Glycoconj J (2008) 25:685–701
DOI 10.1007/s10719-008-9129-5

The expression patterns of β1,4 galactosyltransferase I and V mRNAs, and Galβ1-4GlcNAc group in rat gastrocnemius muscles post sciatic nerve injury

Meijuan Yan · Chun Cheng · Fei Ding · Jing Jiang · Lingling Gao · Chunlin Xia · Aiguo Shen

Received: 18 October 2007 / Revised: 20 March 2008 / Accepted: 26 March 2008 / Published online: 30 May 2008

Abstract Glycosylation is one of the most important post-translational modifications. It is clear that the single step of β1,4-galactosylation is performed by a family of β1,4-galactosyltransferases (β1,4-GalTs), and that each member of this family may play a distinct role in different tissues and cells. β1,4-GalT I and V are involved in the biosynthesis of N-linked oligosaccharides and play roles in sciatic nerve regeneration after sciatic nerve injury. In the present study, the expression of β1,4-galactosyltransferase (β1,4-GalT) I, V mRNAs and Galβ1-4GlcNAc group were examined in rat gastrocnemius muscles after sciatic nerve crush and transection. Real time PCR revealed that β1,4-GalT I and V mRNAs expressed at a high level in normal gastrocnemius muscles and decreased gradually from 6 h, reached the lowest level at 2 weeks, then restored gradually to relatively normal level at 4 weeks after sciatic nerve crush. In contrast, in sciatic nerve transection model, β1,4-GalT I and V mRNAs decreased gradually from 6 h, and remained on a low level at 4 weeks in gastrocnemius muscles after sciatic nerve transection. *In situ* hybridization indicated that β1,4-GalT I and V mRNAs localized in numerous myocytes and muscle satellite cells under normal conditions and at 4 weeks after sciatic nerve crush, and in a few muscle satellite cells at 4 weeks after sciatic nerve transection. Furthermore, lectin blotting showed that the expression level of the Galβ1–4GlcNAc group decreased from 6 h, reached the lowest level at 2 weeks, and restored to relatively normal level at 4 weeks after sciatic nerve crush. RCA-I lectin histochemistry demonstrated that Galβ1–4GlcNAc group localized in numerous membranes of myocytes and muscle satellite cells in normal and at 4 weeks after sciatic nerve crush, and in a few muscle satellite cells at 2 and 4 weeks after sciatic nerve transection. These results indicated that the expressions of β1,4-GalT I, V mRNAs and Galβ1–4GlcNAc group were involved in the process of denervation and reinnervation, which suggests that β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group may play an important role in the muscle regeneration.

M. Yan · C. Cheng · F. Ding · J. Jiang · L. Gao · A. Shen
The Jiangsu Key Laboratory of Neuroregeneration,
Nantong University,
Nantong, Jiangsu Province 226001, People's Republic of China

M. Yan · C. Xia (✉)
Lab of Aging and Nervous Diseases & Cytoneurobiology Unit,
Soochow University,
Suzhou 215123, People's Republic of China
e-mail: xclcnb@yahoo.com.cn

M. Yan (✉) · A. Shen (✉)
Nantong University,
19 Qi-Xiu Road,
Nantong, Jiangsu Province 226001, People's Republic of China
e-mail: ymz@ntu.edu.cn
e-mail: shag@ntu.edu.cn

Keywords β1,4-Galactosyltransferases I and V · Galβ1 · 4GlcNAc group · Sciatic nerve injury · Real-time PCR · *In situ* hybridization · RCA

Abbreviations

β1,4-GalTs	β1,4-galactosyltransferases
Glcβ1,4NAc	galactose-β1,4-*N*-acetylglucosamine
DIG	digoxigenin
β2-M	β2-microglobulin
HRP	horseradish peroxidase
RCA-I	Ricinus communis agglutinin-I
RT	room temperature
RT-PCR	reverse transcription polymerase chain reaction

SD	Sprague-Dawley
SDS	sodium dodecyl sulfate
CBB	Coomassie Brilliant Blue

Introduction

Skeletal muscle atrophy induced by denervation after peripheral nerve injures is quite common in clinical practice, but the recovery is often incomplete despite physical rehabilitation that takes a long period of time. Some researches have found skeletal muscle atrophy after sciatic nerve injury [33, 37]. Skeletal muscle atrophy due to denervation was an integration of innutrition atrophy with wasting atrophy. The optimal scheme to prevent muscle atrophy was to achieve the reinnervation. Because irreversible denaturation had generated in the denervated muscle before the regenerated axon migrated to the motor end-plate, a delay in the rate and extent of the muscle atrophy was crucial for restoration of the muscle functional capacities. Since its molecular mechanisms are not clear, therapeutic and preventative strategies for skeletal muscle atrophy after nerve injures still belongs to the most challenging and difficult medical reconstructive problems.

β1,4-Galactosyltransferase I (β1,4-GalT I) is the first mammalian glycosyltransferase cDNA to be cloned and the first mammalian glycosyltransferase to have its crystal structure resolved [25]. β1,4-GalT I is a type II membrane-bound glycoprotein, which has been found on the plasma membrane of different types of cells, where it serves as a cell surface adhesion molecule mediating various cell–cell and cell–matrix interactions such as sperm–egg binding, cell spreading, migration and neurite outgrowth [10, 15, 29]. Indirect immunofluorescence staining showed that β1,4-GalT I localized at the growth cone during neurite outgrowth; blocking β1,4-GalT-I activity with its antibody or consuming its substrates in laminin by pregalactosylation inhibited neurite outgrowth and elongation from PC12 cells, sensory neurons, and chick dorsal root ganglia (DRG) [10, 30, 31]. Overexpression of β1,4-GalT-I in PC12 cells remarkably enhanced neurite formation and elongation [10]. All these results suggest that β1,4 GalT-I is involved in the neurite outgrowth. β1,4-Galactosyltransferase V (β1,4-GalT V) is a constitutively expressed enzyme that can effectively galactosylate the GlcNAcβ1-6Man group of the highly branched N-glycans. It has 37% identity to β1,4-GalT I [23] and is involved in the biosynthesis of N-linked oligosaccharides like β1,4-GalT I [7, 14, 17]. Our previous study has showed that β1,4-GalT I and β1,4-GalT V are involved in the nerve injury [26, 27]. β1,4-GalT I and β1,4-GalT V transcripts have been shown to be present in human normal skeletal muscle [17, 21, 23, 24]. What effects of β1,4-GalT I and β1,4-GalT V exerts on skeletal muscle atrophy induced by various factors such as disuse, microgravity, disease, aging, or denervation are unknown. To achieve these objectives, identifying β1,4-GalT I and β1,4-GalT V expression levels during muscle denervation and reinnervation establish an important prerequisite to the more thorough researches.

The Galβ1-4GlcNAc structure is mostly found in the outer chain moieties of N-glycans, and it is formed by a sequential action of uridine diphosphate (UDP)-GlcNAc: *N*-acetylglucosaminyltransferases and UDP-Gal: GlcNAc β1,4-galactosyltransferase. The synthesis of Galβ1-4GlcNAc 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 [12]. Preliminary studies suggested that β1,4-GalT I and V were involved in the biosynthesis of N-linked oligosaccharides [7, 14, 17]. Which effects Galβ1-4GlcNAc exerts on skeletal muscle after sciatic nerve injury is unknown, so we focused on the Galβ1-4GlcNAc group to study its expression change in gastrocnemius muscles during denervation and reinnervation.

Based upon this, we constructed sciatic nerve crush and sciatic nerve transection models. Sciatic nerve crush model leads to functional denervation of muscles with a variety of degenerative changes in muscle morphology and biochemistry that subsist until the regeneration of injured nerve takes place. Reinnervation and regeneration of skeletal muscles result in restoration, to some degree, of the original muscle fiber structure and contractile activity accompanied by a reversal of some previous biochemical alterations [5, 18–20]. In contrast, sciatic nerve transection leads to denervation of muscles and muscle atrophy. Then we analyzed the expression patterns of β1,4-GalT I, β1,4-GalT V mRNAs and Galβ1-4GlcNAc group in gastrocnemius in these two models by using real-time PCR and *in situ* hybridization. The relationship between expression levels of β1,4-GalT I, β1,4-GalT V mRNAs, Galβ1-4GlcNAc group and gastrocnemius muscles during denervation and reinnervation was discussed in an attempt to contribute to a better understanding of the molecular mechanisms for muscle atrophy and regeneration during denervation-reinnervation.

Materials and methods

Animals

Ninety Sprague Dawley (SD) rats, weighing from 180 to 220 g, were provided by the Experimental Animal Center

of Nantong University. All animals were kept under standardized laboratory conditions in an air-conditioned room with free access to food and water. All the animal tests were carried out in accordance with the US National Institute of Health (NIH) Guide for the Care and USE of Laboratory Animals published by the US National Academy of Sciences (http://oacu.od.nih.gov/regs/index.htm) and approved by the Administration Committee of Experimental Animals, Jiangsu Province, China.

Methods

All animals were divided into three groups randomly, the normal group (control group), the sciatic nerve crush group and the transection group, which received crush or transection on the right sciatic nerve and were sacrificed at different time (6 h, 12 h, 1 day, 2 days, 1 week, 2 weeks, 4 weeks) after crush or transection, while the control group received no injury. Six rats in an operated group per time point and in control group were used.

Surgical procedure

Animals of control and injury group were deeply anesthetized with a cocktail of xylazine (10 mg/kg), ketamine (95 mg/kg) and acepromazine (0.7 mg/kg) administered intraperitoneally. In the sciatic nerve crush group, the right sciatic nerves were crushed three consecutive times, ten seconds each crush by using the same forceps, the sciatic nerves on the left side were left intact and thus used as internal controls, at the same time the sciatic nerves of the control group, were not operated. In the transect group, the right sciatic nerves at the region of mid-thigh were cut. To segregate sciatic nerves permanently, a piece of 0.5 cm nerve tissue was removed. All animals were kept in the same condition. On post-operative time 6 h, 12 h, 1 day, 2 days, 1 week, 2 weeks and 4 weeks, the experimental rats were anesthetized with the above cocktail, exposed the chest chamber. Rats were perfused transcardially with 250 ml normal saline followed by 250 ml of 4% polyfomaldehyde in 0.1 M phosphate buffer (pH 7.4), taking out the gastrocnemius muscles. All these tissues were fixed in the same 4%PA for 12 h, then transferred to the 30% sucrose 0.1 M PBS until the tissues were sinked. The dissected gastrocnemius muscles of three rats in each group were subjected to cryosectioning (transverse and longitudinal sections) for *in situ* hybridization and RCA-I histochemistry, cryostat sections were cut at a thickness of 10 μm. The sections were prepared and used for these studies as previously reported [16].

For real time PCR or lectin blot analysis, middle region of gastrocnemius muscles were taken from each injured rat. The individual nerves were weighed followed by storing at −80°C until homogenization.

