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Neuroscience Letters 327 (2002) 45–48

Neuroscience Letters

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Expression of β-1,4-galactosyltransferase II and V in rat injured sciatic nerves

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Received 4 February 2002; received in revised form 8 April 2002; accepted 9 April 2002

Abstract

β-1,4-Galactosyltransferases II and V (β-1,4-GalT II and V) are involved in the biosynthesis of N-linked oligosaccharides as β-1,4-GalT I which plays important roles in promoting neuron outgrowth. In the present paper, it was illustrated that β-1,4-GalT II and V were localized mainly in Schwann cells of lesion sciatic nerves by in situ hybridization. Northern blot showed that the expression of β-1,4-GalT II increased gradually at both stumps of injured nerves, while that of β-1,4-GalT V decreased at proximal stumps but increased and reached its peak on the third day post-operation at distal stumps, before it declined. The different expression of β-1,4-GalT II and V in Schwann cells suggested that they would affect the different galactosylation of glycoproteins in injured nerves regeneration. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: β-1,4-Galactosyltransferase II and V; Peripheral nerves regeneration; Schwann cells

During the course of the development and regeneration of the mammalian nervous system, the changes of glycosylation in the neuronal surface have been observed by using lectins, anti-carbohydrate antibodies and carbohydrate-specific toxins [1,14]. These changes suggested that carbohydrates on the cell surface play important roles in the neural tissue formation. The Gal β1 → 4GlcNAc structure is mostly found in the outer chain moieties of *N*-glycans, and formed by a sequential action of uridine diphosphate (UDP)-GlcNAc:*N*-acetylglucosaminyltransferases and UDP-Gal:GlcNAc β-1,4-galactosyltransferase (β-1,4-GalT) [6]. The ablation of the mouse gene encoding *N*-acetylglucosaminyltransferase I resulted in the absence of hybrid-type and complex-type N-linked oligosaccharides and embryonic lethality of the mice with severe defects in the neural tube formation [11]. However, in the β-1,4-GalT I-knockout mouse in which the galactosylation of glycoproteins was impaired, no apparent defects in the organ genesis were observed except the growth retardation, abnormal of differentiation epithelial cells and neonatal lethality of the mice [3]. Polysialic acid and human natural killer cell carbohydrate (HNK-1, CD57) attached to Gal β1 → 4GlcNAc group, and both of them were expressed on the outer chain of N-linked oligosaccharides and attached to several neural cell recognition molecules, including neural cell adhesion molecule (N-CAM) involved in the development and regeneration of nervous system [9,10]. These structures were present without reduction in the glycoprotein of β-1,4-GalT I-knockout mouse brain [8]. This suggested that carbohydrate antigens containing the galactose residues expressed on oligosaccharides should be synthesized by other β-1,4-GalT(s) in the nervous system.

Recently, several human genes encoding novel proteins with β-1,4-GalT activities have been cloned [2]. Preliminary studies suggested that β-1,4-GalT II and V were involved in the biosynthesis of N-linked oligosaccharides as β-1,4-GalT I [6,10,13]. Nakamura et al. have cloned β-1,4-GalT II and V cDNA from the mouse brain, and found both of them had different expression patterns in the development of brain compared with that of β-1,4-GalT I. During the development of mouse brain, it was found β-1,4-GalT I was expressed mainly in the mid-embryonic stage, and the level of β-1,4-GalT II transcript remained constant and that of the β-1,4-GalT V transcript increased after birth [13,18]. Nakamura et al. inferred that β-1,4-GalT II and V were major enzymes which galactosylated N-linked oligosaccharides in the mouse brain [13].

We found that the expression of β-1,4-GalT I and Gal β1 → 4GlcNAc containing glycan structure changed during

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PII: S0304-3940(02)00381-6

the degeneration of peripheral nerves and their synthesis would play important roles in maintaining normal nerves' functions and promoting injured nerves to regenerate (to be published). In the present study, expression changes of β-1,4-GalT II and V were investigated after rats' sciatic nerves were injured by in situ hybridization and Northern blot.

The model of injured peripheral nerves was carried out on six groups of 12 adult female Sprague–Dawley rats (180–220 g) [12,16]. Experimental rats were deeply anesthetized with a cocktail of xylazine (10 mg/kg), ketamine (95 mg/kg) and acepromazine (0.7 mg/kg) administered intraperitoneally. Then the sciatic nerves at the region of midthigh were cut. In order to transect sciatic nerves permanently, a piece of 0.5 cm nerve tissue was removed. After survival times of 6, 12 and 18 h, and 1, 2, 3, 7, 14 and 21 days post-axotomy, the rats were anesthetized with the above cocktail. The proximal and distal stumps of injured sciatic nerves were dissected for further experiments.

