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Research report

The differential expression patterns of messenger RNAs encoding Nogo-A and Nogo-receptor in the rat central nervous system

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Abstract

Nogo-A and Nogo-receptor have been considered to play pivotal roles in controlling axonal regeneration and neuronal plasticity. We investigated the total distribution of Nogo-A and Nogo-receptor mRNAs in the adult rat central nervous system using in situ hybridization histochemistry. Nogo-A is abundantly expressed in both neurons and oligodendrocytes throughout the central nervous system. Interestingly, we could not find any neuron which lacks Nogo-A mRNA expression, indicating that Nogo-A mRNA is universally expressed in all neurons. In contrast, Nogo-R mRNA expression was very restricted. Nogo-R mRNA was expressed in the olfactory bulb, hippocampus, tentia tecta, some amygdala nuclei, cerebral cortex, some thalamic nuclei, medial habenular, whereas we could not detect it in the other regions. Interestingly, we did not detect Nogo-R mRNA in monoaminergic neurons, which are known to have high regenerative capacity, in the substantia nigra, ventral tegmental area, locus caeruleus, and raphe nuclei. In addition, although neurons in the reticular thalamus and cerebellar nuclei are also known to show high capacity for regeneration, Nogo-R mRNA was not detected there. These data indicate that Nogo-A and Nogo-R mRNAs were differentially expressed in the central nervous system, and suggest that the lack of Nogo-R expression in a given neuron might be necessary to keep its high regenerative capacity.

Theme: Development and regeneration
Topic: Regeneration

Keywords: In situ hybridization; Nogo-A; Nogo-66 receptor; CNS myelin inhibitor; Regeneration

1. Introduction

Nogo-A has been discovered as a protein in myelin, which impedes axonal regeneration [3,4,7,18]. This protein is expressed by central nervous system (CNS) myelin-forming oligodendrocytes but not by peripheral Schwann cells [8,11,25], and can be observed in immunoelectron micrographs at the innermost adaxonal and outermost myelin membranes [8,25]. This expression pattern is well coincident with the fact that in the adult CNS axon regeneration is highly restricted [19]. In addition to its localization in oligodendrocytes, Nogo-A is also expressed in a range of central and peripheral neurons [8,11,25], strongly suggesting that this protein has additional functions in the CNS. Nogo-A is divided into three regions separated by two hydrophobic segments. Its large N-terminal domain (N-nogo) and small C-terminal domain (C-nogo) reside in the cytoplasmic space, whereas a short stretch of 66 amino acids (Nogo-66) forms an extracellular loop. Interestingly, both N-nogo and Nogo-66 have been reported to inhibit axons in vitro independently [4–6].

Recently, Nogo-66 receptor (Nogo-R) has been identified [4,7]. Transfection of Nogo-R gene into retinal ganglion cells at a developmental stage when they otherwise are

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unresponsive to Nogo-66 promotes growth cone collapse in response to exogenously applied Nogo-66 [4]. As Nogo-R is a glycosyl posphatidylinositol (GPI) anchored protein, it needs the interaction of p75, which is known as the receptor for the neurotrophin family of nerve growth factor [24]. In addition, Mi et al. [15] have reported that a new protein LINGO-1 is a component of the Nogo-R/p75 signaling complex, and plays a role to modulate intracellular Rho activity, which is important in cytoskeletal regulation. Although Nogo-R is not completely dependent on Nogo-A, the interaction of both proteins is considered to play pivotal roles in the inhibition of axon regeneration and other additional phenomena in the CNS.

The distributions of Nogo-A and Nogo-R have been reported using immunohistochemisty and in situ hybridization [4,8,9,11–13,21,25,26]. Despite the importance of these proteins in the CNS, these reports mainly deal with their expressions in the restricted areas. In addition, they are sometimes very contradictory. For example, in the cerebellum, Fornier et al. [4] and Hunt et al. [9] reported that Nogo-R mRNA was expressed in Purkinje neurons; however, Josephson et al. [12] did not detect Nogo-R mRNA in Purkinje neurons. In addition, Hunt et al. [9] reported that cerebellar nuclei expressed Nogo-R mRNA, whereas Josephson et al. [12] did not. It is, thus, necessary to perform more wide and detailed investigations of the expression patterns of Nogo-A and Nogo-R in the adult rat CNS.