The measurement of sciatic function index, SFI

To do the walking track test, we must investigate the activity, wound, spread claw, spirit of the rat after the right sciatic nerve crush or transection, then we can calculate the SFI based on the following formula:

$$\mathrm{SFI} = 38.3(\mathrm{EPL} - \mathrm{NPL})/\mathrm{NPL} + 109.5(\mathrm{ETS} - \mathrm{NTS})/\mathrm{NTS} + 13.3(\mathrm{EIT} - \mathrm{NIT})/\mathrm{NIT} - 8.8$$

The factors that contributed to this formula were print-length factor (PLF), toe-spread factor (TSF), and intermediary toe-spread factor (ITF). E and N in the formula represent experimental side and normal side, respectively. Zero value indicates normal or completely recovery, and the −100 accounts for the sciatic nerve was crushed or transected completely. The sciatic functional index offer the peripheral nerve investigator a noninvasive quantitative assessment of hindlimb motor function in the rat with selective hindlimb nerve injury [2, 32].

Quantitative analysis of β1, 4-GalT I and V transcripts in gastrocnemius muscles by real time PCR

The concept and validation of real-time quantitative PCR have been described previously [35]. real time PCR primers for β1,4-GalT-I, V and β2-microtubulin (β2-M) were designed corresponding to the coding region of the genes as follows: β1,4-GalT-I primers, sense 5′- TATTTGCATCCAGTCTTT CAGC -3′ and antisense 5′- CAGCTTAGCTCGATTAAA CATGG -3′; β1,4-GalT V primers, sense 5′-GGCATAGT GAACACCTACCTCTT-3′ and antisense 5′-TTCAGGCT GCTGTTCCTCTTG-3′; β2-M primers, sense 5′-GTCTT TCTACATCCTGGCTCACA-3′ and antisense 5′-GACGG TTTTGGGCTCCTTCA-3′. PCR probe for GalT I, V and β2-M were designed corresponding to the coding region of the genes as follows: β1,4-GalT-I probe, 5′(FAM)-TCCAGCCTGATTGATGACGTAGATGC -(TAMRA)3′; β-1,4-GalT-V probe, 5′FAM-CTGCGGACCACCTGCTCG TACACCT-TAMRA3′ and β2-M probe, 5′(FAM)-CACC CACCGAGACCGATGTATATGCTTGC-(TAMRA)3′. The reaction mixes included 1×PCR buffer, 20 mM magnesium chloride, 0.2 mM deoxyNTP, and 10 nmol TaqMan probe with a pair of 10 nmol β1,4-GalT I primers, 10 nmol β1,4-GalT V primers or 10 nmol β2-M primers and probe of each molecule respectively. We have used the relative standard curve method to determine the β1,4-GalT I and V transcripts levels in gastrocnemius muscles. β2 microtubulin (β2-M)

was used as an endogenous control to normalize the expression level of the transcripts. Standard curves for β2-M were constructed using serial dilutions of cDNA from different time gastrocnemius muscle. Standard curves and experimental conditions were amplified in quadruplet. The standard curves for the β1,4-GalT I, V and β2-M (standards) were constructed from respective mean Ct value, and the linear equation was derived using the Sequence Detection Systems(SDS) software (PE Biosystems, Norwalk, CT). The amount of template in the cDNA pool of the respective experimental conditions was then determined by applying the mean Ct value of that reaction in the equation of the standard curve. The expression levels of β1, 4-GalT I and V mRNAs were normalized to the respective β2-M value. The normalized values of control and gastrocnemius muscle injured samples were compared.

Real-time PCR detection of PCR product accumulation was performed in a Rotor Gene 3000 Detector (Perkin-Elmer/Applied Biosystems, Foster City, CA, USA). Primers for the β1,4-GalT I, V and β2-M were used at a final concentration of 900 and 50 nM respectively, whereas the probes were used at a final concentration of 200 nM. Amplification was done in a 20 μl final volume, under the following cycling conditions: The PCR cycling was initiated by activating the Taq polymerase for 2 min at 93°C; thereafter, two-step PCR was run for 40 cycles. Each PCR was denatured for 10 s, at 93°C, followed by annealing and elongation for 60°C, 40 s, and 40°C, 10 s. The housekeeping gene β2-M was targeted for an internal control gene. The target gene (β1, 4-GalT I, V) data were normalized by corresponding β2-M data, and the mean value of duplicate sample extracts was used for determining the relative RNA levels. The relative RNA level was calculated as the ratio of the sample mean-value to the baseline sample mean-value. β1,4-GalT I, V and β2-M templates were amplified in separate wells.

The real time PCR of other glycosyltransferases including B4GalT-II, III, IV, VI and VII were performed, EvaGreen (Biotium Inc, Hayward, CA, USA) served as a dye that binds to amplified DNA to emit fluorescence during reactions. EvaGreen recently emerged as an optimal green fluorescent DNA dye for qPCR, which has equal or better sensitivity compared with SYBR Green I. The reaction mixture of 25 μl contained 12.5 μl of EvaGreen TM qPCR Master Mix (Biotium Inc), 1 μl of primers (10 mM) and 1 μl of template cDNA, and 10.5 μl of double distilled water. The β2-M gene served as an internal control for expression levels of target growth factor genes. Primers were synthesized to amplify a gene segment of β2-M: 5′-GTCTTTCTACATCCTGGCTCACT-3′ and antisense 5′-GACGGTTTTGGGCTCCTTCA-3′. The primers were designed based on sequences from the Genebank database. The primers for other glycosyltransferases genes are as follows; left primers were listed first followed by right primers. β1,4GalT-II: 5′-CTATTGGCTCCACTATCTGC-3′ and 5′-TGGGCTCGTTATGTTTGT-3′, β1, 4GalT-III: 5′-AGGGTTCGCCTGGCTGGGAT-3′, β1, 4GalT-IV: 5′-GGAGAACTGGGACTGCTT-3′, 5′-CACTGT GATGTTGGCGTAT-3′, β1, 4GalT-VI: 5′- ACAACCGTT CAATCGTGC-3′, 5′-CTGTTCCAAAGGTCGTCA-3′, β1, 4GalT-VII: 5′-ATGCTCCATCTTCCACCTC-3′, 5′-CTGCCCGATTGAACCTGA-3′. After an initial incubation for 15 min at 95°C, the reactions were carried out for 40 cycles at 95°C for 15 s and 60°C for 45 s (florescence collection). By setting the threshold at the level at the middle steady portion of reaction cycles versus florescence curve, the Ct values of target genes were calculated using customized software (Rotor Gene Analysis Software 6.0, Corbett Research Pty Ltd.). To account for variability in total RNA input, the expression of the transcriptions were normalized to β2-M gene to standardize comparison.

Preparation of probes for *in situ* hybridization

The probes for *in situ* hybridization were prepared by reverse transcription polymerase chain reaction (RT-PCR, KIT from TaKaRa Biotech) using rat brain total RNA. The first-strand cDNA fragments were prepared by reverse transcription using 5 μg total RNA. The cDNA fragments were amplified by PCR with sense primer 5′-TACA ACTGCTTTGTGTTCAGTGATG-3′ and antisense primer 5′-GCAGGCTAAACCCGAACTTG-3′ specific for rat β1, 4-GalT I, and with sense primer 5′-GGCATAGTGA ACACCTACCTCTT-3′ and antisense primer 5′-TTCA GGCTGCTGTTCCTCTTG -3′ for rat β1,4-GalT-V. The PCR products were sequenced by a laser fluorescent DNA sequencer (Pharmacia). RT-PCR amplified cDNA fragments of β1, 4-GalT I and V were prepared and used as the probes for *in situ* hybridization: amplified cDNA fragments were subcloned into pGEM®-T Easy (Promega). Plasmids were linearized with the restriction enzymes ApaI and SacI. The digoxigenin (DIG) labeled sense and antisense β1, 4-GalT I, V RNA probes were produced using SP6 and T7 RNA polymerase, respectively, by *in vitro* transcription according to its protocol (Boehringer Mannheim) [36]. The labeling efficiency was measured by dot blot analysis with a DIG detection kit (Boehringer Mannheim).

In situ hybridization

In situ hybridization was performed as previously described [18]. In brief, sections were incubated in 10 μg/ml proteinase K for 15 min at 37°C and treated with 0.2 M

HCl for 10 min, and 0.25% (*v*/*v*) acetic anhydride/0.1 M triethanolamine [pH 8.0] for 15 min. The sections were then prehybridized in the following solution: 50% (*v*/*v*) formamide, 10% (*w*/*v*) dextran sulfate, 5×SSC (standard saline citrate, 150 mM NaCl, 15 mM sodium citrate), 0.5% (*w*/*v*) sodium dodecyl sulfate (SDS), 5×Denhardt's reagent, and 200 μg/ml of yeast tRNA in DEPC-treated water at 42°C for 60 min and hybridized at 42°C for 16 h by adding digoxigenin-labeled sense or antisense probe in a humidified chamber. After hybridization, the slides were washed in 50% formamide /2×SSC at 42°C for 20 min three times and treated with 10 μg/ml of RNase A in buffer (10 mM Tris–HCl [pH 7.6], 500 mM NaCl, and 1 mM EDTA) at 37°C for 30 min. Washed in 2×SSC at 42°C for 20 min and in 0.2×SSC at 42°C for 20 min twice, the sections were then immersed in 7.5 U/ml of an FITC-conjugated anti-DIG- antibody (Roche).

Combined *in situ* hybridization for β1,4-GalT I, β1,4-GalT V mRNAs and immunohistochemistry for pax7

The sections were air-dried, hybridized with β1,4-GalT I or β1,4-GalT V probes (10 pmol/ml), and then rinsed for 30 min in 1×SSC, at 42°C, and in 0.2×SSC at 42°C for 20 min twice, followed by 3×10 min in 0.05 mol/L PBS at room temperature (RT). Next, the sections were preincubated in 1%BSA(bovine serum album with 0.1% Triton X-100) for 30 min at RT, followed by incubation overnight at 4°C with a monoclonal antibody directed against pax7 (R&D systems) diluted 1:400 in 1% BSA. Then, the sections were rinsed 4×10 min in 0.05 mol/L PBS at RT and incubated for 1 h at RT with TRITC-conjugated secondary antibody (1:300, Jackson immunolab) and DAPI (1:200, sigma). Finally, development of the *in situ* hybridization signal was performed as described above.

Lectin blotting

Detection of Galβ1–4GlcNAc was accomplished by using the lectin RCA I in a blotting protocol. Gastrocnemius muscles were homogenized in 10 mM Tris–HCl buffer (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA, and centrifuged at 600 g/min at 4°C for 10 min. The supernatant was then centrifuged at 8,000 g/min at 4°C for 20 min. The resulting pellet was defatted with acetone three times. The membrane proteins thus obtained were solubilized in 50 mM Tris–HCl buffer (pH 6.8) containing 5% β-mercaptoethanol and 2% sodium dodecyl sulfate (SDS) by incubating at 100°C for 5 min, and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Blots were treated with 25 mM H_2SO_4 at 80°C for 60 min to remove sialic acid residues according to the method described previously [11]. *Psathyrella velutina* lectin (PVL), which interacts with β-*N*-acetylglucosamine-terminated glycans. Then lectin blot analysis was conducted using *Ricinus communis* agglutinin (RCA)-I or PVL as described previously [22].