Since the gene of rat β-1,4-GalT II and V had not yet been cloned and the nucleotide sequences of β-1,4-GalT were highly conserved in eukaryotes [15], reverse transcription-polymerase chain reaction (RT-PCR, QIAGEN) was utilized to detect the existence of β-1,4-GalT II and V in mice sciatic nerves. The first-strand cDNA was prepared from total RNA from Balb/C mice sciatic nerves. The cDNA fragments were amplified by PCR using primers 5′-tggtcatcgaattcacctca-3′, 5′-atcctcagaaactgggcctt-3′ for mouse β-1,4-GalT II (GenBank accession number: AB019541) and 5′-tgcagttcgccttctatgtg-3′, 5′-gttcaggttgtt-gaggccat-3′ for mouse β-1,4-GalT V (GenBank accession number: AB004786). PCR products were sequenced by DNA sequencing.

RT-PCR amplified cDNA fragments of mice β-1,4-GalT II and V were prepared as probes for further studies of Northern blot and in situ hybridization. For Northern blot analysis, amplified cDNA fragments of β-1,4-GalT II (481 bp: 322–802) and V (503 bp: 725–1227) were radio-labeled with [α-^{32}P]deoxyadensoine triphosphate (dATP) (Amersham Pharmacia) using a Prime-A-Gene random primer labeling kit (Promega) according to its protocol [18]. For in situ hybridization analysis, amplified cDNA fragments were subcloned into pGEM-T Easy (Promega). Plasmids were linearized with restriction enzymes of *Apa*I and *Sal*I and then the sense and antisense digoxigenin (DIG) labeling probes were generated with the respective SP6 and T7 RNA polymerase by in vitro transcription according to its protocol (Boehringer Mannheim) [12,16]. The labeling efficiency was quantitated by dot blotting using a DIG detection kit (Boehringer Mannheim).

Expression changes of β-1,4-GalT II and V in injured sciatic nerves were analyzed by Northern blot. Total RNA was isolated from above dissected rats' sciatic nerves using Trizol reagent (Gibco/BRL) according to its protocol. RNA was transferred to Hybond-N + nylon membrane and cross-linked by ultraviolet (UV) irradiation using a GS Gene Linker UV chamber (Bio-Rad). Northern blot analysis was performed as described previously [18], using [α-^{32}P]dATP-labeled β-1,4-GalT II and V fragments as probes. [α-^{32}P]dATP-labeled glyceraldehyde-3-phosphate dehydrogenase fragment (1–759 bp) was used as a control.

With the method of in situ hybridization, the cell type where β-1,4-GalT II and V were localized could be determined. Longitudinal and transverse sections were prepared as reported [12,16]. The specificity of the DIG-labeled antisense probes was examined by control experiments using RNase A (20 μg/ml) prior to incubation in hybridization buffer, or substituting with sense probe or omitting the probe in hybridization solution, or adding a 100-fold excess of unlabelled probe to the labeled probe in hybridization buffer.

It was found that β-1,4-GalT II and V existed at mice sciatic nerves by RT-PCR (Fig. 1). Changes of two β-1,4-GalTs in rats' injured sciatic nerves were studied by Northern blot. The integrated density of blots was scanned, digitized and analyzed using ONE-Dscan software (Scananalytics, Billerica; data not shown). The expression of β-1,4-GalT II at both stumps of injured nerves increased gradually, reached its peak on the second week post-operation, before it declined. The expression of β-1,4-GalT decreased at proximal stumps but increased and reached its peak on the third day post-operation at distal stumps, before it declined (Fig. 2).

It was also found by in situ hybridization that β-1,4-GalT II and V mRNA were localized mainly in the myelin (arrow), which consisted of Schwann cells in peripheral nerves (Figs. 3 and 4). Immunohistochemistry experiments on sections of injured sciatic nerves were tested by S100 antibodies which were the marker of Schwann cells, as the positive control (arrow; Fig. 4E). Based on these results, we

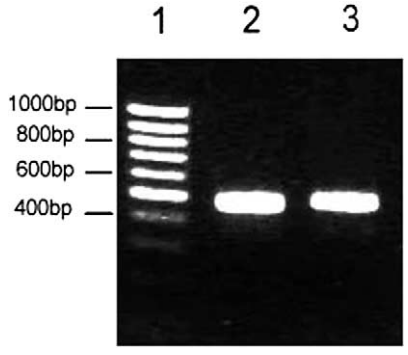

Fig. 1. The existence of β-1,4-GalT II and V mRNA in mice sciatic nerves were studied by RT-PCR. The first-strand cDNA was prepared from 5 μg total RNA of Balb/C mice (18–22 g) sciatic nerves. PCR was carried out with the initial denaturing step at 95 °C for 3 min, then 30 cycles at 95 °C for 30 s, 60 °C for 45 s, and 72 °C for 45 s and a further extension at 72 °C for 10 min. Amplified cDNA fragments of mouse β-1,4-GalT II (481 bp: 322–802) and β-1,4-GalT V (503 bp: 725–1227) were confirmed by DNA sequencing. 1, 100 bp DNA ladder; 2, amplified fragment of β-1,4-GalT II; 3, amplified fragment of β-1,4-GalT V.

