



LOTUS, a New Natural Agent Providing a Regenerative Brain Environment

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Abstract

After spinal cord injury (SCI), primates, such as humans, hardly ever recover the affected motor function. The primary cause of limited neuronal regeneration in the central nervous system is the interaction between axon growth inhibitors such as Nogo protein and Nogo receptor-1 (NgR1), a common receptor of these inhibitors. We have previously shown that lateral olfactory tract usher substance (LOTUS) identified in the developing brain contributes to axon tract formation by antagonizing Nogo-NgR1 mediated signaling. Furthermore we also found that the binding of LOTUS to NgR1 blocks thisNgR1-mediated axon growth inhibition. We therefore hypothesized that LOTUS may promote neuronal regeneration by antagonizing NgR1. To address this issue, we examined functional and histological recovery after SCI in wild type, lotus- deficient (LOTUS- KO) and LOTUS over expressing transgenic (LOTUS- TG) mice. We found that LOTUS- KO mice lost spontaneous functional recovery after SCI, whereas LOTUS-TG mice increased functional recovery when respectively compared with wild type mice. These findings suggest that LOTUS promotes neuronal regeneration after SCI by blockade of NgR1 function and is useful as a natural agent for clinical treatment of SCI. We are currently attempting to administer LOTUS after SCI by protein injection, gene transfection or transplantation of LOTUS over expressing neuronal stem cells.

After spinal cord injury (SCI), primates, such as humans, hardly ever recover the affected motor function. The limited neuronal regeneration is primarily caused by a non-permissive environment of the central nervous system (CNS) and decreased axon regrowth activity. It has been considered that this inhibitory environment for axon regrowth is mainly caused by interaction of axon growth inhibitors with their common receptor, Nogo receptor-1 (NgR1). It is well known that the 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.

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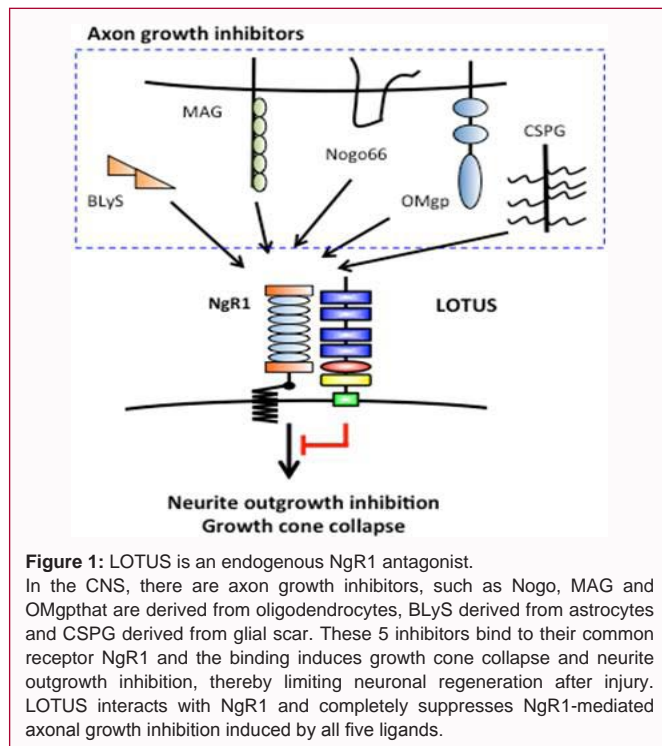
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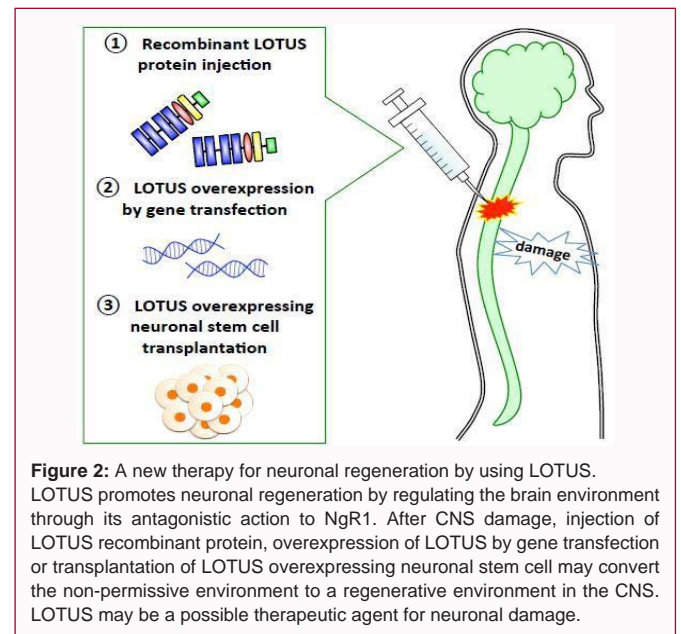
Previous studies have shown that inhibition of NgR1 activity promotes functional recovery in animal models of CNS injury [1,-3]. Targeting of NgR1 function clearly showed that locomotor function after SCI was improved by NgR1 gene deletion in these mice [1]. Nogo, MAG and OMgp triple-knockout mice exhibited greater axonal regrowth and improved locomotion after SCI [2]. Nogo-66 antagonist peptide (NEP1-40) administration to rat SCI models resulted in significant corticospinal tract (CST) axon regrowth, and improved motor function [3]. Deletion of NgR1 also enhanced structural plasticity and spontaneous functional recovery [4]. Furthermore, the inhibitory reagent of semaphorin-3A also induced neuronal regeneration after SCI [5]. These studies suggest that inhibition of axon growth inhibitors and/or NgR1 promotes functional recovery after SCI and may be therapeutic targets for a conducive brain environment for neural regeneration.

