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Galectin-1 plays essential roles in adult mammalian nervous tissues. Roles of oxidized galectin-1

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Previous data have suggested that galectin-1 is expressed widely in nervous tissues at embryonic stages but becomes restricted mainly to peripheral nervous tissues with maturation. Though the expression is intense in adult mammalian peripheral neurons, there had been no report about functions of galectin-1 there. Recently we discovered a factor that enhanced peripheral axonal regeneration. The factor was identified as oxidized galectin-1 with three intramolecular disulfide bonds and showed no lectin activity. Oxidized recombinant human galectin-1 (rhGAL-1/Ox) showed the same nerve growth promoting activity at very low concentrations (pg/ml). rhGAL-1/Ox at similarly low concentration was also effective in *in vivo* experiments of axonal regeneration. Moreover, the application of functional anti-rhGAL-1 antibody strongly inhibited the axonal regeneration *in vivo* as well as *in vitro*. Since galectin-1 is expressed in the regenerating sciatic nerves as well as in both sensory neurons and motor neurons, these results suggest that galectin-1 is secreted into the extracellular space to be oxidized, and then, in its oxidized form, to regulate initial repair after axotomy. The administration of oxidized galectin-1 effectively promoted functional recovery after sciatic nerve injury *in vivo*. Oxidized galectin-1, hence, appears to play an important role in promoting axonal regeneration, working as a kind of cytokine, not as a lectin. Recent reports indicated additional roles of cytosolic galectin-1 in neural diseases, such as ALS. Furthermore galectin-1 has been proved to be a downstream target of ΔFosB. In hippocampus of rat brain, expression of ΔFosB is induced immediately after ischemia-reperfusion, suggesting that galectin-1 may also play important roles in central nervous system after injury.
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Abbreviations: **rhGAL-1: recombinant human galectin-1; rhGAL-1/Ox: Oxidized recombinant human galectin-1; DRG: dorsal root ganglion; CNTF: ciliary-derived neurotrophic factor; LIF: leukemia inhibitory factor; IGF-I: insulin-like growth factor-I; ALS: amyotrophic lateral sclerosis.**

1. Introduction

Galectin-1 is widely distributed in the rodent brain during development. Although it has been revealed that galectin-1 plays an important role in forming the neural network of the olfactory bulb [1–3], there is no report on its role in other regions. Galectin-1 null mice show deficiency only in the olfactory network [4] and can grow up to adults without any other specific deficiency. After maturation the distribution of galectin-1 is restricted mainly to peripheral neurons [5–7]. Though its expression was intense, there had been no report about its importance in peripheral nervous systems.

On the other hand, there have been many studies on neurotrophic factors in peripheral nerve regeneration. Neurotrophins (nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, neurotrophin-4) as well as ciliary-derived neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) have been thought to be potent factors to promote initial axonal growth after peripheral axotomy, but recent works have put this into question [8–14]. Unlike the neurotrophic factors mentioned above, insulin-like growth factor-I (IGF-I) expression was significantly increased after transection of the sciatic nerve [15,16] and perfusion of anti-IGF-I antibodies inhibits the regeneration of crushed sciatic nerves [17]. However, since IGF-I mRNA content at the site of injury does not increase until 4–6 days after nerve crush [18], the factors that prompt the

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axons to send out processes in peripheral nerves sooner after axotomy are not well understood.

To find factors that initiate the early axonal regrowth we introduced two specific strategies. One is a novel *in vitro* assay system based on a dorsal root ganglion (DRG) with associated nerve stumps cultured in collagen gel [19–21]. This *in vitro* model preserves the three-dimensional structure of the nervous tissues that enables cell-cell interactions and thus is more like an *in vivo* assay. Another strategy is that we focused on factors from organs such as kidney or liver. These organs secrete various physiologically essential factors, and it is also expected that these organs may secrete important factors to promote axonal regeneration after damage because the cross talk between the organs and nervous system is essential for repair after damage of the organs. We thus screened the culture supernatants of cells derived from kidney or liver using the *in vitro* assay. This led us to discover oxidized galectin-1 as a possible regulator of initial axonal regeneration after injury in peripheral nerves.

2. Distribution of galectin-1 in mammalian nervous system

As mentioned above, galectin-1 is localized in the central and peripheral nervous systems during development and becomes mainly restricted to peripheral nervous tissues with maturation [5–7], although some recent studies have revealed galectin-1 in adult central nervous tissues also [22,23].

In peripheral nervous tissues, galectin-1 is expressed in sensory neurons, motor neurons, and olfactory neurons [1,19,24]. Galectin-1 mRNA persists in DRG neurons at later developmental stages and is maintained in adult sensory neurons. The mRNA is also present in motoneurons in the spinal cord and brain stem [24]. Immunohistochemical study for galectin-1 in normal rats also showed immunoreactivity in DRG neurons and their axons as well as in the spinal cord, where motoneurons and their axons were specifically positive [19]. Galectin-1 immunoreactivity was observed in regions containing Schwann cells and regenerating axons [19]. In the olfactory system, galectin-1 is expressed by ensheathing cells both in olfactory nerve and within the nerve fiber layer of the olfactory bulb of the embryonic and adult rat. In the adult rat, galectin-1 was preferentially expressed by olfactory ensheathing cells in the nerve fiber layer of the ventromedial and lateral surfaces of the olfactory bulb [3].

