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PERSPECTIVE

Nogo-A and its functions beyond axonal inhibition: the controversial role of Nogo-A in Parkinson's disease

Nogo-A belongs to the reticulon family (RTN4) and is generally assumed to be one of the most potent myelin associated neurite outgrowth inhibitors in the central nervous system (CNS). Together with other inhibitors such as the myelin associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (OMgp), several semaphorins and ephrins as well as chondriotin sulphate proteoglycans, Nogo-A contributes to a nonpermissive environment in the brain and spinal cord. Based on their seminal observation that neurons grown in close proximity to CNS derived oligodendrocytes showed a robust decrease in neurite outgrowth and number of processes, Schwab and Caroni (1988) postulated for nonpermissive substrate properties present in the CNS myelin. In further studies, they identified a high molecular weight component from the CNS myelin called NI-250 which was later renamed Nogo-A. In the course it could be demonstrated that Nogo-A is expressed on the surface of oligodendrocytes and is responsible for the inhibition of axonal sprouting. Since the nonpermissive environment plays a very important role in brain injuries and neurodegenerative diseases, Nogo-A and its receptors were extensively studied.

Nogo-A has several ligand domains that interact with different receptors. One of these ligand domains is the so called Nogo-66 domain that binds to the Nogo-receptor 1 (NgR1). NgR1 is a glycosylphasphatidylinositol anchored membrane protein, which does not contain a transmembrane domain and needs therefore other proteins to transfer the signal further into the cytosol. Thus, NgR1 forms a complex with Leucine rich repeat and Ig domain containing 1 (LINGO-1) and tumor necrosis factor family member (TROY) and/or low affinity nerve growth factor receptor (p75). p75 is cleaved sequentially upon activation by alpha and gamma secretase thereby releasing an intracellular signaling peptide that activates the Rho GTPase pathway, which leads to inhibition of axonal growth (Saha et al., 2014). On the other hand, Nogo-66 can bind to the paired immunoglobulin-like receptor (PirB), however, its mechanisms are not well investigated. Another Nogo-A ligand domain is the so called Δ–20 domain, which binds to the sphingosine-1-phosphate receptor 2 that also leads to activation of the Rho GTPase pathway (Schwab and Strittmatter, 2014). Although Nogo-A signals through different receptors, all pathways converge into the Rho GTPase pathway, yet, Nogo-A causes different effects (**Figure 1**).

Given the neurite growth inhibitory properties of Nogo-A, suppressors of Nogo-A and Nogo receptor function including anti-Nogo-A antibodies and peptides blocking the NgR1 complex were broadly investigated in the setting of spinal cord injury in which axons do not regenerate spontaneously. Rodents and monkeys with a large spinal cord injury showed significant recovery of locomotion, balance and fine forepaw or finger movements within 2–4 weeks of treatment with different interventions to neutralize Nogo-A or antagonize its receptors (Schwab and Strittmatter, 2014). Moreover, human anti-Nogo-A antibodies (ATI 355 from Novartis Pharma, Switzerland; Ozanezumab and GSK1223249 from GlaxoSmithKline gsk plc, UK) have been developed and passed the clinical trial phase I in patients with acute spinal cord injury, amyotrophic lateral sclerosis (ALS) and multiple sclerosis (Schmandke et al., 2014). ALS is a neurodegenerative disease that selectively destroys upper and lower motoneurons and Nogo-A is ectopically expressed in muscles of ALS patients at levels that correlate with the severity of the clinical symptoms.