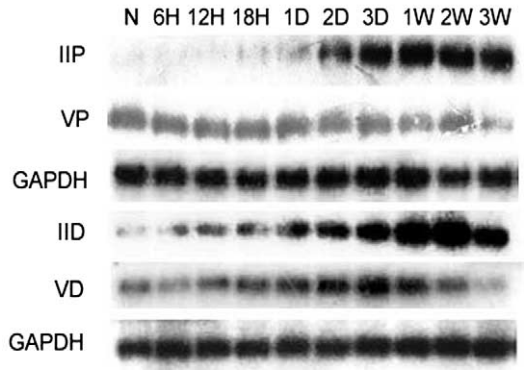

Fig. 2. Expression changes of β-1,4-GalT II and V in rats' injured sciatic nerves were studied by Northern blot. RNA concentration was calculated by measuring UV light absorbance at 260 nm. Each RNA sample (30 μg) was denatured and subjected to electrophoresis in 1% agarose gel containing 2.2 M formaldehyde. IIP, VP: expression of β-1,4-GalT II and V in proximal stumps, respectively; IID, VD: expression of β-1,4-GalT II and V in distal stumps, respectively; N, normal sciatic nerves; H, D, W, hours, days and weeks after lesion, respectively.

inferred that mRNA of β-1,4-GalT II and V were centralized in Schwann cells of injured peripheral nerves.

As we have known, Schwann cells de-differentiate and proliferate during the first 3 weeks after axotomy of peripheral nerves [4]. Activated and myelin-forming Schwann cells switch their functions from myelination of electrically active axons to the creation of a favorable growth environment for nerve regeneration by decreasing their synthesis of myelin-related protein and glycolipids and increasing cell adhesion molecules such as N-CAM, L1 and *N*-cadherin to support neurite outgrowth [5,7]. The synthesis of Gal β1 → 4GlcNAc group is the basis of the synthesis of HNK-1 carbohydrate and polysialic acid which are the foundation of the modification of several nerve regeneration associated molecules such as L1 and N-CAM [6,10]. The Gal β1 → 4GlcNAc structure is found in the outer chain moieties of *N*-glycans [10]. Further glycosylation of this group brings about a variety of carbohydrate antigens including ABO blood group determinants, I and i antigens, polysialic acid and HNK-1 carbohydrate, etc. [6]. Polysialic acid is expressed predominantly on N-CAM and modulates the binding between N-CAM proteins, and thus regulates the neurons interaction [10]. Highly sialylated N-CAM plays important roles in neuron regeneration [17]. HNK-1 carbohydrate epitope is the characteristic of Schwann cells and is found on several neural cell recognition molecules, including N-CAM, L1, P0 and myelin associated glycoprotein, and it has been suggested to be involved in the migration of neural and neural crest cells, neurite outgrowth, transient cell-to-cell and cell-to-laminin interaction [6,8]. After peripheral nerves were injured, the different expression of β-1,4-GalT II and V in Schwann cells would affect the different galactosylation of glycoproteins during the degeneration and regeneration of peripheral nerves. Although the acceptor-specificities of β-1,4-GalT II and V have not been well established, the presence of N-linked oligosaccharides and the different galactosylation of N-linked oligosaccharides may determine the subsequent glycosylation including the expression of polysialic acid and HNK-1 carbohydrate. Further studies by analyzing individual β-1,4-GalT II- and V-knockout mice may elucidate yet unknown functions of