In central nervous tissues of adult mammal, galectin-1 seems to be expressed in the restricted sites using the antibody for galectin-1. They are the retinal pigment epithelium, outer limiting membrane, and outer plexiform layer in bovine and rat retinas [22]. In the adult rat brain, galectin-1 mRNA is expressed in pineal gland and in neurons in nuclei of cranial nerves, red nucleus, locus ceruleus and cerebeller nucleus [23]. The expression level of the mRNA is upregulated in motoneurons in facial nucleus after transection of facial nerve [23].

3. Oxidized galectin-1 as an axonal regeneration promoting factor

3.1. Identification and characterization

There should be a factor to regulate initial repair in peripheral nerves after axotomy. For the identification of the factor, we introduced an *in vitro* model (Figure 1), which consists of DRG explants with the associated spinal nerve stump [19], instead of cultured single neurons. Because this *in vitro* model preserves the three-dimensional structure of the cells that enables cell-cell interactions, applied factors are thought to work on the sites which are constructed from axons and non-neuronal cells, including Schwann cells, fibroblasts, and perineurial cells. The target site is thus analogous to the proximal site of *in vivo* axotomized nerve stumps. As previously mentioned, we searched the activity from the cultured supernatants of cells derived from kidney or liver using the *in vitro* nerve regeneration model. The cultured medium of COS1 cells (a cell line derived from kidney) contained an activity promoting axonal regeneration from the transected nerve site of the explant cultures. The COS1-secreted activity was purified by a combination of ultrafiltration, ion-exchange chromatography, gel filtration, reversed phase chromatography and SDS-PAGE. In extraction experiments, the activity was eluted from a SDS-PAGE gel slice corresponding to a Mr 14,000-protein band only. Approximately 200 ng of the protein was obtained from about 300 L of COS1 cultured supernatant. The analysis of an internal amino acid sequence of the active protein indicated that it was identical to galectin-1 [19].

It is well known that galectin-1 is a homodimer with a subunit molecular mass of 14.5 kDa and exhibits β-galactoside binding activity only in the reduced form. However, the galectin-1 was in the oxidative environments when it was purified from the culture supernatant of COS1 cells. And the activity was eluted in the fraction corresponding to Mr of around 14 kDa by gel filtration. Furthermore, this action of the galectin-1 was apparent at concentrations (pg/ml) substantially two orders lower than those at which lectin effects of galectin-1 on neuronal cells *in vitro* were apparent [2]. So, we investigated the relation between structure and axonal regeneration-promoting activity of galectin-1. To characterize the secreted galectin-1, recombinant human galectin-1 secreted into the culture supernatant of transfected COS1 cells was purified under nonreducing conditions and subjected to structural analysis. The analysis revealed that the secreted proteins exist as an oxidized form of galectin-1 containing three intramolecular disulfide bonds (Cys^{2}-Cys^{130}, Cys^{16}-Cys^{88}, Cys^{42}-Cys^{60}) [20] (Figure 2).

Recombinant human galectin-1 (rhGAL-1) and a galectin-1 mutant (CSGAL-1) in which all six cysteine residues were replaced by serine, were expressed in and purified from *Escherichia coli* for further analysis; the purified rhGAL-1 was subjected to oxidation, which induced the same pattern of disulfide linkages as that observed in the galectin-1 secreted in the COS1 cultured supernatant. Whereas the oxidized rhGAL-1