RCA-I lectin histochemistry

RCA-I lectin histochemistry was performed as reported previously [6, 7]. Briefly, sections were treated with 0.03 U/ml neuraminidase at 37°C for 5 h to remove sialic acid residues and incubated overnight with FITC-conjugated RCA-I in a moist chamber at 4°C. After three more washes with PBS/Tween, sections were mounted in 1:1 PBS: glycerol and coverslipped. Sections were examined by epifluorescent microscopy (Leica; Wetzlar, Germany).

Combined RCA-I lectin histochemistry and immunohistochemistry for pax7

The sections were treated with 0.03 U/ml neuraminidase at 37°C for 5 h to remove sialic acid residues and incubated overnight with FITC-conjugated RCA-I in a moist chamber at 4°C. Then the sections were preincubated in 1%BSA(bovine serum album with 0.1% Triton X-100) for 30 min at RT, followed by incubation overnight at 4°C with a monoclonal antibody directed against pax7 (R&D systems) diluted 1:400 in 1% BSA. Then, the sections were rinsed 4×10 min in 0.05 mol/l PBS at RT and incubated for 1 h at RT with TRITC-conjugated secondary antibody (1:300, Jackson immunolab) and DAPI (1:200, sigma). After three more washes with PBS/Tween, sections were mounted in 1:1 PBS: glycerol and coverslipped. Sections were examined by epifluorescent microscopy (Leica; Wetzlar, Germany).

β1,4-GalT I and β1,4-GalT V expression plasmids were injected intramuscular after sciatic nerve crush

β1,4-GalT I and β1,4-GalT V expression plasmids were constructed as previously [27, 28]. Rats were injected intramuscularly using β1,4-GalT I and β1,4-GalT V expression plasmids followed by electroporation as previously reported [13] after sciatic nerve crush on every other day.

Statistical analysis

At least three repetitive real time PCR assessments were performed, and for each assessment all groups were tested in quadruplicate. All data were given in terms of relative values and expressed as mean±standard error. One-way

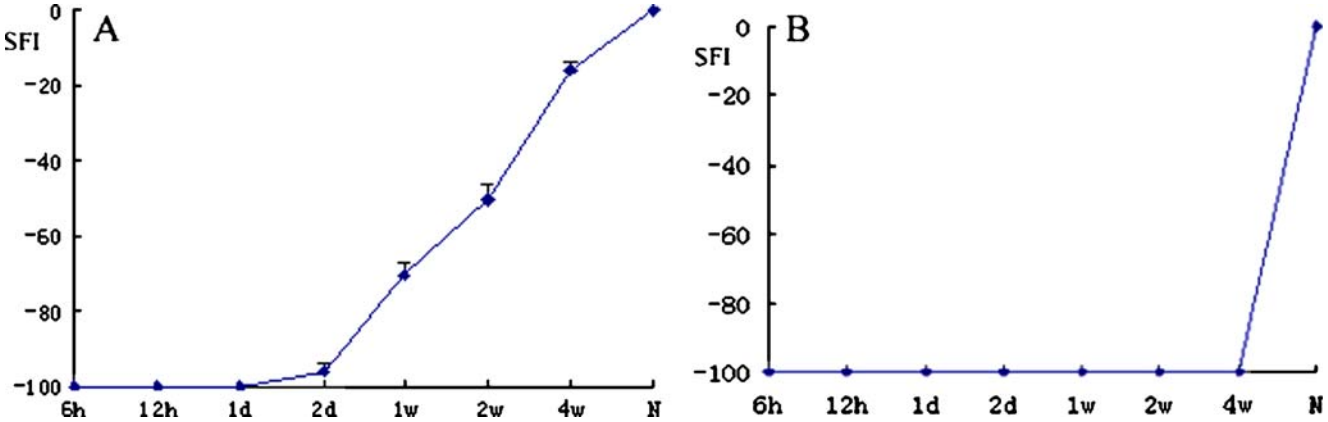


Fig. 1 The sciatic function index (SFI) values of the rats at different time points post sciatic nerve injury. **A** sciatic nerve crush model, **B** sciatic nerve transection model. *N* normal sciatic nerve, *h* hour, *d* day, *w* week

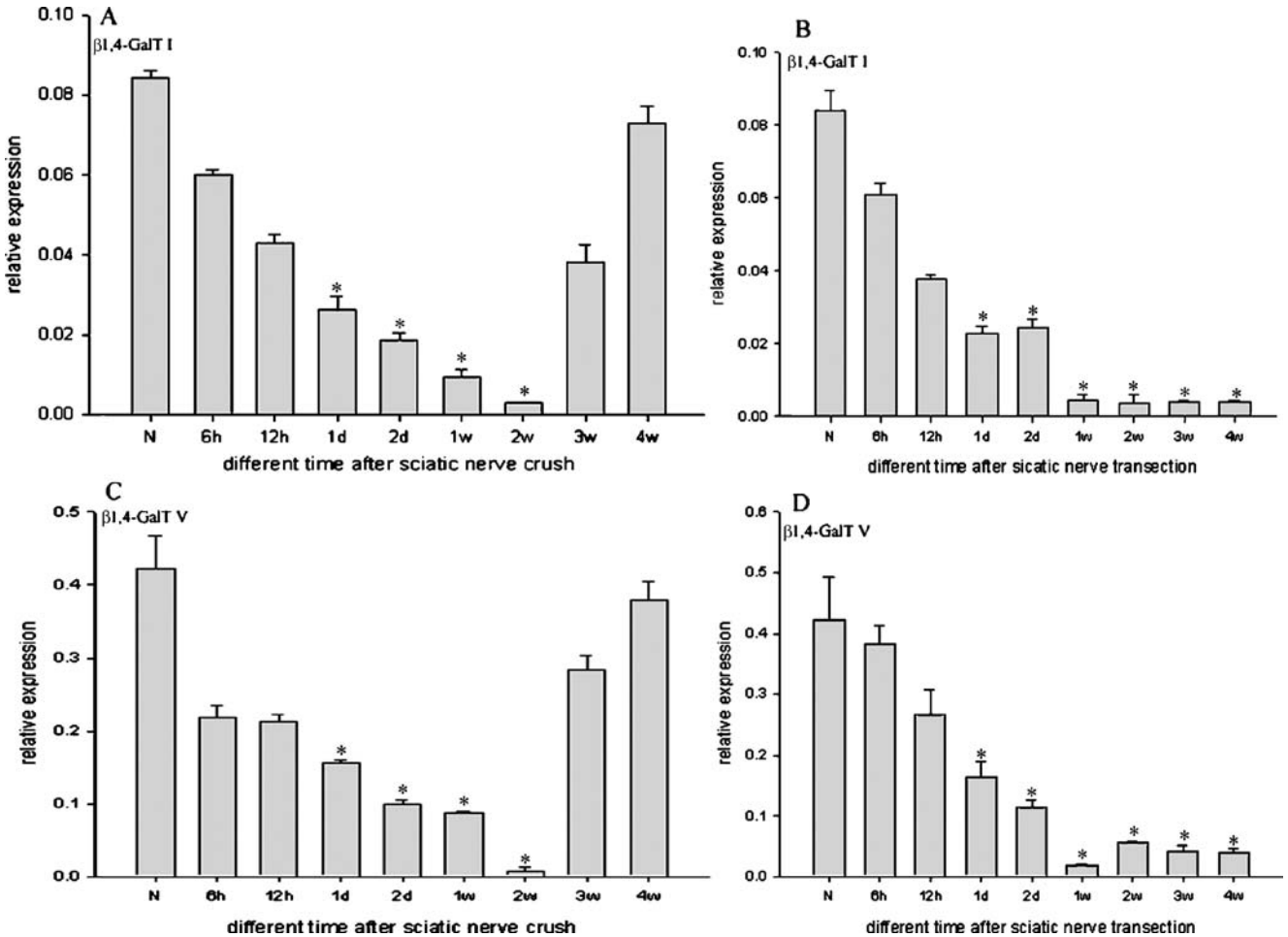


Fig. 2 Quantification of β1,4-GalT I and V mRNAs in gastrocnemius muscles at different time points post sciatic nerve injury. **A** and **C** sciatic nerve crush model, **B** and **D** sciatic nerve transection model. **A** and **B** Quantification of β1,4-GalT I mRNA in gastrocnemius muscles at different time points post sciatic nerve injury, **C** and **D** Quantification of β1,4-GalT V mRNA in gastrocnemius muscles at different time points post sciatic nerve injury. β1,4-GalT I and V transcripts were measured by real time PCR. The data (means±SEM) were presented as the ratio of β1,4-GalT I and V mRNAs to β2-M mRNA, and the experiments were tripled under the same condition. *$P<0.05$ indicates a statistically significant difference compared with any other group. *N* normal sciatic nerve, *h* hour, *d* day, *w* week

ANOVA was used to compare differences between the operated groups and the control group. All statistical analyses were conducted with a STATA 7.0 software package (Stata Corp., College Station, TX, USA), and all significance levels were set at $P<0.05$.

Results

The measurement of SFI post sciatic nerve injury

Neurological recovery was analyzed by the sciatic function index (SFI), which characterized hindlimb use and foot and toe positions, and by the pinch reflex. The SFI evaluates crucial aspects of locomotion involving recovery of hindlimb sensory and motor function, nonlesioned animals have an SFI of zero (±10). The present study showed that the SFI value was normal before lesion, -100 at 6 h, 12 h and 1 day after crushing the sciatic nerve, then increased gradually from 2 days, and reached near the normal level at 4 weeks. By contrast, SFI value was −100 at any time after sciatic nerve transaction (Fig. 1). This phenomenon showed that the sciatic nerve crush and transection model were successful and indicated that the sciatic nerve function could recover at the defined time after crush and could not recover after transection.

Quantitative analysis of β1,4-GalT I to V transcripts in rat gastrocnemius muscles by real time PCR

We determined the tissue distribution and expression levels of rat β1,4-GalT I and V transcripts by the real time PCR method, which is a sensitive and accurate assay system. The expression levels of β1,4-GalT I and V mRNAs in gastrocnemius muscles were shown as relative values to the β2-M transcript to be able to compare to each other. In sciatic nerve crush model, β1,4-GalT I mRNA expressed at a high level in normal gastrocnemius muscles and decreased gradually from 6 h, reached the lowest level at 2 weeks, then restored gradually to relatively normal level at 4 weeks after sciatic nerve crush (Fig. 2A). In contrast, in sciatic nerve transection model, β1,4-GalT I mRNA decreased gradually from 6 h, and remained on a low level at 4 weeks in gastrocnemius muscles after sciatic nerve transection (Fig. 2B). Similarly, β1,4-GalT V mRNA expressed at a high level in normal gastrocnemius muscle and decreased gradually from 6 h, reached the lowest level at 2 w, and then increased gradually to relatively normal level at 4 w after sciatic nerve crush (Fig. 2C). In addition, β1,4-GalT V mRNA decreased gradually from 6 h, and remained a low level in gastrocnemius muscles at 4 w after sciatic nerve transection like β1,4-GalT I mRNA (Fig. 2D).