2. Materials and methods

2.1. In situ hybridization

Six male Wistar rats (SLC, Shizuoka, Japan) weighing approximately 150 g were decapitated under diethylether anesthesia. All experiments conformed to the Guidelines for Animal Experimentation at Hamamatsu University School of Medicine on the ethical use of animals and all efforts were made to minimize the number of animals used and their suffering. The fresh brains and nervous tissues were quickly removed and immediately frozen on powdered dry ice. Serial sections (20 μm thick) were cut on a cryostat, thaw-mounted onto silan-coated slides, and stored at −80 °C. After being warmed to room temperature, slide-mounted sections were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 15 min (all steps were performed at room temperature unless otherwise indicated), rinsed three times (5 min each) in 4× SSC (pH 7.2) (1× SSC contains 0.15 M sodium chloride and 0.015 M sodium citrate), and dehydrated through a graded ethanol series (70–100%). The sections were then defatted with chloroform for 5 min, and immersed in 100% ethanol (twice for 5 min each time) before being subjected to hybridization. Hybridization was performed by incubating the sections with a buffer [4× SSC, 50% deionized formamide, 0.12 M phosphate buffer (pH 7.2), Denhardt's solution (Nakarai, Kyoto, Japan), 2.5% tRNA (Roche, Tokyo, Japan), 10% dextran sulfate (Sigma, Tokyo, Japan)] containing [^{35}S]dATP (1000–1500 Ci/mmol (37–555 TBq/mmol; New England Nuclear, Boston, MA, USA))-labeled probes (1–2× d.p.m./ml, 0.2 ml/slide) for 24 h at 41 °C. After hybridization, the sections were rinsed in 1× SSC (pH 7.2) for 10 min, followed by rinsing three times in 1× SSC at 55 °C for 20 min each time. The sections were then dehydrated trough a graded ethanol series (70–100%). After film exposure for 7 days at room temperature, the sections were coated with Kodak NBT-2 emulsion (Kodak, Rochester, NY, USA) diluted 1:1 with water. The sections were then exposed at 4 °C for 2 weeks in a tightly sealed dark box. After being developed in D-19 developer (Kodak), fixed with photographic fixer, and washed with tap water, the sections were counterstained with thionine solution to allow morphological identification.

2.2. Oligonucleotide probes

Two antisense oligo cDNA probes (AS1 and AS2) and a sense oligo cDNA probe (S, complementary to AS1) for each Nogo-A and Nogo-receptor were designed based on the corresponding rat sequences [3,4] and synthesized commercially (Takara, Tokyo, Japan). The sequences of the probes are as follows:

Nogo-A-AS1, GCTCTGGAGCTGTCCTTCACAGGTTCTGGGGTACTGGGGAAAGAAGCA;
Nogo-A-AS2, AGTCTTCTCTGTTATAATTTGGGCCTTCCTTTCTTCTATT;
Nogo-A-S, TGCTTCTTTCCCCAGTACCCCAGAACCTGTGAAGGACAGCTCCAGAGC;
Nogo-R-AS1, GTCATGCCGGAATCTCACCATCCTGTGGCTGCACTCAAAT;
Nogo-R-AS2, GCAAACAGGTAGAGGGTCATGAGTCGGCCAAGGTCCCGGA;
Nogo-R-S, ATTTGAGTGCAGCCACAGGATGGTGAGATTCCGGCATGAC.

Computer-assisted homology searches (NCBI-BLAST) showed that each probe has less than 50% homology with any sequences contained in the gene banks. The probes were labeled at the 3′ end using [^{35}S] dATP (1000–1500 Ci/mmol (37–55.5 Tbq/mmol); New England Nuclear) and terminal deoxynucleotidyl transferase (Takara) to obtain a specific activity of approximately $1.4–2.0\times10^{9}$ d.p.m./μg.

2.2.1. Control studies

We carried out control competition experiments for the four antisense probes using a 100-fold excess of one of the unlabeled probes together with the corresponding labeled probe, and other controls involving RNase A pretreatment just before hybridization. These experiments showed no positive signals for any of the probes.