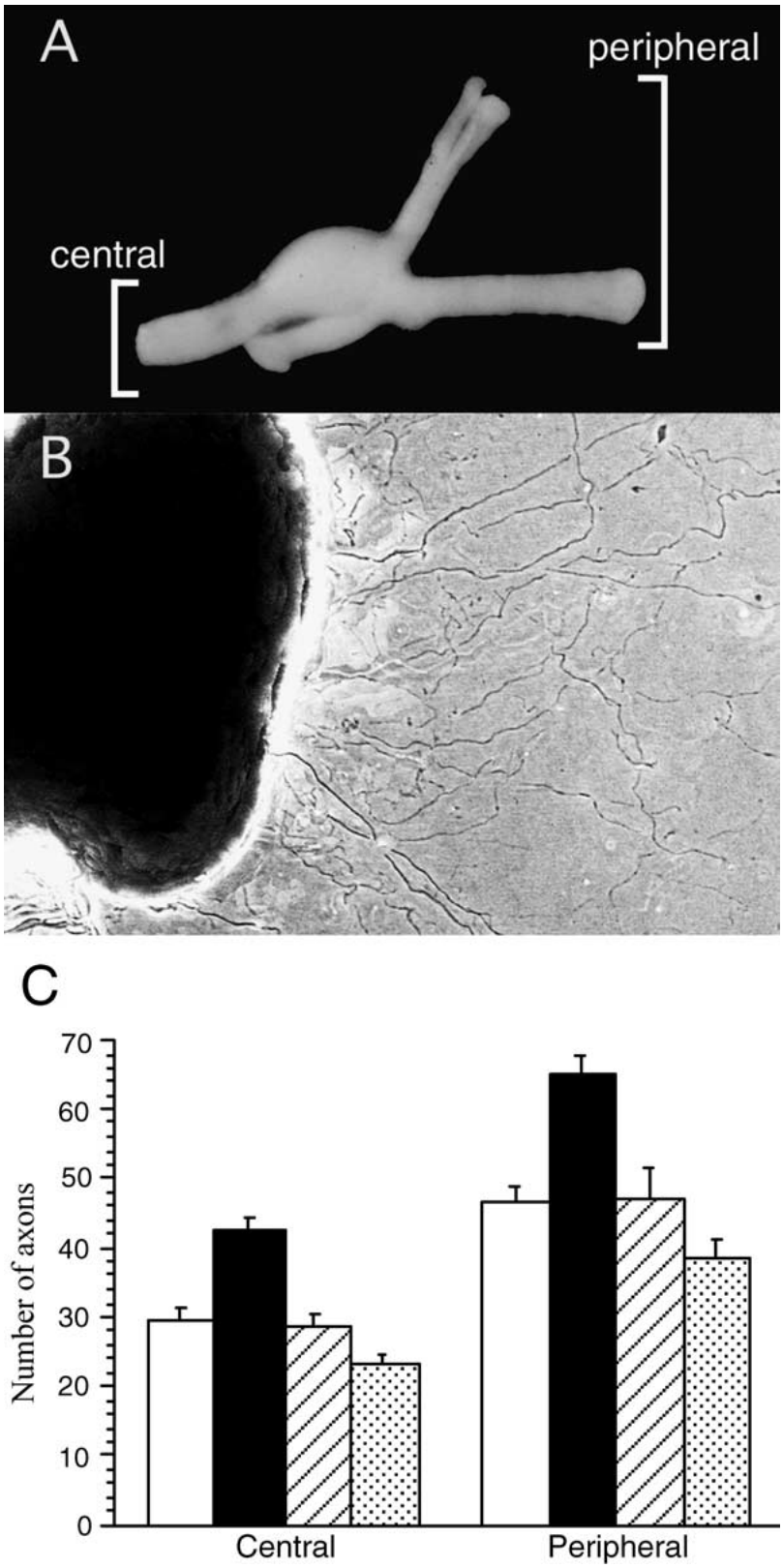

Figure 1. *In vitro* model for axonal regeneration assay. Activity of axonal regeneration was detected with a three dimensional culture of adult rat DRG explant. A: DRG explant with associated nerve stumps. B: Regenerating axons from the peripheral nerve stump. C: The numbers of regenerating axons at both central and peripheral nerve stumps of DRG explants were determined after culture for 6 days. Bars indicate control (□), 50 pg/ml rhGAL-1 (■), 3 μg/ml control IgG (▨), and 3 μg/ml anti-rhGAL-1 IgG (▩).