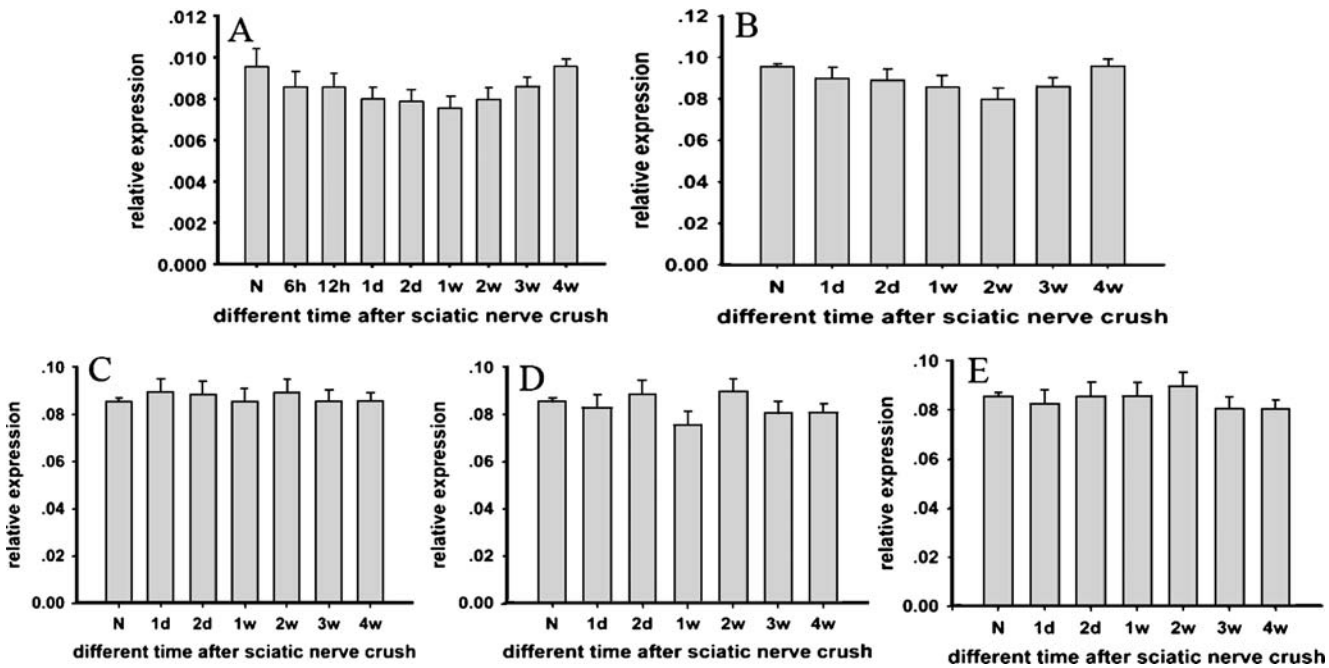


Fig. 3 Quantification of β1,4-GalT II, III, IV, VI and VII mRNAs in gastrocnemius muscles at different time points post sciatic nerve crush. **A** β1,4-GalT II, **B** β1,4-GalT III, **C** β1,4-GalT-IV, **D** β1,4-GalT VI, **E** β1,4-GalT VII. β-1,4 GalTs transcripts were measured by real time PCR. The data (means±SEM) were presented as the ratio of β1,4-GalT I and V mRNAs to β2-M mRNA, and the experiments were tripled under the same condition. *N* normal sciatic nerve, *h* hour, *d* day, *w* week

The same results were obtained in three independent experiments. These results indicated that β1,4-GalT I and V mRNAs expressions were influenced by the sciatic nerve injury, and they decreased in gastrocnemius muscles during denervation and restored gradually during reinnervation.

To verify these expression patterns are specific or not specific for β1,4GalT-I, -V mRNAs and Galβ1, 4GlcNAc group, the real time PCR of other glycosyltransferases including β1,4GalT-II, III, IV, VI and VII after sciatic nerve injury were performed, the expression levels of β1,4-GalT II, III, IV, VI and VII mRNAs in gastrocnemius muscles were shown as relative values to the GAPDH to be able to compare to each other. In sciatic nerve crush model, β1,4-GalT II, III, IV, VI and VII mRNAs expressed at similar levels in normal gastrocnemius muscles and other time points after sciatic nerve crush (Fig. 3). There were not

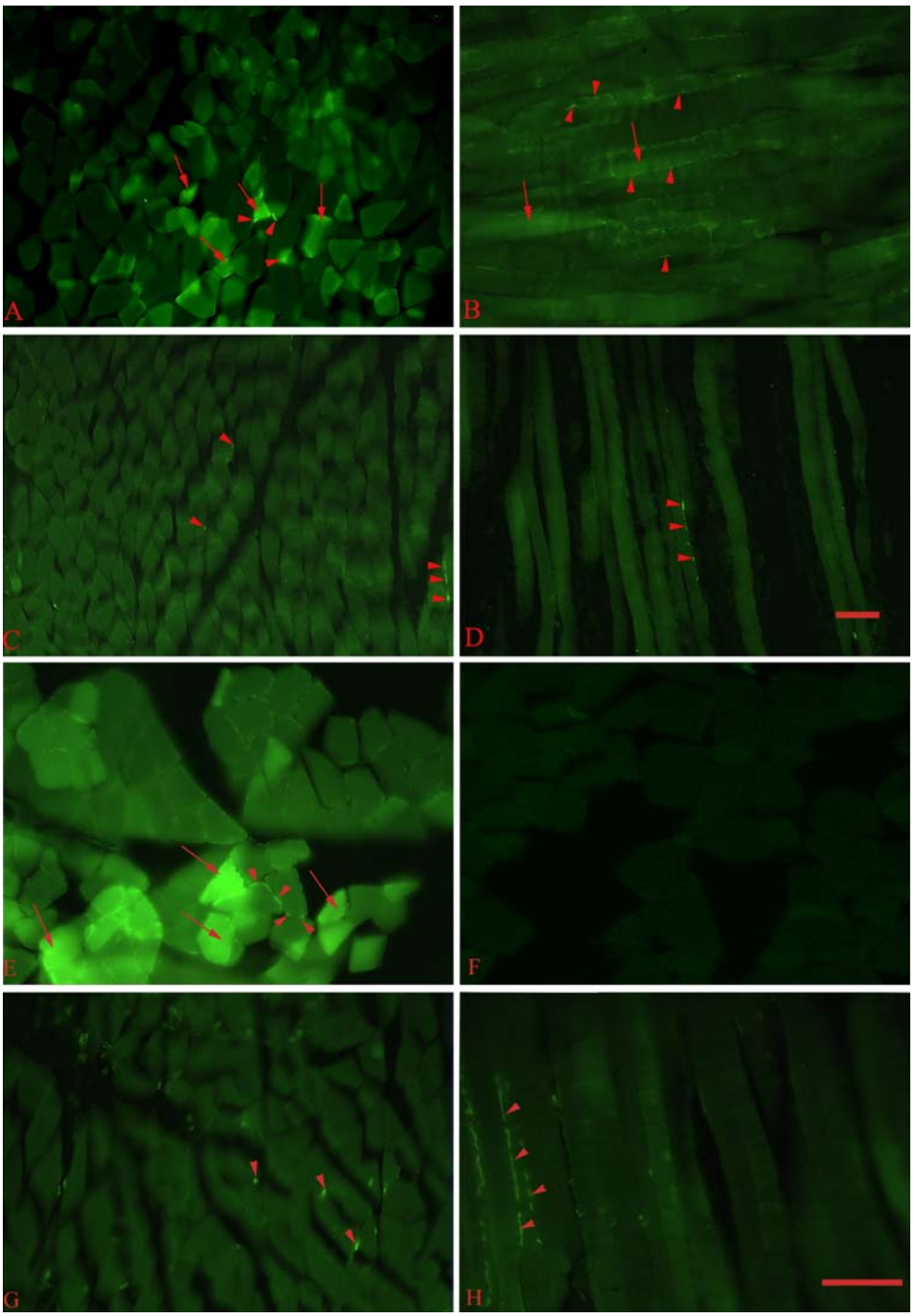


Fig. 4 The expression of β1, 4-GalT I mRNA in gastrocnemius muscles after sciatic nerve injury. **A** and **B** normal gastrocnemius muscles, **C** and **D** 2 weeks gastrocnemius muscles after sciatic nerve crush, **E** and **F** 4 weeks gastrocnemius muscles after sciatic nerve crush, **G** and **H** 4 weeks gastrocnemius muscles after sciatic nerve transection. *In situ* hybridization was performed on sections of rat gastrocnemius muscles using β1,4-GalT I cRNA probe and FITC-conjugated anti-digoxigeninantibody. *Green* represent β1,4-GalT I positive signal. *Arrows* represent myocytes, *arrowheads* represent muscle satellite cells. **A**, **C**, **E**, **F**, and **G** transverse sections, **B**, **D**, and **H** longitudinal sections, **F** digoxigenin-labeled β1,4-GalT I sense riboprobe showed background staining level. **A**, **B**, **C**, and **D** scale bars = 20 μm. **E**, **F**, **G**, and **H** scale bar = 50 μm

significant differences among every group. In sciatic nerve transection model, similar results were obtained (data not shown). These results indicated that these expression patterns of β1,4GalT-I, -V mRNAs and Galβ1, 4GlcNAc group during sciatic nerve injury are specific.

Localization of β1,4-GalT I and V mRNAs expression in gastrocnemius muscles

Due to the distinct temporal expression of β1,4-GalT I mRNA in muscles after sciatic nerve injury, β1,4-GalT I mRNA expressed at a high level in normal gastrocnemius muscles, reached the lowest level at 2 weeks, then restored to relatively normal level at 4 weeks after sciatic nerve crush, and remain a low level at 4 weeks after sciatic nerve transection. To reveal the distribution of β1,4-GalT I transcript in gastrocnemius muscles, *in situ* hybridization was performed with gastrocnemius muscles at 2 and 4 weeks after sciatic nerve crush and at 4 weeks after sciatic nerve transection to complete the analysis of β1,4-GalT I mRNA expression. The more accurate localization of β1,4-GalT I mRNA expression in muscles as studied by *in situ* hybridization was in good agreement with the general expression pattern seen with real time PCR.

β1,4-GalT I mRNA in normal and at 4 weeks after sciatic nerve crush gastrocnemius muscles was expressed widely both in the transverse and longitudinal sections, there were a lot of positive signals in gastrocnemius muscles (Fig. 4A,B,E). In contrast, the expression of β1,4-GalT I mRNA in gastrocnemius muscles at 2 weeks after sciatic nerve crush was at a low level, and only few positive cells were detected (Fig. 4C–D). Similarly, in transection model, β1,4-GalT I mRNA was expressed at a low level at 4 weeks after sciatic nerve transection (Fig. 4G–H). Digoxigenin-labeled β1,4-GalT I sense riboprobe showed background staining level (Fig. 4F).