In parallel, it has been reported that supply of neurotrophic factors gives rise to promotion of neuronal regeneration, for example, brain-derived neurotrophic factor provides neuronal protection and significantly enhances motor axonal regeneration after spinal nerve root injury [6]. Different neurotrophic factors such as hepatocyte growth factor can also promote nerve regeneration [7], therefore treatment of combinations with several neurotrophic factors may prove beneficial. Transplanted human iPS cell-derived oligodendrocyte precursor cells have been shown to contribute to remyelination of demyelinated axons and functional recovery after SCI [8]. Thus, it is considered that blockade of NgR1-mediated axon growth inhibition, promotion of axonal regrowth by supply of neurotrophic factors and repair of neural network by cell transplantation are needed for neuronal regeneration.

Why do neurons grow and form a network in the developing brain while expressing Nogo and



NgR1? Discovery of LOTUS may address this paradox. In 2011, we discovered a neural circuit formation factor, named as lateral olfactory tract (LOT) usher substance (LOTUS). LOTUS is expressed in healthy neurons and contributes to the formation of the LOT axonal bundle. LOTUS binds to NgR1 and suppresses NgR1-mediated axonal growth inhibition induced by the binding of its ligand Nogo to NgR1, and we thus identified LOTUS as an endogenous NgR1 antagonist [9]. In dorsal root ganglion (DRG) neurons that express little LOTUS, over expression of LOTUS completely suppressed growth cone collapse and neurite outgrowth inhibition by blocking NgR1 function induced by all five types of its ligand [10,11] (Figure 1). We therefore hypothesized that the level of LOTUS expression may regulate neuronal regeneration activity by increasing and decreasing its antagonistic action to NgR1. To address this issue, we first examined functional and histological recovery after SCI in wild type and *lotus*-deficient (LOTUS-KO) mice. Rodents such as mice and rats show incomplete but substantial spontaneous motor recovery after SCI, however, the factors associated with this spontaneous improvement remained completely unknown. We found that LOTUS-KO mice showed remarkable delayed spontaneous functional recovery of behavioral and histological outcome when compared with wild type mice [12]. This result suggests that LOTUS plays a prominent role in the spontaneous motor recovery in rodents. We also found that LOTUS expression level was down-regulated in the injured site of wild type mice about one week after SCI. The down-regulation of LOTUS expression may be associated with decreased locomotion activity after SCI. It is thus possible that a non-permissive environment in the CNS for neuronal regeneration may be attributed to decreased LOTUS expression level. Therefore, we hypothesized that the supply of LOTUS could compensate for the loss of regenerative activity and eventually promote functional recovery after SCI. We then generated transgenic mice that overexpressed LOTUS in neurons (LOTUS-TG mice) and examined the influence of LOTUS overexpression on the functional recovery after SCI. We found definitive evidence for continued locomotion recovery in LOTUS-TG mice after the



recovery had reached a plateau in wild type mice, indicating that LOTUS overexpression promotes functional recovery after SCI [12].

Similarly, we also found that LOTUS improved motor function in another CNS injury model, which was ischemia by middle cerebral artery occlusion. In this model, CST axon fibers sprouting from the non-ischemic side to the ischemic side were drastically increased in LOTUS-TG mice when compared with wild type mice [13]. These findings suggest that LOTUS enhances neuronal plasticity of CST neurons and improves motor function. Recently, we also found that the soluble form of LOTUS protein has same antagonistic activity against NgR1 (unpublished data). Furthermore, LOTUS promotes axonal growth not only by its antagonistic action to NgR1 function, but also by promoting intrinsic neurite outgrowth activity (unpublished data). Therefore, it is very possible to induce neuronal regeneration by utilizing both of LOTUS physiological functions, which are its antagonistic action towards NgR1 function and its neurite outgrowth activity. Therefore, LOTUS administration by recombinant protein injection or gene transfection may be successful in promoting functional recovery after nerve injury. Moreover, there is little possibility of side-effects as LOTUS is an endogenous protein abundantly expressed in the healthy CNS.

Thus, LOTUS is a good candidate for providing a regenerative brain environment and promoting axonal regrowth. We are currently attempting to administer LOTUS to injured CNS by injection of purified recombinant LOTUS protein, LOTUS gene transfection of *lotus* gene or transplantation of LOTUS overexpressing neuronal stem cells as a novel therapy for neuronal regeneration in both acute and chronic phase injury (Figure. 2). Treatment combinations with other drug targets which LOTUS does not act on such as by injection of Sema3A inhibitor [5], pleiotrophin, or a chondroitin sulfate proteoglycan inhibitor [14], and rehabilitation may be more successful in future therapy.

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