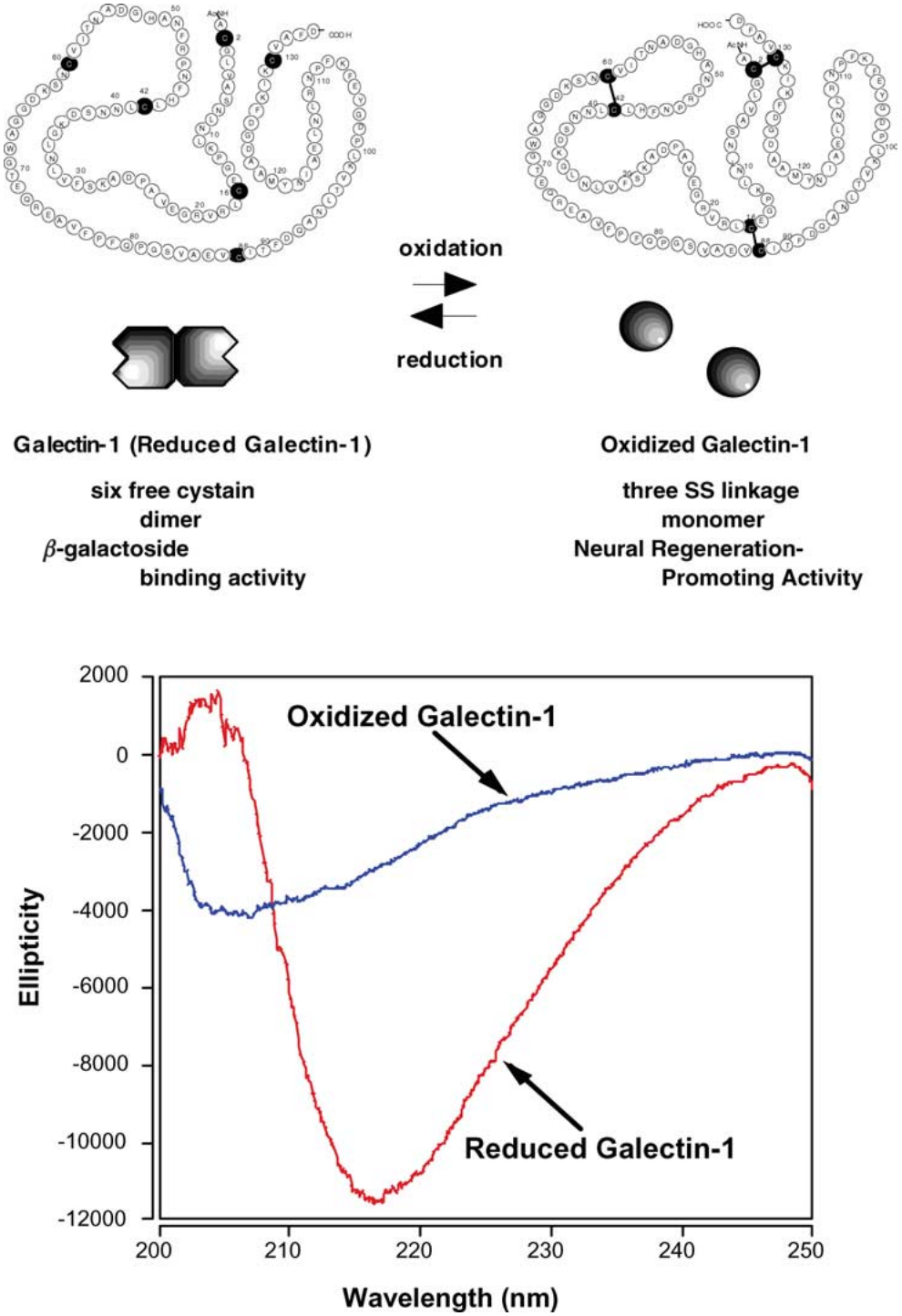

Figure 2. Structure of oxidized galectin-1. Secondary structure features of oxidized galectin-1 and reduced rhGAL-1 were obtained by measuring circular dichroism from 250 to 200 nm.

(rhGAL-1/Ox) did not show the lectin activity, it exhibited marked axonal regeneration-promoting activity in a dose-dependent manner in the DRG explant model. In contrast, CSGAL-1 did not promote axonal regeneration but induced marked hemagglutination even under nonreducing conditions [20] (Figure 3). Furthermore we showed that galectin-3, which possesses a typical lectin activity in solution under nonreducing conditions, did not promote axonal regeneration in our *in vitro* models (Figure 3) as well as *in vivo* nerve transection model [19], though it has been reported that galectin-3 promotes neural cell adhesion and neurite growth of dissociated DRG neurons [25].

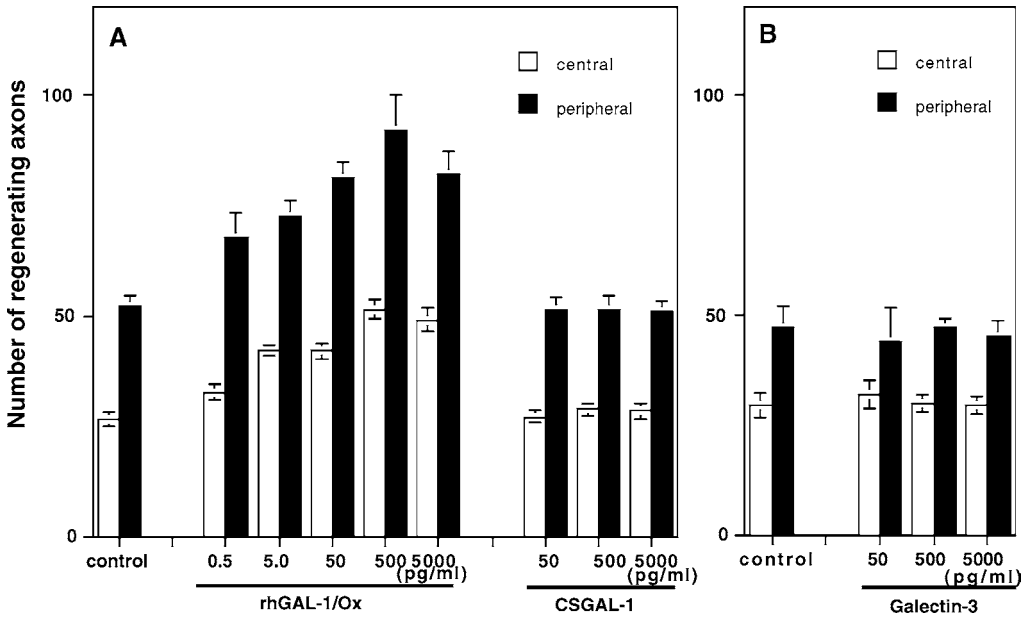

Figure 3. Oxidized rhGAL-1 enhanced axonal regeneration from the transected nerve stumps of adult rat DRG, but CSGAL-1 and galectin-3 lacked the axonal regeneration-promoting activity.

The lower axonal regeneration seen in the control preparation (Figure 1C, open bar) may be due to endogenous galectin-1, which is highly expressed in peripheral nerve as mentioned above. To test this possibility we applied a rabbit polyclonal antibody to rhGAL-1 (stippled bar) and a control antibody (striped bar). The anti-galectin-1 antibody significantly inhibited the axonal regeneration supporting a role for endogenous galectin-1.

In conclusion, these findings indicate that galectin-1 promotes axonal regeneration only in the oxidized form containing three intramolecular disulfide bonds, not in the reduced form that exhibits lectin activity. This oxidized form of galectin-1 is not a member of galectin family, because it lacks β-galactoside binding ability, and therefore we call the factor oxidized galectin-1 in order to distinguish it from galectin-1 having the lectin activity.