To further verify β1,4-GalT I mRNA positive cells were muscle satellite cells, sections were subjected to combined *in situ* hybridization for β1,4-GalT I mRNA and immunohistochemistry for muscle satellite cells marker pax7. The present study showed that β1,4-GalT I mRNA positive cells were pax7 positive, which suggests that β1,4-GalT I mRNA is located in muscle satellite cells (Fig. 5).

According to the distinct temporal expression of β1,4-GalT V mRNA in gastrocnemius muscles after sciatic nerve injury, it decreased gradually and reached the lowest expression level at 2 weeks after sciatic nerve crush and 4 weeks after sciatic nerve transection. To reveal the distribution of β1,4-GalT V transcript in gastrocnemius muscles, *in situ* hybridization was performed with muscles after sciatic nerve crush 2 weeks and transection 4 weeks to complete the analysis of β1,4-GalT V expression. The more accurate localization of β1,4-GalT V mRNA expression in

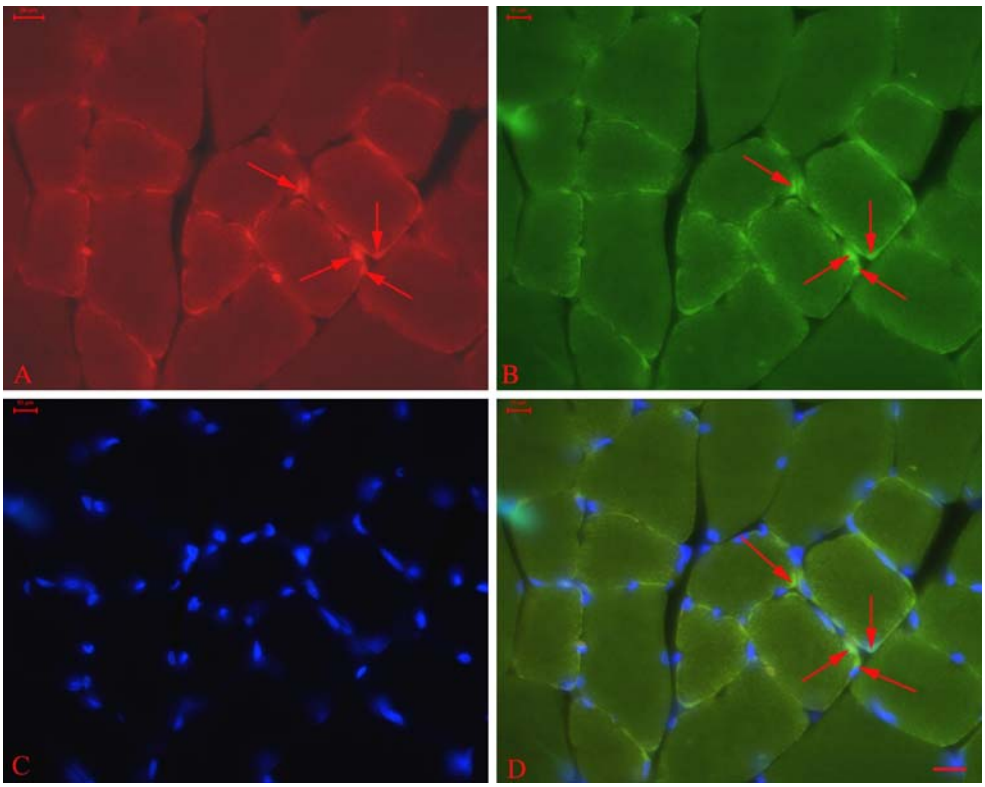


Fig. 5 The expression of β1, 4-GalT I mRNA in muscle satellite cells. Combined *in situ* hybridization and immunohistochemistry was performed on sections of rat gastrocnemius muscles at 4 weeks after sciatic nerve crush using β1,4-GalT I cRNA antisense probe and antibody pax7 specific for muscle satellite cells. **A** pax7, a marker of muscle satellite cells was stained with *red*, **B** β1,4-GalT I mRNA was stained with *green*, and **C** DAPI, a marker of nucleus was stained with *blue*. **D** merged picture of **A**, **B** and **C**. Scale bar=20 μm

gastrocnemius muscles as studied by *in situ* hybridization was in good agreement with the general expression pattern seen with real time PCR.

Similar to β1,4-GalT I, the expression of β1,4-GalT V mRNA in normal gastrocnemius muscles was widespread both in the transverse and longitudinal sections, there were a lot of positive signals in gastrocnemius muscles (Fig. 6A–B). β1,4-GalT V was expressed mainly in gastrocnemius muscles at 4 weeks after sciatic nerve crush (Fig. 6E–F), While low level of β1,4-GalT V mRNA at 2 weeks after sciatic nerve crush (Fig. 6C–D) and at 4 weeks after sciatic nerve transection (Fig. 6G–H).

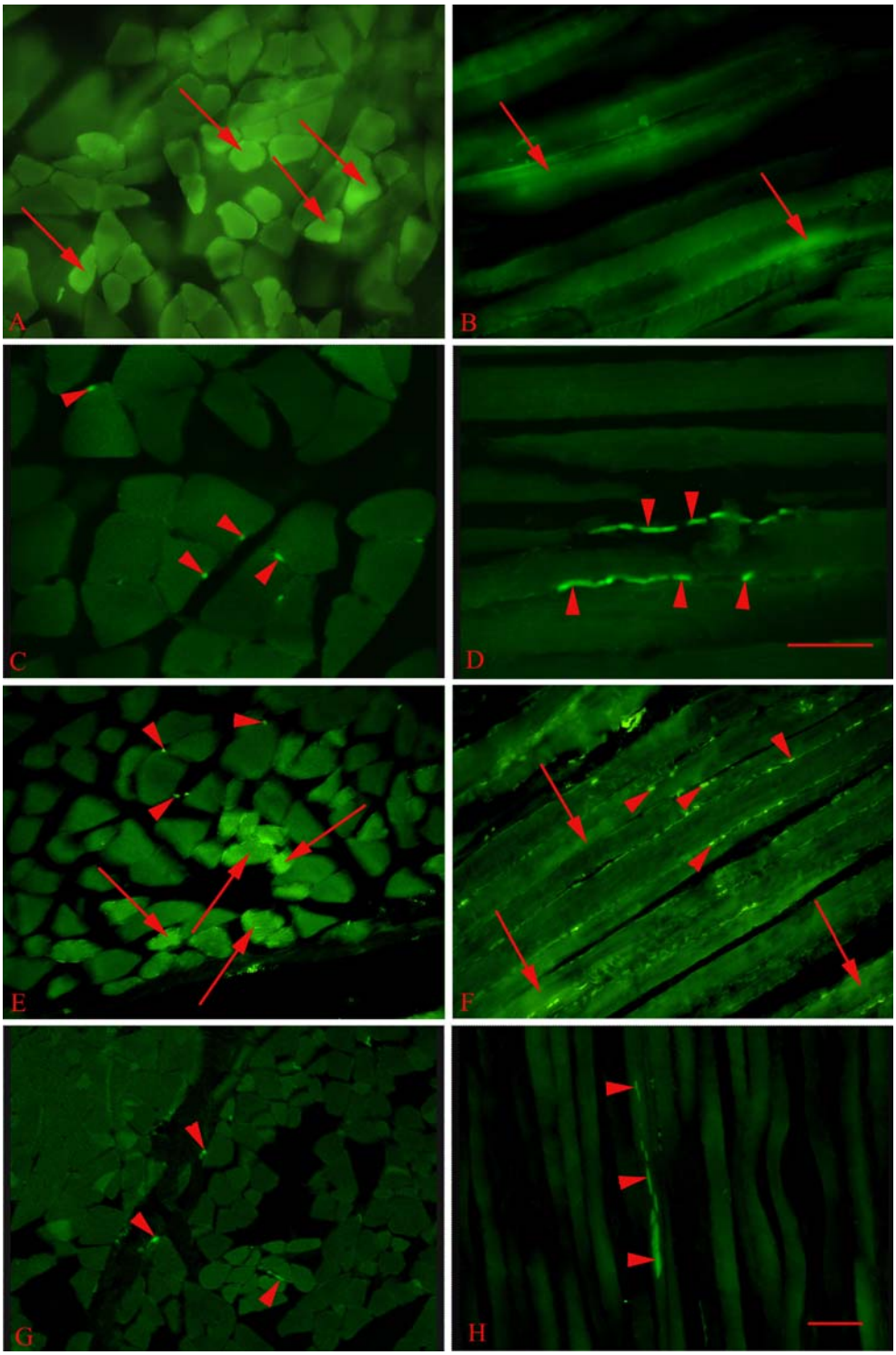


Fig. 6 The expression of β1, 4-GalT V mRNA in gastrocnemius muscles after sciatic nerve injury. **A** and **B** normal gastrocnemius muscles, **C** and **D** 2 weeks gastrocnemius muscles after sciatic nerve crush, **E** and **F** 4 weeks gastrocnemius muscles after sciatic nerve crush, **G** and **H** 4 weeks gastrocnemius muscles after sciatic nerve transection. *In situ* hybridization was performed on sections of rat gastrocnemius muscles using β1,4-GalT V cRNA probe and FITC-conjugated anti-digoxigenin antibody. *Green* represent β1, 4-GalT V positive signal. *Arrows* represent myocytes, *arrowheads* represent muscle satellite cells. **A**, **C**, **E** and **G** transverse sections, **B**, **D**, **F** and **H** longitudinal sections, **A**, **B**, **C** and **D** scale bars=50 μm. **E**, **F**, **G** and **H** scale bars=20 μm

