PERSPECTIVE

Lateral olfactory tract usher substance (LOTUS) protein, an endogenous Nogo receptor antagonist, converts a non-permissive to permissive brain environment for axonal regrowth

It is well known that primates, including humans, hardly recover motor function after spinal cord injury (SCI) when compared with non-primate mammals such as rodents. This limited functional recovery is in part due to a non-permissive environment of the central nervous system (CNS) inhibiting axonal regrowth. This inhibitory environment for axonal regrowth is mainly caused by interaction of axon growth inhibitors with their common receptor, Nogo receptor-1 (NgR1). Axon regrowth inhibitors such as Nogo proteins, myelin associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (OMgp) and B lymphocyte stimulator (BLyS) are derived from glial cells in damaged brain.

Previous studies have demonstrated that inhibition of NgR1 activity promotes functional recovery in animal models of CNS injury (GrandPré et al., 2002; Kim et al., 2004; Cafferty et al., 2010). Administration of NEP1–40 peptide, a Nogo-66 antagonist, to rat SCI models resulted in significant axon regrowth in corticospinal tract (CST), and improved motor function (GrandPré et al., 2002). Targeting of NgR1 function showed that deletion of the NgR1 gene in these mice improved motor function following SCI (Kim et al., 2004). Targeting multiple axon regrowth inhibitors also showed greater axonal regrowth and improved motor function after SCI in Nogo, MAG and OMgp triple-knockout mice (Cafferty et al., 2010). Gene targeting of NgR1 also enhanced structural plasticity and spontaneous functional recovery (Cafferty et al., 2010). As such, inhibition of NgR1 function promotes functional recovery in motor activity after SCI and may be one of a main therapeutic approach for neural regeneration.

In parallel, supplying neurotrophic factors such as brain-derived neurotrophic factor to an environment provides neuronal protection and enhances motor axonal regeneration (Novikov et al., 1997) Therefore, treatment with various combinations of neurotrophic factors may have a greater impact on neuronal regeneration after SCI. Transplantation of human iPS cell-derived oligodendrocyte precursor cells has been shown to contribute to remyelination of demyelinated axons (Kawabata et al., 2016). Thus, significant functional recovery requires repair of neural networks not only by inhibiting NgR1 function, but also by supplying neurotrophic factors and cell transplantation.

The discovery of LOTUS addresses the paradox of why neurons extend their neurites and form a neural network in the developing brain while expressing Nogo and NgR1. A neural circuit formation factor, named lateral olfactory tract (LOT) usher substance (LOTUS) was discovered in our laboratory in 2011. Expressed LOTUS in healthy neurons contributes to the LOT axonal bundle formation through antagonism of Nogo-NgR1 interaction. We therefore identified LOTUS as an endogenous NgR1 antagonist (Sato et al., 2011). LOTUS is a potent inhibitor of NgR1, as overexpression of LOTUS completely suppressed growth cone collapse and neurite outgrowth inhibition. This was achieved by blocking NgR1 function induced by all five types of its ligand in dorsal root ganglion (DRG) neurons that express little LOTUS (Kurihara et al., 2014, 2017) (**Figure 1**). LOTUS is abundantly expressed in many regions of the CNS. However, it was observed that neurons hardly regenerate when a potent NgR1 antagonist, LOTUS, is expressed in the CNS. Why is this so? We found that LOTUS expression levels drastically decreased at the injured site in wild type mice about one week after SCI. The down-regulation of LOTUS expression may be associated with perturbation of recovery in motor activity after SCI. We thus proposed that decreased LOTUS expression may give rise to a non-permissive environment in the CNS for neuronal regeneration. Therefore, we hypothesized that the level of LOTUS expression may regulate neuronal regrowth activity by increasing and decreasing its antagonism to NgR1. To address this issue, we first compared functional and histological recovery after SCI in wild type mice to that in *lotus*-knocking-out (LOTUS-KO) mice. It is well established that rodents such as mice and rats show incomplete but substantial spontaneous motor recovery after SCI. However, the factors involved in this spontaneous improvement remained elusive. We found a remarkable delayed spontaneous functional recovery of behavioral and histological outcome in LOTUS-KO mice when compared with wild type mice (Hirokawa et al., 2017). The data thus suggest that LOTUS is a factor associated with spontaneous motor recovery in rodents. We then speculated that the supply of LOTUS could compensate for the loss of regenerative activity due to decreased LOTUS expression and eventually promote functional recovery with neuronal regeneration after SCI. To examine this possibility, we generated transgenic mice (LOTUS-TG mice) that overexpressed LOTUS specifically in neurons and examined the effect of LOTUS overexpression on functional recovery after SCI. Definitive evidence for continued recovery of motor activity in LOTUS-TG mice after the recovery had reached a plateau in wild type mice. Although LOTUS expression level was down-regulated from the level of overexpression after SCI, the down-regulated level of LOTUS expression in LOTUS-TG mice was almost similar to the level in healthy wild type mice. These findings do suggest that the supply of LOTUS promotes functional recovery after SCI (Hirokawa et al., 2017).