For assessment of the monomeric-dimeric structure of oxidized or reduced galectin-1 in a physiological solution, absolute molecular mass was determined by a combination of size-exclusion HPLC and multiangle laser light scattering. The retention volume of rhGAL-1/Ox was larger than that of reduced rhGAL-1. The absolute molecular mass of the oxidized protein was calculated as 14.5 kDa, likely corresponding to a monomeric structure, whereas that of the reduced protein was 30 kDa, likely corresponding to a dimeric structure. Differences in secondary structural features between oxidized and reduced galectin-1 were probed by measurement of circular dichroism (CD) from 250 to 200 nm. The CD spectrum of the oxidized form exhibited a small negative signal around 205 to 210 nm, whereas that of the reduced form showed a broad negative peak around 215 to 220 nm (Figure 2). This shift in the CD spectrum is indicative of a substantial change in secondary structure induced by the formation of disulfide bonds. The X-ray crystal structure study of bovine galectin-1 in complex with *N*-acetyllactosamine showed that the integrity of the dimer is maintained by the anti-parallel β-sheet interactions across the monomer [26]. The CD analysis showed that the secondary structure of rhGAL-1/Ox differed from that of reduced rhGAL-1, which could not construct the dimer. These results indicate that disulfide bond formation alters the structure of galectin-1 in such a way as to confer the ability to promote axonal regeneration.

Numerous studies on the biological effects of galectin-1 have been described. Most of these studies were performed under reducing conditions, and the effects of galectin-1 were inhibited by lactose. The biological effects of galectin-1 were therefore assumed to depend on its ability to bind β-galactoside. On the other hand, some reports show that galectin-1 acts as a cytostatic factor and regulator of cell growth, and the growth-inhibitory effect is not inhibited by lactose [27–30]. A study by site-directed mutagenesis indicated that galecin-1 possesses a growth inhibitory site, which is not part of the β-galactoside binding site [31]. It has been shown that the oxidative loss of lectin activity correlates with the formation of disulfide bonds [32]. Our study, however, represents the first demonstration that the oxidized galectin-1 promotes axonal regeneration, and that this activity is strictly dependent on the oxidized structure of the molecule.

3.2. Oxidized galectin-1 plays essential roles in axonal regeneration after peripheral nerve injury

Regeneration-promoting effects of rhGAL-1/Ox from crushed mouse sciatic nerves into freeze-killed nerves where no living cell exists was examined after continuous delivery of 2.5 ng

per hour of rhGAL-1/Ox (0.5 μl/h) to the proximal stumps and analyzed by electron microscopy [19]. These nerve crush experiments suggest that rhGAL-1/Ox promoted axonal regeneration as well as Schwann cell migration into the acellular nerve.

Further analysis of the effect of rhGAL-1/Ox on neural regeneration used the *in vivo* nerve-transection plus tubulization model [19]. Neural regeneration from the nerve-transection site of the peroneal nerve into a grafted silicone tube filled with collagen gel was examined in four different conditions: control, gel with 5 ng/ml rhGAL-1/Ox, gel with 30 μg/ml anti-rhGAL-1 IgG, and gel with 30 μg/ml control IgG. At 10 days after the operation, double immunostaining of longitudinal sections of frozen regenerated tissues taken from the silicone tubes was carried out with anti-neurofilament and anti-S-100 antibodies. These findings suggest that rhGAL-1/Ox enhanced both number and growing rate of regenerating axons resulting in a promotion of Schwann cell migration (Figure 4, Table 1). A similar role of endogenous galectin-1 in this axonal regeneration was indicated by the effect of treating the collagen gel with anti-rhGAL-1; Figure 4C shows that Schwann cell migration remained at the region near to the cut edge of the proximal nerve stump and that other cells migrated beyond the Schwann cells. These findings were confirmed by the analysis of enhancement of axonal regeneration by rhGAL-1/Ox performed in cross sections by measuring the number of NF-positive axons in two regions, 0.5 mm and 1.0 mm distal to a transected site. The number of regenerating axons was increased by the treatment with rhGAL-1 and reduced by anti-rhGAL-1 (Table 2). These findings are similar to the results from the *in vitro* model suggesting that the *in vitro* model is comparable to the initial stages of nerve repair.

The essential roles of oxidized galectin-1 in peripheral nerve regeneration were strongly supported by another experimental model. Fukaya *et al*. investigated the effects of oxidized galectin-1 on regeneration of rat spinal nerves using acellular autografts (containing no viable cells) and allografts (containing no cell membranes) with special attention to the relationship between axonal regeneration and Schwann cell migration [33]. Immunohistochemically, endogenous galectin-1 was expressed in dorsal root ganglion (DRG) neurons, spinal cord motoneurons and Schwann cells in normal sciatic nerves. Administration of rhGAL-1/Ox, (5 ng/ml) promoted axonal regeneration from motoneurons as well as from DRG neurons; this was confirmed by a fluorogold tracer study. Anti-rh-gal-1 antibody (30 μg/ml) strongly inhibited axonal regrowth. Pretreatment of allografts with rhGAL-1/Ox stimulated the migration of Schwann cells not only from proximal stumps but also from distal stumps into the grafts, resulting in accelerated axonal regeneration. Moreover, Schwann cell migration preceded the axonal growth in the presence of exogenous rhGAL-1/Ox in the grafts. These results strongly suggest that local administration of exogenous rhGAL-1/Ox promotes the migration of Schwann cells followed by axonal regeneration from both motor and sensory neurons, and that oxidized galectin-1 is a key factor in the initial stages of axonal regeneration.