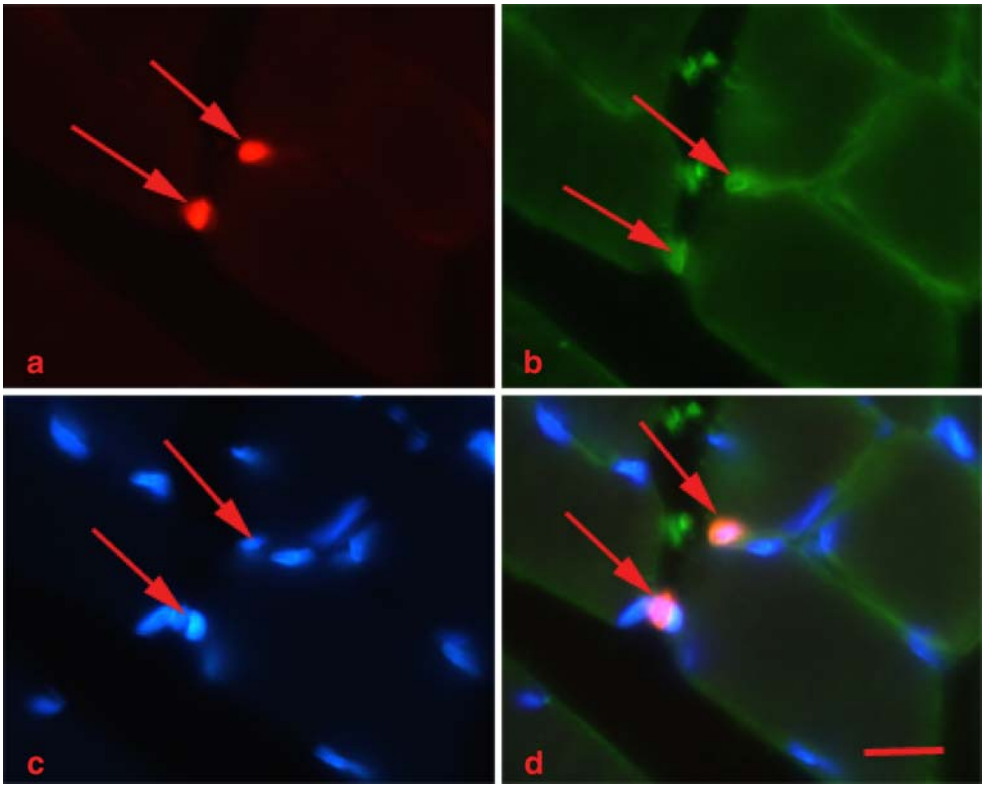


Fig. 7 The expression of β1,4-GalT V mRNA in muscle satellite cells. Combined *in situ* hybridization and immunohistochemistry was performed on sections of rat gastrocnemius muscles at 4 weeks after sciatic nerve crush using β1,4-GalT V cRNA antisense probe and antibody pax7 specific for muscle satellite cells. **A** pax7, a marker of muscle satellite cells was stained with *red*, **B** β1,4-GalT V mRNA was stained with *green*, **C** DAPI, a marker of nucleus was stained with *blue*. **D** merged picture of **A**, **B** and **C**. Scale bar=20 μm

To further verify that β1,4-GalT V mRNA positive cells were muscle satellite cells, sections were subjected to combined *in situ* hybridization for β-1,4-GalT V mRNA and immunohistochemistry for muscle satellite cells marker pax7. The present study showed that β1,4-GalT V mRNA positive cells were pax7 positive, which suggests that β1,4-GalT V mRNA is located in muscle satellite cells (Fig. 7).

Lectin blot analysis of glycoproteins in gastrocnemius muscles

Since the gene expression levels of β1,4-GalT I and V in gastrocnemius muscles changed after sciatic nerve injury, as revealed in Fig. 2, we investigated whether the galactosylation of membrane glycoproteins of gastrocnemius

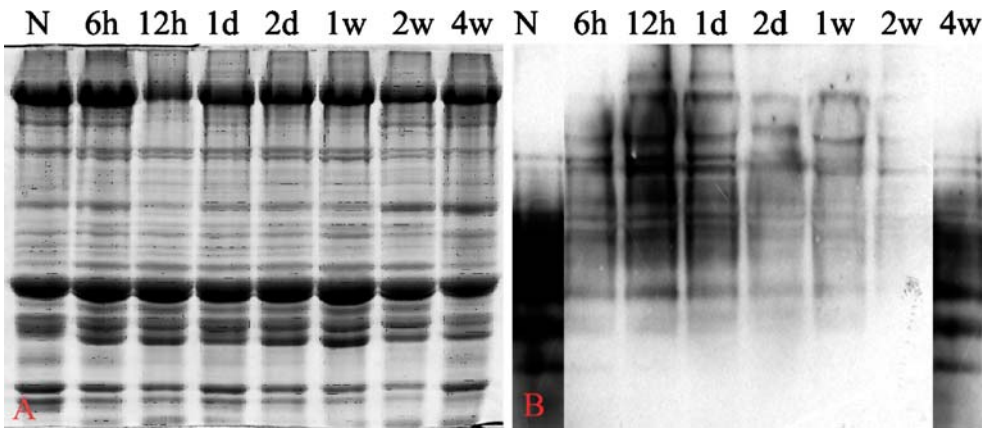


Fig. 8 Lectin blot analysis of gastrocnemius muscles Gal β-1,4GlcNAc group after sciatic nerve crush and transection. The blots were stained CBB, RCA-I after acid treatment and then visualized as described in 'Materials and methods'. Samples were prepared from normal, 6 h, 12 h, 1 day, 2 days, 1 week, 2 weeks, 4 weeks gastrocnemius muscles after sciatic nerve crush. **A** CBB staining of gastrocnemius muscles protein, **B** lectin blot analysis of gastrocnemius muscles Gal β1-4GlcNAc group

muscles also changed during this process. When blotted membranes were stained with Coomassie Brilliant Blue (CBB), they showed almost similar staining patterns (CBB in Fig. 8A), indicating that rat gastrocnemius muscles after sciatic nerve crush contain similar protein components regardless of the injury time.

Since some of the galactose residues are sialylated, blots were treated with acid to remove sialic acid residues and then incubated with peroxidase-conjugated RCA-I, which interacts with oligosaccharides terminating with the Galβ1-GlcNAc group [1]. The binding patterns of protein bands to RCA-I were different among the different samples. The overall lectin binding decreased from 6 h and reached the lowest level at 2 weeks, then restored to relatively normal level at 4 weeks after sciatic nerve crush. Since the RCA-I binding disappeared upon treatment of blots either with diplococcal β-galactosidase which cleaves the Galβ1-4GlcNAc linkage or with N-glycanase, the lectin binding observed is considered to be soley for the β1-4-linked galactose residues attached to N-linked oligosaccharides [17].

Lectin blots were performed with Psathyrella velutina lectin (PVL) which interacts with β-*N*-acetylglucosamine-terminated oligosaccharides. The binding patterns of protein bands to PVL were similar among the different samples (Fig. 9). These results indicated that β-*N*-acetylglucosamine-terminated oligosaccharides were not affected by sciatic nerve injury.

Analysis of Galβ1-4GlcNAc in gastrocnemius muscles by RCA-I lectin histochemistry

Since lectin blot showed that the expression of Galβ1-4GlcNAc changed in gastrocnemius muscles after sciatic nerve injury, RCA-I lectin histochemistry was performed to find out the distribution of Galβ1-4GlcNAc group in gastrocnemius muscles. The present study showed that Galβ1-4GlcNAc group was mainly localized in the membranes of myocytes and muscle satellite cells in normal gastrocnemius muscles (Fig. 10A–B), a few positive signals were found in some muscle satellite cells at 2 weeks after sciatic nerve crush (Fig. 10C–D), 2 and 4 weeks after sciatic nerve transection (Fig. 10G–J), and a lot of positive signals were found in gastrocnemius muscles at 4 weeks after sciatic nerve crush (Fig. 10E–F). These results indicated that Galβ1-4GlcNAc was involved in the normal and reinnervated gastrocnemius muscles.

To further verify Galβ1-4GlcNAc positive cells were muscle satellite cells, sections were subjected to combined RCA-I lectin histochemistry and immunohistochemistry for muscle satellite cells marker pax7. The present study showed that Galβ1-4GlcNAc positive cells were pax7 positive, which suggests that Galβ1-4GlcNAc is located in muscle satellite cells (Fig. 11).

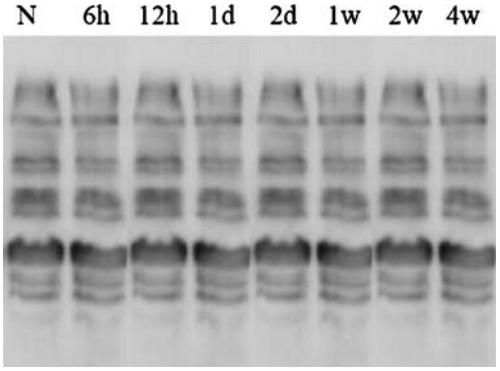


Fig. 9 PVL lectin blot analysis of gastrocnemius muscles β-*N*-acetylglucosamine-terminated oligosaccharides group after sciatic nerve crush. Samples were prepared from normal, 6 h, 12 h, 1 day, 2 days, 1 week, 2 weeks, 4 weeks gastrocnemius muscles after sciatic nerve crush

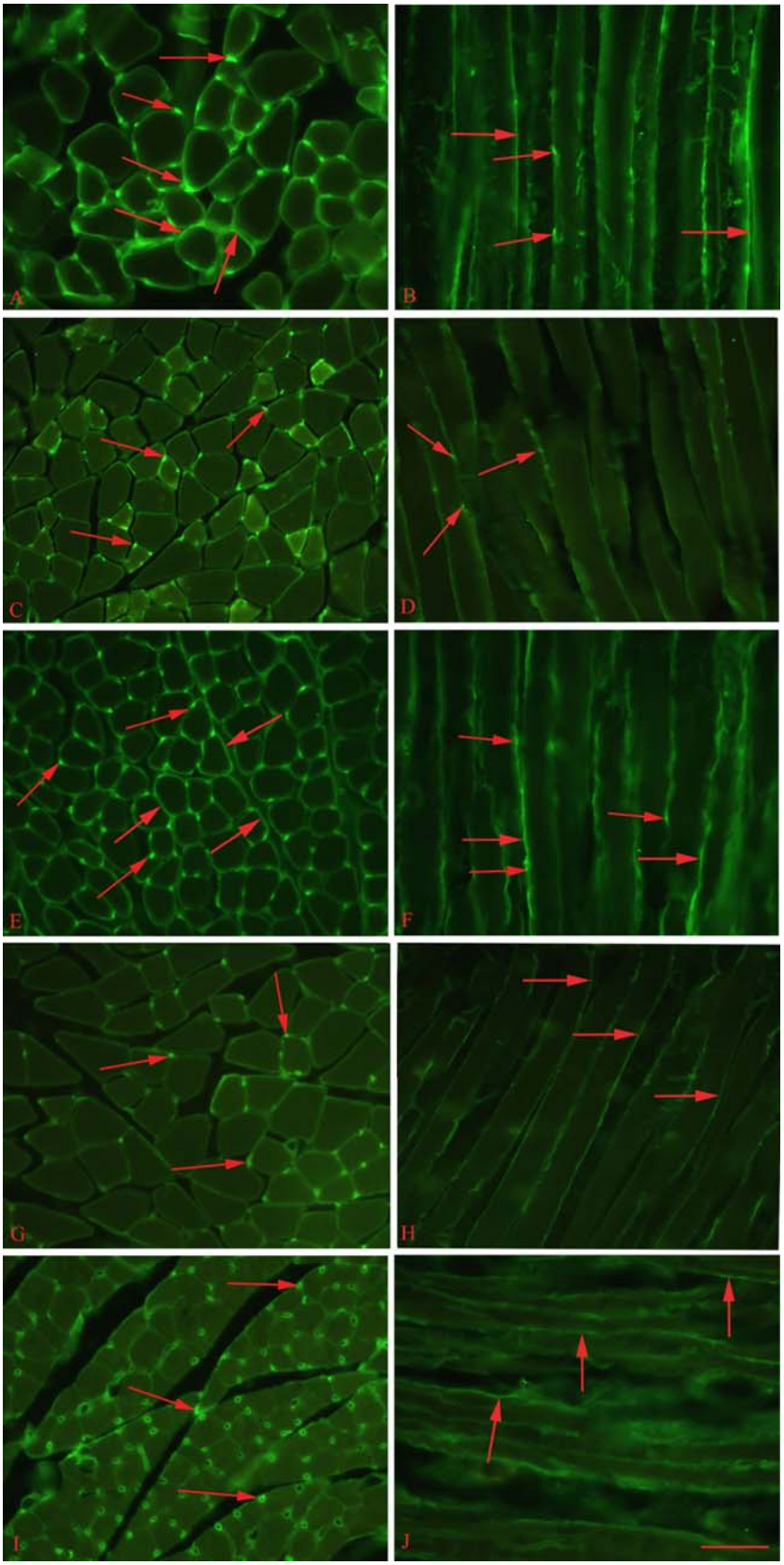
The measurement of SFI after β1,4GalT-I and V expression plasmids being injected intramuscular

To investigate the involvement of β1,4GalT-I and V in the process of muscle denervation and reinnervation SFI were measured after β1,4GalT-I or V expression plasmids being injected intramuscular during sciatic nerve crush. The present study showed that SFI value was normal before lesion, −100 at 6 h, 12 h and 1 day after crushing the sciatic nerve, then increased significantly at 2 and 3 weeks, then reached near the normal level at 4 weeks (Fig. 12). Compared with the results without β1,4-GalT-I or V expression plasmids, SFI were improved obviously after β1, 4GalT-I or V expression plasmids being injected. These results indicated that β1,4GalT-I and V were involved in the process of muscle denervation and reinnervation.

