2002;10:358-365.
12. Kim JE, Bonilla IE, Qiu D, Strittmatter SM. Nogo-C is sufficient to delay nerve regeneration. Mol Cell Neurosci 2003;23:451-459.
13. Chen Y, Tang X, Cao X, Chen H, Zhang X. Human Nogo-C overexpression induces HEK293 cell apoptosis via a mechanism that involves JNK-c-Jun pathway. Biochem Biophys Res Commun 2006;348:923-928.
14. Novak G, Tallerico T. Nogo A, B and C expression in schizophrenia, depression and bipolar frontal cortex, and correlation of Nogo expression with CAA/TATC polymorphism in 3'-UTR. Brain Res 2006;1120:161-171.
15. Chen Y, Tang X, Zhang X, Zhuang L. New mutations of Nogo-C in hepatocellular carcinoma. Mol Biol Rep 2009;36:377-380.
16. Acevedo L, Yu J, Erdjument-Bromage H, Miao RQ, Kim JE, Fulton D, *et al*. A new role for Nogo as a regulator of vascular remodeling. Nat Med 2004;10:382-388.
17. Rodriguez-Feo JA, Hellings WE, Verhoeven BA, Moll FL, de Kleijn DP, Prendergast J, *et al*. Low levels of Nogo-B in human carotid atherosclerotic plaques are associated with an atheromatous phenotype, restenosis, and stenosis severity. Arterioscler Thromb Vasc Biol 2007;27:1354-1360.
18. Yu J, Fernández-Hernando C, Suarez Y, Schleicher M, Hao Z, Wright PL, *et al*. Reticulon 4B (Nogo-B) is necessary for macrophage infiltration and tissue repair. Proc Natl Acad Sci USA 2009;106:17511-17516.
19. Wright PL, Yu J, Di YP, Homer RJ, Chupp G, Elias JA, *et al*. Epithelial reticulon 4B (Nogo-B) is an endogenous regulator of Th2-driven lung inflammation. J Exp Med 2010;207:2595-2607.
20. Di Lorenzo A, Manes TD, Davalos A, Wright PL, Sessa WC. Endothelial reticulon-4B (Nogo-B) regulates ICAM- 1-mediated leukocyte transmigration and acute inflammation. Blood 2011;117:2284-2295.
21. Li Q, Qi B, Oka K, Shimakage M, Yoshioka N, Inoue H, *et al*. Link of a new type of apoptosis-inducing gene ASY/Nogo-B to human cancer. Oncogene 2001;20:3929-3936.
22. Tambe Y, Isono T, Haraguchi S, Yoshioka-Yamashita A,Yutsudo M, Inoue H. A novel apoptotic pathway induced by the drs tumor suppressor gene. Oncogene 2004;23:2977-2987.
23. Gao L, Utsumi T, Tashiro K, Liu B, Zhang D, Swenson ES, *et al*. Reticulon 4B (Nogo-B) facilitates hepatocyte proliferation and liver regeneration in mice. Hepatology 2013; 57:1992-2003.
24. Men R, Wen M, Dan X, Zhu Y, Wang W, Li J, *et al*. Nogo-B: a potential indicator for hepatic cirrhosis and regulator in hepatice stellate cell activaion. Hepatol Res 2014 Mar 10. doi: 10.1111/hepr.12324. [Epub ahead of print]
25. Sutendra G, Dromparis P, Wright P, Bonnet S, Haromy A, Hao Z, *et al*. The role of Nogo and the mitochondria-endoplasmic reticulum unit in pulmonary hypertension. Sci Transl Med 2011;3:88ra55.
26. Pan JW, Zheng X, Yang PY, Qin YW, Rui YC, Ma LP, *et al*. Different expressions of Nogo-B1 and Nogo-B2 in mouse heart microvascular endothelial cell dysfunction induced by lysophosphatidylcholine. Microvasc Res 2006;72:42-47.
27. Teng RJ, Rana U, Afolayan AJ, Zhao B, Miao QR, Konduri GG. Nogo-B Receptor Modulates Angiogenesis Response of Pulmonary Artery Endothelial Cells Through eNOS Coupling. Am J Respir Cell Mol Biol 2014 Feb 25. [Epub ahead of print] http://dx.doi.org/10.1165/rcmb.2013-0298OC
28. Miao RQ, Gao Y, Harrison KD, Prendergast J, Acevedo LM, Yu J, Hu F, *et al*. Identification of a receptor necessary for Nogo-B stimulated chemotaxis and morphogenesis of endothelial cells. Proc Natl Acad Sci USA 2006; 103:10997-11002.
29. Li M, Song J. Nogo-B receptor possesses an intrinsically unstructured ectodomain and a partially folded cytoplasmic domain. Biochem Biophys Res Commun 2007;360: 128-134.
30. Harrison KD, Miao RQ, Fernandez-Hernándo C, Suárez Y, Dávalos A, Sessa WC. Nogo-B receptor stabilizes Niemann-Pick type C2 protein and regulates intracellular cholesterol trafficking. Cell Metab 2009;10:208-218.
31. Wang B, Zhao B, North P, Kong A, Huang J, Miao QR. Expression of NgBR is highly associated with estrogen receptor alpha and survivin in breast cancer. PLoS One 2013 Nov 4;8(11):e78083.
32. Teng RJ, Eis A, Bakhutashvili I, Arul N, Konduri GG. Increased superoxide production contributes to the impaired angiogenesis of fetal pulmonary arteries with in utero pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2009;297: L184-L195.
33. Teng RJ, Du J, Xu H, Bakhutashvili I, Eis A, Shi Y, *et al*. Sepiapterin improves angiogenesis of pulmonary artery endothelial cells with in utero pulmonary hypertension by recoupling endothelial nitric oxide synthase. Am J Physiol Lung Cell Mol Physiol 2011; 301:L334-L345.
34. Afolayan AJ, Eis A, Teng RJ, Bakhutashvili I, Kaul S, Davis JM, *et al*. Decreases in manganese superoxide dismutase expression and activity contribute to oxidative stress in persistent pulmonary hypertension of the newborn. Am J Physiol Lung Cell Mol Physiol 2012;303:L870-L879.