Importantly, Nogo-A is expressed not only on oligodendrocytes but also in many subpopulations of neurons. In most of the studies assessing the distribution of Nogo-A in the CNS *in situ* hydridization methods were used for the detection of *Nogo-A* mRNA that, however, did not allow for a precise identification of the single cellular phenotype. Only recently neuronal expression was addressed on the protein level (Schmandke et al., 2014; Schawkat et al., 2015). Nogo-A expression found in neurons is involved in plasticity of the CNS, including the hippocampal formation and the cerebral cortex (Schmandke et al., 2014). Furthermore, Nogo-A was detected in the cerebellum mostly in Purkinje cells, in neurons of the red nucleus and in striatal cholinergic interneurons (Schawkat et al., 2015). It is rather surprising to realize that so far only little is known on the Nogo-A expression in the nigro-striatal system, which is critically involved in the pathogenesis of Parkinson's disease (PD). PD is a neurodegenerative disorder mainly characterized by a progressive loss of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc) that leads to a depletion of dopamine in the striatum. Notably, we recently demonstrated that half of all tyrosine hydroxylase (TH) positive DAergic neurons co-express Nogo-A in the adult rat SNc (Schawkat et al., 2015) (**Figure 2**). In addition, we identified subpopulations of these co-localizing cells as DAergic neurons projecting to the striatum. In line with this notion, in a rat model of PD, the number of nigral Nogo-A positive neurons was significantly reduced both 1 week and 1 month after the 6-OHDA lesions. Interestingly, this drop in Nogo-A positive neurons occurred only during the first week after the lesion and remained stable thereafter. Moreover, at 1 week after the lesion, more DAergic neurons co-expressing Nogo-A were affected by the lesion than Nogo-A-negative DAergic neurons. However, at the later time point, a higher portion of the surviving DAergic neurons co-expressed Nogo-A (Schawkat et al., 2015). This hints to the idea that Nogo-A is dynamically regulated in response to the insult, leading to a neuroprotection of DAergic neurons co-expressing Nogo-A in the later course of PD. Indeed, Kurowska and co-workers reported that 6-OHDA lesions in Nogo-A knock-out mice showed no difference of surviving DAergic neurons up to 4 weeks after the lesions, while after 8 weeks, a tendency towards a more pronounced death of DAergic neurons in the SNc was observed. Surprisingly and despite the known effects of Nogo-A on neurite growth inhibition, in these Nogo-A knock-out mice no regeneration of the nigrostriatal DAergic fibers has been detected. Moreover, intracellular Nogo-A has been proposed to initiate branching of midbrain DAergic neurons (Kurowska et al., 2014). Yet, another group noticed that knocking out Nogo-A or NgR1 does not lead to a specific phenotype or to neurological deficits. Moreover, these Nogo-A or NgR1 knock-out animals reveal only modest regenerative capacity compared to antagonization or neutralization of Nogo-A signaling. This hints to the idea that other factors compensate for the loss of Nogo-A for example Nogo-B, MAG or OMgp (Teng and Tang, 2005).

Roughly 10% of all PD cases are caused by genetic mutations mainly associated with mitochondrial function, protein degradation, synaptic functions, growth factor functions and importantly also with axon guidance. Hence, several recent studies investigated the possible involvement of Nogo-A signaling genes in the context of PD. The most extensively tested gene is the Nogo-receptor interacting protein LINGO-1. LINGO-1 is upregulated in animal models of PD and by binding to the epidermal growth factor receptor (EGFR) it hampers the survival of DAergic neurons. Blocking of LINGO-1 resulted in increased EGFR levels and increased survival of DAergic neurons (Mi et al., 2013). Moreover, LINGO-1 knock-out mice showed increased DAergic neuron survival and reduced behavioral abnormalities compared to wild type animals (Inoue et al., 2007). All these experimental findings suggest that LINGO-1 could be associated with PD. Two clinical studies accomplished in China and Germany, however, were unable to find a significant correlation of LINGO-1 and risk of PD.

Recently, we demonstrated that antagonization of the NgR1 resulted in significantly increased number and morphological complexity of cultured ventral mesencephalic DAergic neurons (**Figure 2**). We furthermore observed a larger culture volume of organotypic

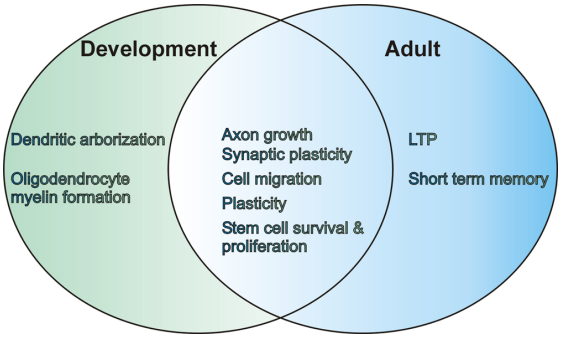

Figure 1 Schematic drawing of the functions of Nogo-A during development and/or in the adult brain.
Nogo-A not only inhibits axonal outgrowth but also regulates dendritic arborization and oligodendrocyte myelin formation during development (left circle) and controls long term depression (LTP) and short term memory formation in the adult brain (right circle). Moreover, Nogo-A modulates synaptic plasticity, cell migration, plasticity and stem cell survival and proliferation throughout life (middle circle).

