Nogo BACE jumps on the exosome

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The protein Nogo-A has been widely studied for its role in inhibiting axonal regeneration following injury to the central nervous system, but the mechanism by which the membrane-bound Nogo-A is presented intercellularly is not fully understood. New research suggests that a highly inhibitory fragment of Nogo-A is generated by the amyloid precursor protein protease BACE1 and presented on the membranes of exosomes following spinal cord injury. This finding represents a new mode through which Nogo-A may exert its effects in the central nervous system.

Traumatic injury of the CNS[2] typically includes damage to neuronal connections and pathways. Although this damage can be repaired through spontaneous axonal regeneration in the peripheral nervous system, this process is inhibited by several molecules in the CNS (1). One of the first inhibitors identified was the protein Nogo-A, by showing that disruption of Nogo-A either with function-blocking antibodies or by genetic ablation of Nogo-A or its receptor, Nogo-Receptor-1 (NgR1) increased axon outgrowth after CNS injury (1). Nogo-A is expressed by both oligodendrocytes and neurons and localizes to the endoplasmic reticulum and on the myelin membrane in close proximity to the axon (1). However, during CNS injury, oligodendrocytes are also damaged, and axons become demyelinated, raising the question of how membrane-bound Nogo-A makes contact with its receptor to initiate downstream actions inhibiting axon regeneration (2). Whereas it is likely that some Nogo-A may be present in myelin debris in the extracellular milieu following spinal cord injury, a new study by Sekine *et al.* (2) helps to solve this mystery by identifying Nogo in another cellular location: the exosome.

Exosomes are small vesicular structures produced through the endosomal pathway. Originally misunderstood as cellular waste bins that dispose of unwanted cellular contents through exocytosis, the past decade has recharacterized the exosome as an important mediator of cellular communication (3). Exosomes demonstrate cargo selectivity and deliver functional RNAs, proteins, and even DNA species to other cells. Exosomes can be targeted to specific cell types through differences in the protein and lipid composition of their membrane, where their contents can have a significant impact on the target cell's behavior (3). An interesting possibility is that exosomes could serve as a novel carrier to convey Nogo-A signals throughout the CNS.

To test this hypothesis, Sekine *et al.* first overexpressed C-terminal Myc-tagged Nogo-A in HEK293T cells. The authors observed the presence of a 24-kDa Nogo fragment in the cell culture medium that was enriched following exosome purification and was present in fractions containing known exosome-associated proteins according to density gradient separation. Pharmacological inhibition or siRNA-mediated down-regulation of β-site amyloid precursor protein–cleaving enzyme 1 (BACE1) reduced the expression of Nogo-24, identifying a key protease for generation of the fragment. Mapping of the proteolytic site revealed that the 24-kDa fragment contains the NgR1-binding domain, Nogo-66. Treatment of exosomes with a nonpermeable biotin reagent capable of labeling a single cysteine residue in the Nogo-66 domain followed by streptavidin immunoprecipitation of unlysed exosomes confirmed its presence on the outer exosomal surface (4). These results confirmed that a potentially functional fragment of Nogo-A is present on exosomes, but is it active?

To find out, the authors performed an *in vitro* scratch injury assay to assess regeneration in the presence of Nogo-24–positive exosomes. Under standard culture conditions, cortical neurons will regenerate into an injury site in the absence of inhibitory cues. The addition of Nogo-24–containing exosomes to WT cortical neurons significantly inhibited regeneration into the injury site compared with exosomes from control cells. However, NgR1$^{-/-}$ neurons were able to regenerate normally in the presence of Nogo-24 exosomes. These results suggest that exosomal Nogo-24 is functional, and its inhibitory effects are mediated through NgR1. Last, the authors found that the Nogo-24 fragment was present in the exosomal fraction of tissue lysates after a spinal cord crush injury of mice, but not in tissues from control mice. These data suggest exosomal release to be a new mechanism by which Nogo may be presented following spinal cord injury (Fig. 1).

Very little is known about exosomal trafficking between cells of the CNS. Exchange of exosomes from oligodendroglia to neurons is triggered by neuronal activity and has been shown to facilitate the transfer of antioxidant proteins, such as superoxide dismutase 1 (SOD1), linked to an increase in neuronal viability in an *in vitro* ischemia model (4). Similarly, Schwann cell–derived exosomes are taken up by axons, promoting regeneration of the sciatic nerve after crush injury (5). The findings of Sekine *et al.* suggest a novel way that exosomes regulate cellular activity in their target cells, through ligand-receptor interactions at the cell surface. It is tempting to speculate about putative advantages of exosome-bound signaling molecules; for instance, exosomal localization may concentrate the ligand more efficiently at the plasma membrane of the recipient cell than passive diffusion. It is possible that the presence of Nogo-24 also helps target the exosomes to the neuronal cell membrane, contributing to the delivery of other cargos. An inter-

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[2] The abbreviations used are: CNS, central nervous system; APP, amyloid precursor protein.