Table 1. Enhancement of schwann cell migration by rhGAL-1/Ox at 10 days after operation in model of peroneal nerve-transection and tubulization

	Distance of migration (mean ± s.e.m.)	
Control	1.2 ± 0.1 mm	($n=12$)
rhGAL-1/Ox	2.0 ± 0.1	($n=11$)
Anti-rhGAL-1	0.6 ± 0.1	($n=9$)
Control IgG	1.2 ± 0.1	($n=6$)

ANOVA: $P<0.0005$: between control and rhGAL-1/Ox; $P<0.05$: between anti-rhGAL-1 and control IgG; $P<0.0001$: between rhGAL-1/Ox and anti-rhGAL-1.

Table 2. Number of regenerating axons at 1.0 mm distal to a transected site in model of peroneal nerve-transection and tubulization

	Number of axons (mean ± s.e.m.)	
Control	52 ± 19	($n=6$)
rhGAL-1/Ox	302 ± 111	($n=7$)
Anti-rhGAL-1	10 ± 5	($n=8$)

Mann-Whitney test: $P<0.05$: between control and rhGAL-1/Ox; $P<0.05$: between control and anti-rhGAL-1; $P<0.005$: between rhGAL-1/Ox and anti-rhGAL-1.

3.3. Oxidized galectin-1 promotes functional recovery in peripheral nerves after freeze/transect axotomy [34]

After having shown that oxidized galectin-1 promotes axonal regeneration from transected-nerve sites in *in vitro* and *in vivo* models, the next study aimed at clarifying whether oxidized galectin-1 also advances restoration of nerve functions after peripheral nerve injury [34]. The sciatic nerve of an adult rat was transected and then 7 mm of the distal nerve section was frozen after being sutured into a proximal stump with four epineurial stitches. This severe frozen injury was expected to verify an axonal regeneration promoting effect of rhGAL-1/Ox in similar injury conditions as mentioned in Section 3.2. Peripheral delivery of oxidized galectin-1 or control solvent to the surgical site was performed with an osmotic pump. The functional recovery was evaluated by measuring the degree of toe spread of the hind paw after holding the rat's back from behind and pushing the paw slightly to the floor. The recovery curves indicated that oxidized galectin-1 administration clearly advanced functional recovery compared with control treatment. The histological study showed that oxidized galectin-1 increased numbers of regenerating myelinated axons in the sciatic nerve after axotomy. There was a tendency that diameters of the myelinated axons were large in oxidized galectin-1 treated group. These results indicate that administration of oxidized galectin-1 to the