LOTUS has also been shown to contribute to neuronal regeneration in another CNS injury model, ischemia by middle cerebral artery occlusion. In this model, CST axon fibers sprouting from the non-ischemic side to the contralateral ischemic side were increased in LOTUS-TG mice when compared with wild type mice (Takase et al., 2017). The data suggest that LOTUS enhances neuronal plasticity of CST neurons and thereby improves motor function after ischemia. LOTUS contains both a membrane-bound form and secreted form. The soluble (secreted) form of LOTUS protein shows the same antagonistic activity against NgR1 and promotes axonal regeneration in optic nerve crush injury of mice (Kawakami et al., 2018). Conversely, LOTUS promotes axonal growth not only by antagonism of NgR1 function, but also by promoting intrinsic neurite outgrowth activity (unpublished data). It is thus possible to induce neuronal regeneration by utilizing both LOTUS functions, which are its antagonism of NgR1 function and its neurite outgrowth promoting action. Therefore, LOTUS administration with recombinant protein injection, LOTUS gene transfection and transplantation of LOTUS overexpressing neuronal stem cells may be useful as a therapeutic agent to promote neuronal regeneration. In addition, there is a low probability of side effects as LOTUS is an endogenous protein abundantly expressed in the healthy CNS.

As such, LOTUS is a strong candidate to convert a non-permissive to permissive brain environment for neuronal regeneration. It is a potential natural agent for providing a regenerative brain environment by inhibition of NgR1 function. We are currently attempting LOTUS administration to injured CNS by injection of purified recombinant LOTUS protein or LOTUS gene transfection in both acute and chronic phase injury (**Figure**

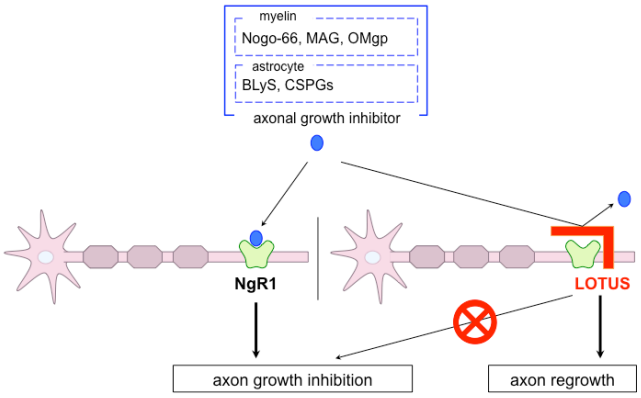

Figure 1 Lateral olfactory tract usher substance (LOTUS) as an endogenous Nogo receptor-1 (NgR1) antagonist.
In the central nervous system (CNS), there are axon growth inhibitors such as Nogo, myelin associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (OMgp), that are derived from oligodendrocytes, and B lymphocyte stimulator (BLyS) and chondroitin sulphate proteoglycans (CSPGs) derived from astrocytes. These 5 inhibitors are the ligands of NgR1 and ligand binding induces axon growth inhibition, thereby limiting neuronal regeneration after injury. LOTUS interacts with NgR1 and completely suppresses NgR1-mediated axonal growth inhibition, thereby promoting axon regrowth.

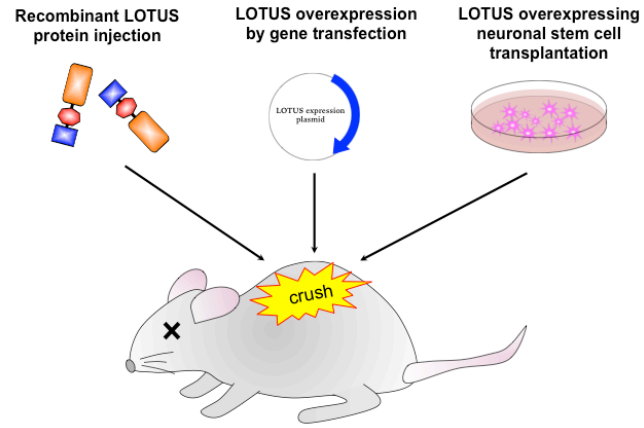

Figure 2 Therapeutic approach to neuronal regeneration by lateral olfactory tract usher substance (LOTUS).
After central nervous system (CNS) damage, injection of recombinant LOTUS protein, overexpression of LOTUS by gene transfection or transplantation of LOTUS overexpressing neuronal stem cell may be useful for future therapy for CNS damage, indicating that LOTUS may convert a non-permissive to permissive environment for neuronal regeneration in the CNS.

2). Combination of treatment with other drug targets on which LOTUS does not act such as Semaphorin 3A inhibitor, pleiotrophin, or a chondroitin sulfate proteoglycan inhibitor, and with rehabilitation may be successful as future therapies. Finally, it is important to develop agents blocking a decrease in LOTUS expression since the decrease of LOTUS expression may be associated with perturbation of functional recovery after SCI.

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Open peer review reports:
***Reviewer 1:** Shaoping Hou, Drexel Univesity College of Medicine, USA.*
***Comments to authors:** The manuscript converts a non-permissive to permissive brain environment for axonal regrowth", is a review about a growth inhibitor in CNS axon regeneration. Authors described the discovery of LOTUS and its major effects on attenuating Nogo receptors. As such, this will help spread knowledge about LOTUS to neuroscience community. Nogo and related factors have been characterized for almost 20 years. Their roles in axon regeneration are much clear: inhibitory factors but not conclusive ones. Therefore, the review should provide not only the positive effects about LOTUS's, but also the limitations. Indeed, all SCI animal models in cited publications were incomplete injury, which rendered difficulties to evaluate real regeneration, sprouting or others based on their tracing techniques and immunostaining.*
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