Discussion

To our knowledge, this is the first report in which the time course of expression changes of β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group in the gastrocnemius muscles

Fig. 10 The expression of Gal β1-4GlcNAc group in gastrocnemius muscles after sciatic nerve injury. **A** and **B** normal gastrocnemius muscles, **C** and **D** 2 weeks gastrocnemius muscles after sciatic nerve crush, **E** and **F** 4 weeks gastrocnemius muscles after sciatic nerve crush, **G** and **H** 2 weeks gastrocnemius muscles after sciatic nerve transection, **I** and **J** 4 weeks gastrocnemius muscles after sciatic nerve transection. RCA-I lectin histochemistry was performed on sections of rat gastrocnemius muscles after sciatic nerve injury using FITC-conjugated anti-RCA antibody. Arrows represent Gal β1-4GlcNAc group positive myocytes. **A**, **C**, **E**, **G** and **I** transverse sections, **B**, **D**, **F**, **H** and **J** longitudinal sections. Scale bars=20 μm ▶


A
B
C
D
E
F
G
H
I
J

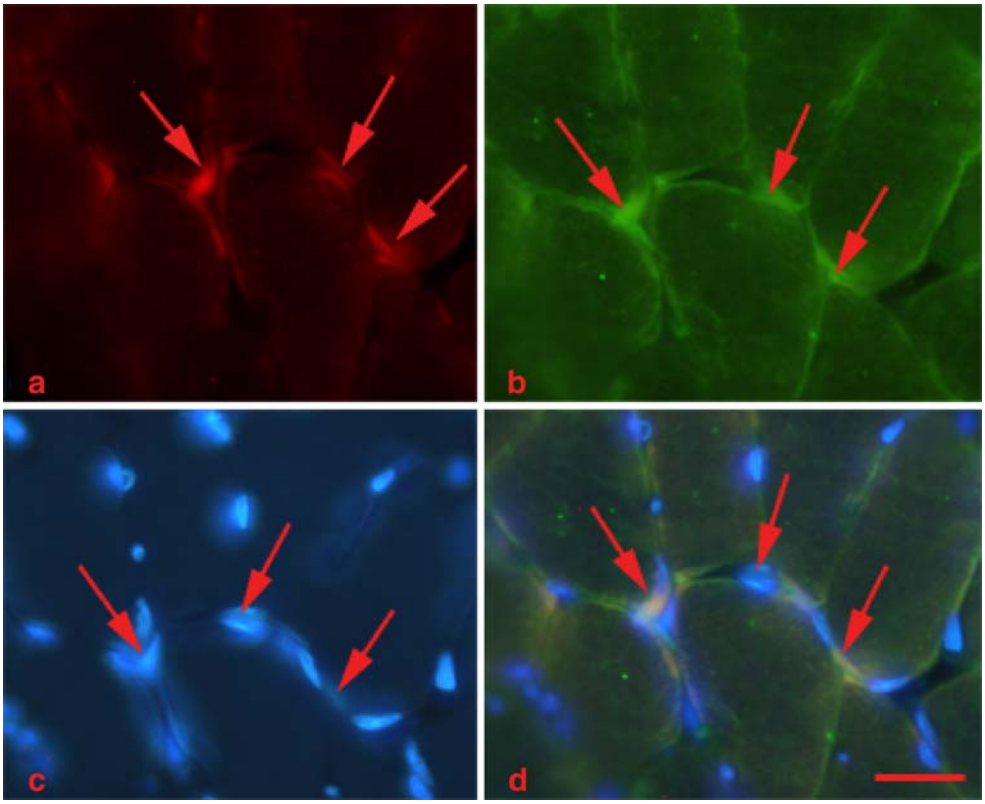


Fig. 11 The expression of Gal β1-4GlcNAc group in muscle satellite cells. RCA-I lectin histochemistry and immunohistochemistry for pax7 was performed on sections of rat gastrocnemius muscles at 4 weeks after sciatic nerve crush. **A** pax7, a marker of muscle satellite cells was stained with *red*, **B** Gal β1-4GlcNAc group was stained with green, and **C** DAPI, a marker of nucleus was stained with *blue*. **D** merged picture of **A**, **B** and **C**. Scale bar=20 μm

after SD rat sciatic nerve injury. Previous researches have shown that pathological changes in peripheral nerve system including damages, inflammation, tumor and denaturation could result in acute, subacute or chronic denervation, which is in turn responsible for the atrophy of target muscles [5, 34]. But the molecular mechanisms of muscular atrophy, due to denervation remain to be elucidated. The purpose of this study was to follow up the time course of changes of β1,4-GalT I and V in the rat gastrocnemius muscles after irreversible denervation induced by sciatic nerve transection and reversible denervation induced by sciatic nerve crush and during the subsequent period of self-reinnervation.

The sciatic nerve crush, with the continuity of the nerve preserved, allows for complete axonal return and restoration of the nerve-muscle interaction. This leads to muscle regeneration. In contrast, the sciatic nerve transection, with continuity of the nerve destroyed, which results in muscle denervation. In the present study, we found that a complete dysfunction of the

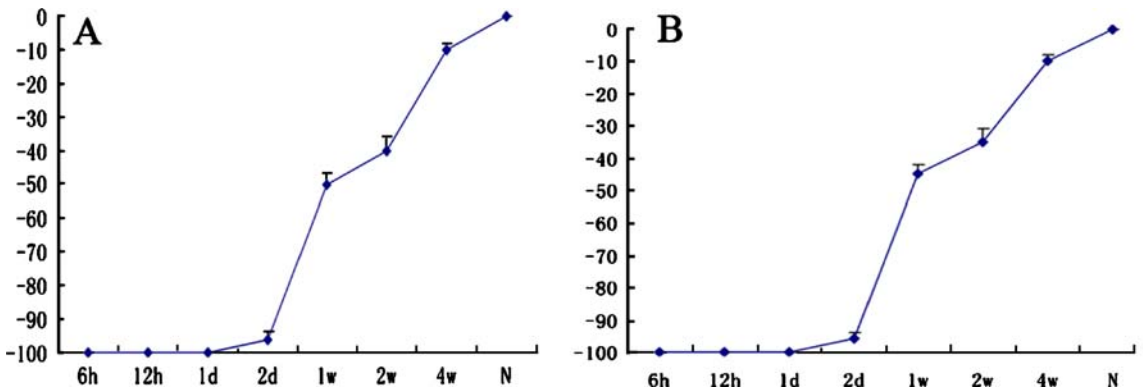


Fig. 12 The sciatic function index (SFI) values of the rats after β1,4-GalT-I and V expression plasmids were directly introduced into rat gastrocnemius muscles at different time points post sciatic nerve crush. **A** β1,4-GalT-I expression plasmids were injected intramuscular after sciatic nerve crush, **B** β1,4-GalT-V expression plasmids were injected intramuscular after sciatic nerve crush. *N* normal sciatic nerve, *h* hour, *d* day, *w* week

sciatic nerve was observed from 6 h to1 day after sciatic nerve crush in terms of the SFI value of −100, then recovered gradually to relatively normal level at 4 weeks. The result is agreement with recent reports showing that a complete dysfunction of the sciatic nerve was observed immediately following sciatic nerve crush in terms of the SFI value of −100 [33]. Afterwards, a steady recovery of locomotive function of the crushed sciatic nerve of the rats was demonstrated in the light of continuous increases in the SFI value until the SFI value of about −16 was reached at 4 weeks post sciatic nerve crush [33]. In contrast, a complete dysfunction of sciatic nerve was investigated from 6 h to 4 weeks after sciatic nerve transection in terms of the SFI value of −100. These results demonstrated that the sciatic nerve crush and transection models were established successfully, and suggested that sciatic nerve crush could lead to sciatic nerve regeneration, but the sciatic nerve transection only resulted in muscles denervation.

Our findings showed that β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group decreased immediately in gastrocnemius muscles after sciatic nerve crush, reached the lowest level at 2 weeks, and then restored gradually to relatively normal level at 4 weeks. By contrast, in the sciatic nerve transection model, β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group decreased from 6 h gradually, and reached a low level at 4 weeks. These results indicated that the expression levels of β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group were involved in the sciatic nerve injury. That is, the expression levels of β1,4-GalT I, V mRNAs and Galβ1,4GlcNAc group decreased in gastrocnemius muscles during denervation, and restored in gastrocnemius muscles during reinnervation. These results indicated that the expression levels of β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group were related to the innervation of muscles. It suggests that upregulating β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group can probably inhibit or delay the skeletal muscle atrophy induced by denervation before the regenerated nerve reached the target organ. This might be of importance in regaining the innervation of the skeletal muscle and maintaining its normal morphology and functions. This study also renders assistance to a thorough understanding of the molecular mechanisms for denervation-induced muscle atrophy and to the development of an effective therapy for denervated muscle diseases.

In sciatic nerve crush model, β1,4-GalT II, III, IV, VI and VII mRNAs expressed at similar levels in normal gastrocnemius muscles and other time points after sciatic nerve crush, which indicated that expression patterns of β1,4-GalT-I and V mRNAs during sciatic nerve injury are specific.

Lectin blotting showed that the higher molecular weight proteins were stained with RCA-I after sciatic nerve crush compared with normal and recovered (4 weeks) stages, which indicated that higher molecular weight proteins containing Galβ1-4GlcNAc groups played important role in the process during sciatic nerve injury. In addition, Lectin blots were performed with Psathyrella velutina lectin (PVL), which interacts with β-*N*-acetylglucosamine-terminated oligosaccharides. The similar binding patterns of protein bands to PVL demonstrated that β-*N*-acetylglucosamine-terminated oligosaccharides were not affected by sciatic nerve injury. These results indicated that expression pattern of Galβ1-4GlcNAc group is specific after sciatic nerve injury.