cultures after NgR1 antagonization, hinting to the idea that this treatment approach goes beyond direct effects on DAergic neurons (Seiler et al., 2013). Indeed, Teng and co-workers reported that Nogo-A neutralization resulted in an up-regulation of growth factors such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor as well as growth-related proteins such as growth associated protein 43 (Teng and Tang, 2005). Particularly the up-regulation of BDNF is in the context of PD very important, as BNDF is a well known neurotrophic factor for DAergic neurons. To what extend indirect mechanisms including changes of neurotrophic factors occur after interfering with NgR1, however, needs still to be elucidated.

Taken together, the role and involvement of Nogo-A in PD is just beginning to evolve. Even though none of the Nogo-A signaling genes has been associated with PD, results from animal studies show that DAergic neurons co-expressing Nogo-A survive better in the later course of PD as well as more pronounced DAergic cell death in PD. Nogo-A knock-out animals hint strongly to a neuroprotective role of Nogo-A in DAergic neurons. Moreover, Nogo-A knock-out mice have a better motor coordination as well as an enhanced locomotor reaction to systemic amphetamine injections compared to wild type mice. Amphetamine increases the release of DA into the synaptic cleft, which leads to hyperactivity of a DA deregulated striatum. These results hint to an altered DA tone in Nogo-A knock-out mice (Willi et al., 2009). The function of Nogo-A in DAergic neurons, however, needs to be further elucidated especially with focus on its signaling. After all, other studies showed that LINGO-1 was increased in animal models of PD and hampered the survival of DAergic neurons. Moreover, antagonization of NgR1 or LINGO-1 led to significant increase in survival of DAgeric neurons, showing that the NgR1 complex has a negative regulatory role on the survival of DAergic neurons. Considering these findings, the potential neuroprotective role of Nogo-A in PD is probably not due to the Nogo-66 domain that signals through the NgR1 complex. Yet, if and how Nogo-A protects DAergic neurons from toxic insults and if there are other myelin associated proteins that act through the NgR1 complex as negative regulator of DAergic neuron survival needs to be further investigated.

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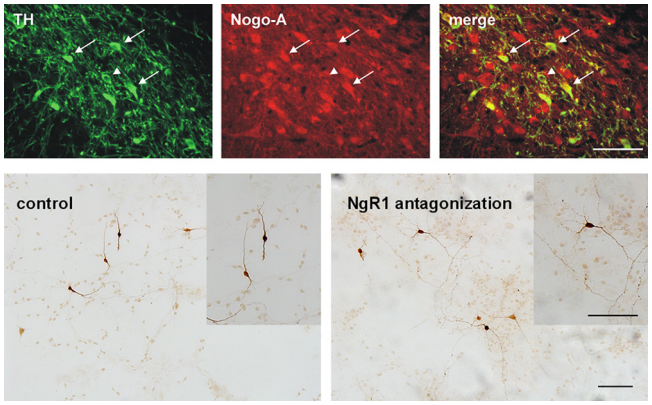

Figure 2 Photomicrograph of the substantia nigra pars compacta (SNc).
SNc cells were stained for the dopaminergic marker tyrosine hydroxylase (TH) and Nogo-A. In the merged image, dopaminergic neurons co-expressing Nogo-A are marked with arrows, whereas dopaminergic neurons that do not express Nogo-A are shown with an arrowhead (upper panel). Photomicrograph of TH positive neurons in primary ventral mesencephalic cultures grown for 7 days with either medium alone (control) or in the presence of a Nogo-receptor 1 (NgR1) antagonist (lower panel). Higher magnifications (inserts) reveal the higher morphological complexity of dopaminergic neurons after NgR1 antagonization. Scale bars: 100 μm.

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