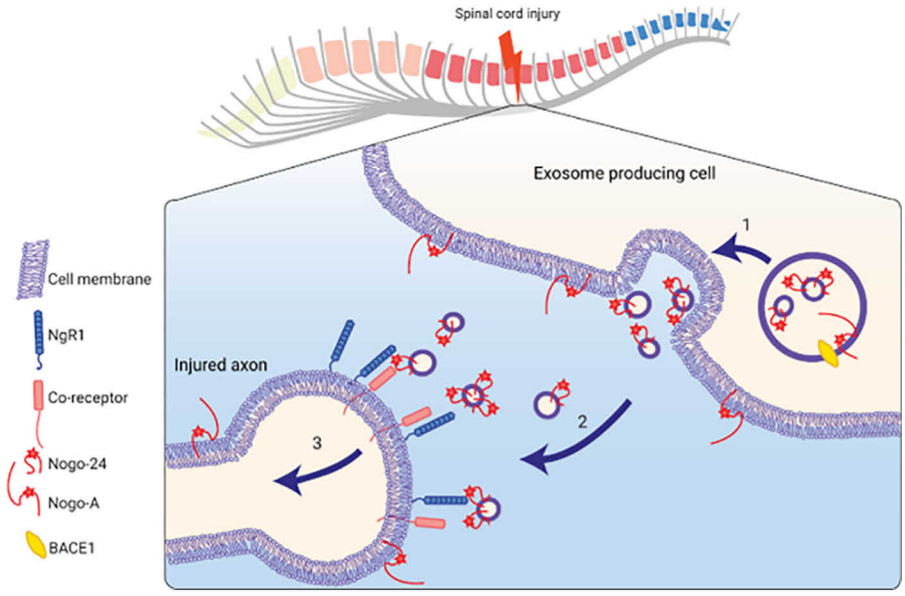

Figure 1. Illustration of how exosomal Nogo-24 may be released after spinal cord injury and signal through NgR1 to inhibit neuron regeneration. *1*, BACE1 cleaves full-length Nogo-A to Nogo-24. Nogo-24–containing exosomes are produced in the endosomal pathway and released when the multivesicular body fuses with the plasma membrane. *2*, exosomes reach the neuronal plasma membrane, where Nogo-24 can interact with NgR1 and its coreceptors. *3*, Nogo-24 binding to NgR1 is transduced through a co-receptor to NgR1 and leads to downstream signaling inhibiting axonal regeneration and sprouting.

esting unanswered question is whether Nogo-24–enriched exosomes have an effect after exosomal uptake independent of signaling through NgR1 and its co-receptors.

Whether Nogo-24 signaling on exosomes exists outside of spinal cord injury remains an open question. Since its original discovery as an inhibitor after CNS injury, we have learned that Nogo-A has important roles in both development of the brain and long-term stability of synapses, evidenced by its expression in nearly all brain regions from development through adulthood (1, 6). A variety of studies have implicated Nogo-A in consolidation of neural circuitry and maintenance of the mature dendritic arbors of adult neurons (6, 7). Neuronal exosomes have previously been implicated in synaptic signaling, where their release is augmented following neuronal activity and contributes to the transfer of proteins between the pre- and post-synapses (8). It is easy to picture a paradigm where release of exosomal Nogo-24 may be augmented through neuronal activity, contributing to the maturation and stabilization of active synapses and neural circuits in the adult brain. Production of Nogo-24 may also be indirectly involved in synaptic maintenance through the modulation of its processing enzyme. Better known for its role cleaving amyloid precursor protein (APP) into the Alzheimer's-associated protein β-amyloid, BACE1 is enriched in the endosome, where exosome production takes place (9). Nogo proteins are known to be negative regulators of β-amyloid production through competition for BACE1 (10). Competition for this enzyme may be modulating the levels of both APP and Nogo-24, further contributing to synaptic regulation in the adult brain.

Ultimately, these possibilities depend on the cell type underlying the production of exosomal Nogo-24. Neurons and oligodendrocytes alike express BACE1 and therefore have the potential to contribute to the exosomal release of Nogo-24 (9). It is equally possible that there may be different cellular sources of Nogo-24, depending on the context, whether in spinal cord injury or homeostatic regulation of synapses. Overall, the new discoveries about this enigmatic protein raise many interesting questions for exosome biologists and neuroscientists alike to consider about the varied mechanisms that cells use to modulate their neighbors' activities.

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