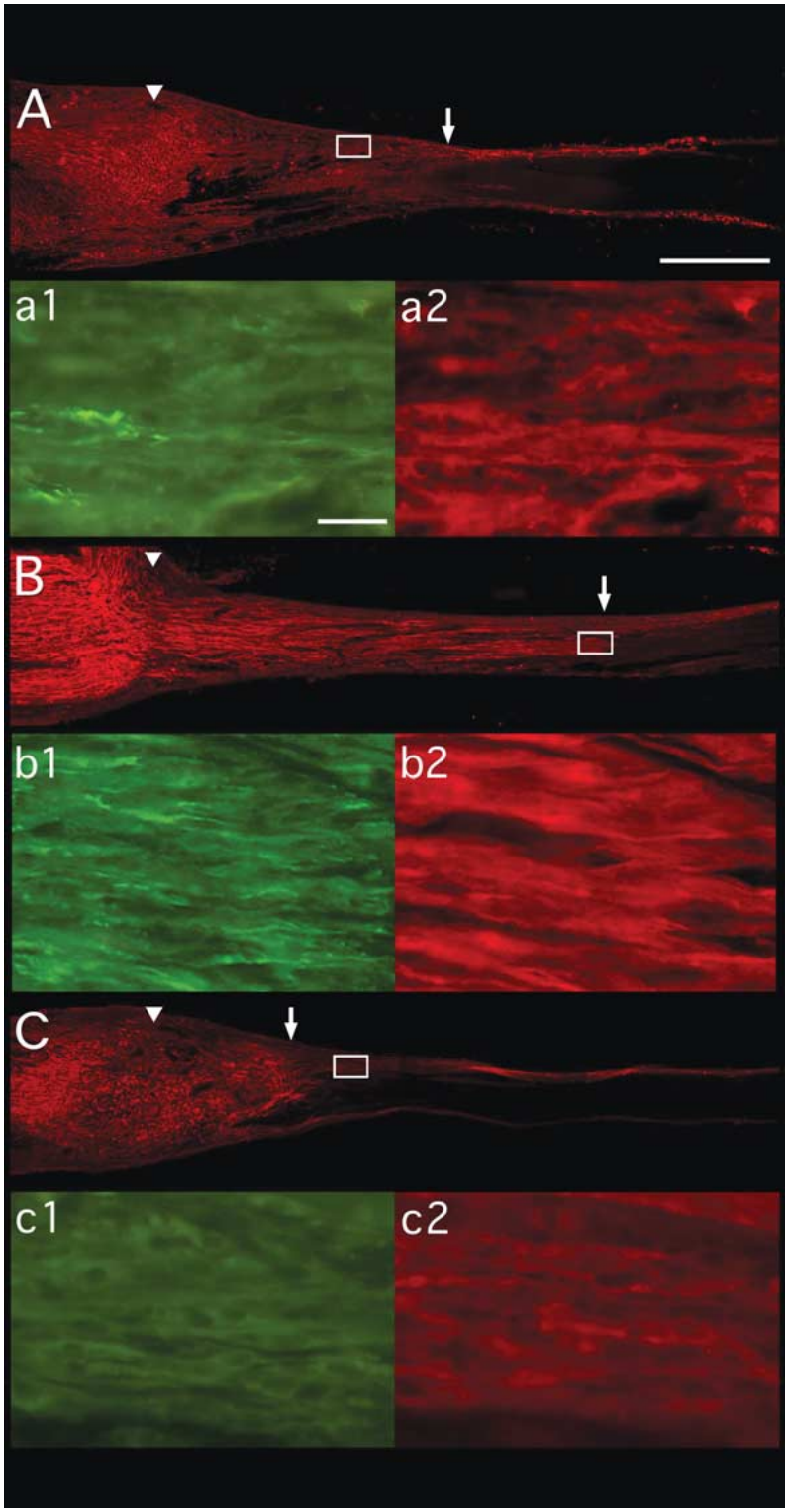

Figure 4. Effect of rhGAL-1 and its antibody on Schwann cell migration and axonal regeneration after nerve transection *in vivo*. Three different collagen gels were examined: A: control, B: gel with 5 ng/ml rhGAL-1, and C: gel with 30 μg/ml antibody to rhGAL-1 IgG. Animals were fixed 10 days after operation. The frozen regenerating tissues were longitudinally sectioned and the sections were double immunostained with antibody against neurofilaments and S-100. A, B, and C were S-100-stained longitudinal sections. Squares in A, B, C were enlarged to a1 and a2, b1 and b2, and c1 and c2, respectively. a1, b1, and c1: NF-staining. a2, b2, and c2: S-100-staining. Arrowheads indicate transected edges and arrows indicate tips of migrating Schwann cells. Bars indicate 500 μm in A, B, C, and 20 μm in a1, a2, b1, b2, c1, and c2.

nerve injury sites *in vivo* promotes rapid restoration of nerve function.

3.4. Oxidized galectin-1 may stimulate non-neuronal cells to initiate axonal regeneration in peripheral nerves after injury

The acceleration of initial repair by oxidized galectin-1 was shown by the application of rhGAL-1/Ox to the two *in vivo* nerve injured models to improve axonal regeneration. Further confirmation of the function was demonstrated by the strong inhibition of axonal regeneration by the treatment with anti-galectin-1 antibody. The treated antibody was thought to bind the free extracellular oxidized galectin-1 to inhibit its attachment to a target cell resulting in the strong inhibition of axonal elongation. These results suggest that oxidized galectin-1 regulates the initial repair after peripheral nerve injury.

We could present from these results the scheme (Figure 5) how galectin-1 regulates initial repair after axotomy in peripheral nerves. Cytosolic galectin-1 localizes in motor neurons, sensory neurons, their axons, and Schwann cells [5,19,24]. After injury, it is secreted via non-classical pathways from growing axons, especially from their growth cones, and reactive Schwann cells to an extracellular space [35,36]. In addition, since growth cones bear high exocytosis activity to secrete endogenous molecules [37], the damaged axons or Schwann cells also provide cytosolic galectin-1 molecules to an extracellular space. Some of the galectin-1 molecules bind to β-galactosides located on cell surfaces, but the others are free from the carbohydrates. These free floating galectin-1 molecules may be oxidized to change their molecular structure by constructing disulfide bonds resulting in loss of lectin activity. Then the oxidized galectin-1 may act as an autocrine or paracrine factor to promote axonal regeneration, functioning more as a cytokine