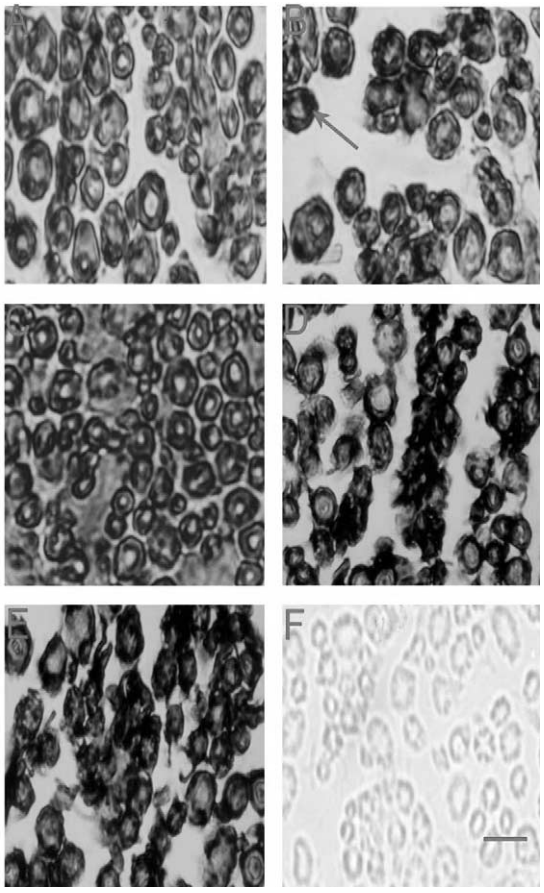

Fig. 3. The expression of β-1,4-GalT II in injured sciatic nerves was studied by in situ hybridization using DIG-labeled riboprobe. Dissected sciatic nerves of three rats of each group were processed for paraffin embedding for in situ hybridization. The analysis was performed on 5-μm sections of injured nerves as described previously [12,16]. In brief, sections were prehybridized in 50% formamide in 4× saline sodium citrate at 58 °C for 30 min and hybridized at 58 °C for 16 h by adding DIG-labeled 0.5 μg/ml sense probe (used as negative control) and antisense probe in the humidified chamber. Sections were then immersed in 7.5 units/ml of the anti-DIG–alkaline phosphatase-conjugated antibody in dilution buffer (10 mM Tris–HCl, pH 7.5, and 150 mM NaCl) for 2 h at 37 °C, then treated with the alkaline phosphatase substrate solution, containing 5-bromo-4-chloro-3-indolyl phosphate and Nitro-Blue tetrazolium at 24 °C for 6 h. Each experiment was repeated three times under identical conditions. (A) The expression of β-1,4-GalT II in normal sciatic nerve. (B,C) The expression in proximal and distal stumps of injured nerve, respectively (2 days after lesion, transverse section). (D,E) The expression in proximal and distal stumps, respectively (2 weeks after lesion). (F) The hybridization using DIG-labeled β-1,4-GalT II sense riboprobe showing background staining. Scale bar, 50 μm.

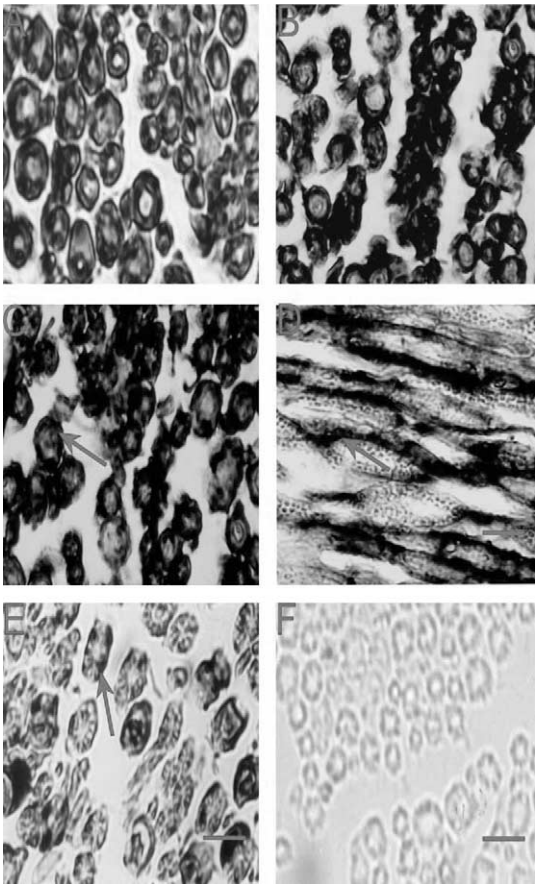

Fig. 4. The expression of β-1,4-GalT V in injured sciatic nerves was studied by in situ hybridization using DIG-labeled riboprobe. (A) The expression of β-1,4-GalT V in normal sciatic nerve. (B,C) The expression in proximal and distal stumps of injured nerve, respectively (2 weeks after lesion, transverse section). (D) The expression in distal stumps of injured nerve, respectively (2 days after lesion, longitudinal section). (E) The localization of S100 antibodies (Sigma; 1:1000) in injured nerves by immunohistochemistry. (F) The hybridization using DIG-labeled β-1,4-GalT V sense riboprobe showing background staining. Scale bar, 50 μm.

the carbohydrates in the degeneration and regeneration of peripheral nerves.

This work is supported by the National Natural Science Grants (39870168, 39970180), PR China and the Grant of Science and Technology Commission of Shanghai Municipality.

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