Muscle satellite cells are primarily quiescent in skeletal muscle, can self-renew [3] and, upon activation, proliferate and further differentiate to become fusion-competent myoblasts and ensure muscle regeneration [4]. Muscle satellite cells can promote a better functional recovery of the atrophic muscle during the reinnervation after denervation [8]. In the present study, β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group were expressed in a few muscle satellite cells at 2 weeks after sciatic nerve, and high levels of β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group were observed in numerous satellites cells of normal gastrocnemius muscle and at 4 weeks after sciatic nerve crush. These results indicated that activated muscle satellite cells after sciatic nerve injury induced expression of β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group, which suggested that β1,4-GalT I, V mRNAs and Galβ1-4GlcNAc group were probably involved in the muscle regeneration.

To investigate the involvement of β1,4-GalT I and V in the process of muscle denervation and reinnervation SFI were measured after β1,4-GalT-I or V expression plasmids being injected intramuscular during sciatic nerve crush. The present study showed that SFI were improved obviously after β1,4 GalT-I or V expression plasmids being injected. These results indicated that β1,4-GalT-I and V were involved in the process of muscle denervation and reinnervation.

To summarize, this study provides the first investigation of expression patterns of β1,4-GalT I, V mRNAs and Galβ1–4GlcNAc group in the rat gastrocnemius muscles during denervation-reinnervation. Our results would be beneficial to an elucidation of molecular mechanisms of denervation-induced muscle atrophy and reinnervation-induced muscle regeneration. This study also raises new possibilities of developing potential targets for therapeutic intervention in skeletal muscle atrophy after nerve injury. These findings suggest that the intact innervation is necessary for maintenance of normal β1,4-GalT I, V mRNAs and Galβ1–4GlcNAc group in gastrocnemius muscles, and upregulation is probably aid to the regeneration of gastrocnemius muscles.

Acknowledgements This work was supported by National Nature Science Foundation of China (Grant No. 30300099, 30772242, 30770488), Natural Science Foundation of Jiangsu Province (BK2003035, BK2006457).

References

1. Baenziger, J.U., Fiete, D.: Structural determinants of Ricinus communis agglutinin and toxin specificity for oligosaccharides. J. Biol. Chem. **254**, 9795–9799 (1979)
2. Bain, J.R., Mackinnon, S.E., Hunter, D.A.: Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast. Reconstr. Surg. **83**, 129–138 (1989)
3. Collins, C.A., Olsen, I., Zammit, P.S., Heslop, L., Petrie, A., Partridge, T.A., Morgan, J.E.: Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell **122**, 289–301 (2005)
4. Ehrhardt, J., Morgan, J.: Regenerative capacity of skeletal muscle. Curr. Opin. Neurol. **18**, 548–553 (2005)
5. Eisenberg, H.A., Hood, D.A.: Blood flow, mitochondria, and performance in skeletal muscle after denervation and reinnervation. J. Appl. Physiol. **76**, 859–866 (1994)
6. Ertl, C., Wrobel, K.H.: Distribution of sugar residues in the bovine testis during postnatal ontogenesis demonstrated with lectin-horseradish peroxidase conjugates. Histochemistry **97**, 161–171 (1992)
7. Furukawa, K., Sato, T.: Beta-1,4-galactosylation of N-glycans is a complex process. Biochim. Biophys. Acta. **1473**, 54–66 (1999)
8. Hawke, T.J., Garry, D.J.: Myogenic satellite cells: physiology to molecular biology. J. Appl. Physiol. **91**, 534–551 (2001)
9. Hayashi, Y., Ikata, T., Takai, H., Takata, S., Sogabe, T., Koga, K.: Time course of recovery from nerve injury in skeletal muscle: energy state and local circulation. J. Appl. Physiol. **82**, 732–737 (1997)
10. Huang, Q., Shur, B.D., Begovac, P.C.: Overexpressing cell surface beta 1.4-galactosyltransferase in PC12 cells increases neurite outgrowth on laminin. J. Cell Sci. **108**, (Pt 2), 839–847 (1995)
11. Kijimoto-Ochiai, S., Hatae, T., Katagiri, Y.U., Okuyama, H.: Microheterogeneity and oligosaccharide chains on the beta chains of HLA-DR, human major histocompatibility complex class II antigen, analyzed by the lectin-nitrocellulose sheet method. J. Biochem. (Tokyo) **106**, 771–777 (1989)
12. Kruse, J., Mailhammer, R., Wernecke, H., Faissner, A., Sommer, I., Goridis, C., Schachner, M.: Neural cell adhesion molecules and myelin-associated glycoprotein share a common carbohydrate moiety recognized by monoclonal antibodies L2 and HNK-1. Nature **311**, 153–155 (1984)
13. Kusumanto, Y.H., Mulder, N.H., Dam, W.A., Losen, M., De Baets, M.H., Meijer, C., Hospers, G.A.: Improvement of *in vivo* transfer of plasmid DNA in muscle: comparison of electroporation versus ultrasound. Drug Deliv. **14**, 273–277 (2007)
14. Liedtke, S., Geyer, H., Wuhrer, M., Geyer, R., Frank, G., Gerardy-Schahn, R., Zahringer, U., Schachner, M.: Characterization of N-glycans from mouse brain neural cell adhesion molecule. Glycobiology **11**, 373–384 (2001)
15. Lu, Q., Shur, B.D.: Sperm from beta 1,4-galactosyltransferase-null mice are refractory to ZP3-induced acrosome reactions and penetrate the zona pellucida poorly. Development **124**, 4121–4131 (1997)
16. Ma, D., Connors, T., Nothias, F., Fischer, I.: Regulation of the expression and phosphorylation of microtubule-associated protein 1B during regeneration of adult dorsal root ganglion neurons. Neuroscience **99**, 157–170 (2000)
17. Nakamura, N., Yamakawa, N., Sato, T., Tojo, H., Tachi, C., Furukawa, K.: Differential gene expression of beta-1,4-galactosyltransferases I, II and V during mouse brain development. J. Neurochem. **76**, 29–38 (2001)
18. Nam, S.Y., Fujisawa, M., Kim, J.S., Kurohmaru, M., Hayashi, Y.: Expression pattern of phospholipid hydroperoxide glutathione peroxidase messenger ribonucleic acid in mouse testis. Biol. Reprod. **58**, 1272–1276 (1998)
19. Nemeth, P.A., Cope, T.C., Kushner, S., Nemeth, P.M.: Spatial arrangement and metabolic capacity of fiber types in self-reinnervated cat muscle. Am. J. Physiol. **264**, C411–C418 (1993)
20. Nemeth, P.M., Turk, W.R.: Biochemistry of rat single muscle fibres in newly assembled motor units following nerve crush. J. Physiol. **355**, 547–555 (1984)
21. Sasaki, N., Manya, H., Okubo, R., Kobayashi, K., Ishida, H., Toda, T., Endo, T., Nishihara, S.: Beta4galt-II is a key regulator of glycosylation of the proteins involved in neuronal development. Biochem. Biophys. Res. Commun. **333**, 131–137 (2005)
22. Sato, T., Furukawa, K., Greenwalt, D.E., Kobata, A.: Most bovine milk fat globule membrane glycoproteins contain asparagine-linked sugar chains with GalNAc beta 1–>4GlcNAc groups. J. Biochem. (Tokyo) **114**, 890–900 (1993)
23. Sato, T., Furukawa, K., Bakker, H., Van den Eijnden, D.H., Van Die, I.: Molecular cloning of a human cDNA encoding beta-1,4-galactosyltransferase with 37% identity to mammalian UDP-Gal: GlcNAc beta-1,4-galactosyltransferase. Proc. Natl. Acad. Sci. U. S. A. **95**, 472–477 (1998)
24. Shaper, N.L., Wright, W.W., Shaper, J.H.: Murine beta 1,4-galactosyltransferase: both the amounts and structure of the mRNA are regulated during spermatogenesis. Proc. Natl. Acad. Sci. U. S. A. **87**, 791–795 (1990)
25. Shaper, N.L., Shaper, J.H., Meuth, J.L., Fox, J.L., Chang, H., Kirsch, I.R., Hollis, G.F.: Bovine galactosyltransferase: identification of a clone by direct immunological screening of a cDNA expression library. Proc. Natl. Acad. Sci. U. S. A. **83**, 1573–1577 (1986)
26. Shen, A., Wang, H., Zhang, Y., Yan, J., Zhu, D., Gu, J.: Expression of beta-1,4-galactosyltransferase II and V in rat injured sciatic nerves. Neurosci. Lett. **327**, 45–48 (2002)
27. Shen, A., Zhu, D., Ding, F., Zhu, M., Gu, X., Gu, J.: Increased gene expression of beta-1,4-galactosyltransferase I in rat injured sciatic nerve. J. Mol. Neurosci. **21**, 103–110 (2003a)
28. Shen, A., Yan, J., Ding, F., Gu, X., Zhu, D., Gu, J.: Over-expression of beta-1,4-galactosyltransferase I in rat Schwann cells promotes the growth of co-cultured dorsal root ganglia. Neurosci. Lett. **342**, 159–162 (2003b)
29. Shi, X., Amindari, S., Paruchuru, K., Skalla, D., Burkin, H., Shur, B.D., Miller, D.J.: Cell surface beta-1,4-galactosyltransferase-I activates G protein-dependent exocytotic signaling. Development **128**, 645–654 (2001)
30. Shur, B.D.: Cell surface beta 1,4 galactosyltransferase: twenty years later. Glycobiology **1**, 563–575 (1991)
31. Shur, B.D., Evans, S., Lu, Q.: Cell surface galactosyltransferase: current issues. Glycoconj. J. **15**, 537–548 (1998)
32. Siconolfi, L.B., Seeds, N.W.: Mice lacking tPA, uPA, or plasminogen genes showed delayed functional recovery after sciatic nerve crush. J. Neurosci. **21**, 4348–4355 (2001)
33. Sun, H., Liu, J., Ding, F., Wang, X., Liu, M., Gu, X.: Investigation of differentially expressed proteins in rat gastrocnemius muscle during denervation-reinnervation. J. Muscle Res. Cell Motil. **27**, 241–250 (2006)

34. White, K.K., Vaughan, D.W.: Age effects on cytochrome oxidase activities during denervation and recovery of three muscle fiber types. Anat. Rec. **230**, 460–467 (1991)
35. Winer, J., Jung, C.K., Shackel, I., Williams, P.M.: Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes *in vitro*. Anal. Biochem. **270**, 41–49 (1999)
36. Xian, C.J., Zhou, X.F.: Neuronal-glial differential expression of TGF-alpha and its receptor in the dorsal root ganglia in response to sciatic nerve lesion. Exp. Neurol. **157**, 317–326 (1999)
37. Zhang, D., Liu, M., Ding, F., Gu, X.: Expression of myostatin RNA transcript and protein in gastrocnemius muscle of rats after sciatic nerve resection. J. Muscle Res. Cell Motil. **27**, 37–44 (2006)