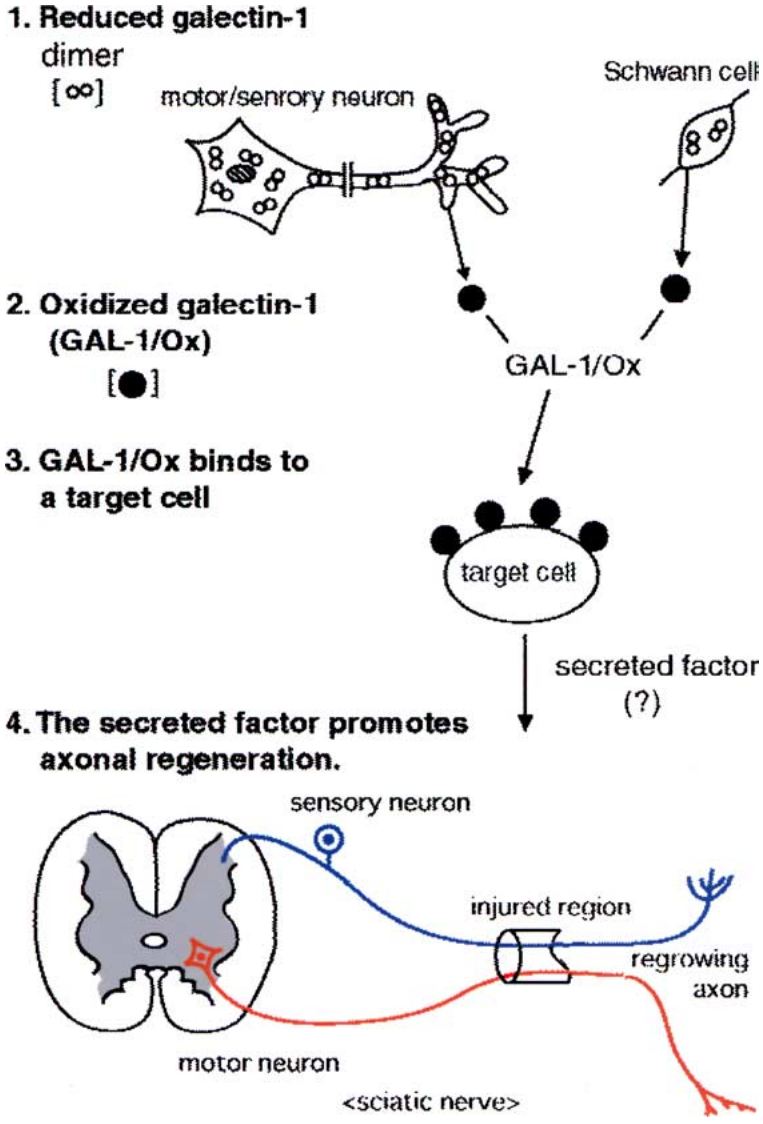

Figure 5. Schematic of how galectin-1 regulates initial repair after axotomy in peripheral nerves.

than as a lectin. Since the factor does not directly work on isolated DRG neurons to promote neurite extension [19], this secreted galectin-1 could not stimulate sprouts themselves, but could influence nonneuronal cells surrounding the axons, including Schwann cells, recruiting macrophages, fibroblasts, and perineurial cells.

Since there have been no studies about the functions of oxidized galectin-1, the previous report [19] may be the first to reveal its possible function. The following questions now need to be addressed. Firstly, does oxidized galectin-1 exist in the extracellular space around injured peripheral nerve sites? Secondly, what are the target cells of oxidized galectin-1 among nonneuronal cells surrounding axons? Thirdly, what is the mechanism by which oxidized galectin-1 promotes axonal regeneration? Recent reports gave possible answered to two of these questions. Macrophages is one target cell for oxidized galectin-1, and an axonal regeneration promoting factor is secreted from macrophages stimulated by oxidized galectin-1 [38]. Furthermore, nerve regeneration experiments in galectin-1 knockout mice [39] will help clarify the roles of the galectin-1 in peripheral nerve regeneration.

The two specific strategies, mentioned in the introduction, of the present study led us to discover a novel factor, oxidized galectin-1, which promotes axonal regeneration. The high activity of this galecin-1 under nonreducing conditions indicates that it may act as a cytokine and not as a lectin. Our study suggests that oxidized galectin-1 plays an important role in peripheral nerve regeneration. These characteristics of oxidized galectin-1 show the possibility that this factor can be applied clinically for restoration of injured peripheral nerves resulting in high functional recovery.

4. Galectin-1 relates to neural diseases

Levels of autoantibodies to galectin-1 were significantly higher in patients with neurological diseases than in healthy controls. This finding can be thought that the concentration of autoantibodies to galectin-1 is possibly associated with impairment of the regulation of the immune system [40]. Galectin-1 also relates to amyotrophic lateral sclerosis (ALS) that is one of the difficult neural diseases to repair. In ALS, abnormal accumulation of neurofilaments induces pathologic changes such as axonal spheroids in the spinal cord, and seems to cause motor neuron degeneration. Kato *et al*. identified a protein along with the neurofilaments in a spinal spheroid of sporadic and familial ALS to be galectin-1 by immunohistochemical analysis with antibody to rhGAL-1 [41]. Another immunohistochemical study revealed that galectin-1 immunoreactivity was reduced in the skin of patients with ALS. The immunoreactive cells were remarkably diminished in the papillary regions, especially in the blood vessels and fibroblasts of the papillary and reticular dermis [42]. Since galectin-1 is intensely expressed in motor neurons and transported into axons and galectin-1 plays important roles in axonal regeneration after injury, these discoveries are expected to become a key to clarify the cause of abnormal accumulation of neurofilaments in ALS.

An immediate early gene, fosB, directs synthesis of FosB and ΔFosB protein. In hippocampus of rat brain, expression of fosB is induced immediately after ischemia-reperfusion, especially in regions of DG and CA3 where most neurons are resistant to the injury. While, in the region of CA1 where delayed neuronal cell death occurs, expression of fosB is induced prior to the cell death. Nakabeppu *et al*. found that ΔFosB expression in rat 3Y1 cells induces morphological differentiation after one round of cell cycle [43]. On the other hand, Rat1a cells expressing ΔFosB underwent delayed cell death after one round of cell cycle [44]. New isoforms of galectin-1, whose expression is altered in these cells, were identified [43]. These results indicate that galectin-1 may play crucial roles in neuronal cell differentiation and survival in central nervous system.

5. Conclusion

It seems that galectin-1 has a variety of biological functions. The functions could vary according to the time, the site and the structure of galectin-1 at the time. This review showed that disulfide bond formation alters the structure of galectin-1 as to confer the novel ability to promote axonal regeneration upon galectin-1. This discovery of essential roles of oxidized galectin-1 opened new insights of galectin-1 to its functions in matured mammalian nervous systems. Recent works indicate that the insights may be extended into an establishment of neural regeneration in a central nervous system and a resolution of cause of neural diseases.

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