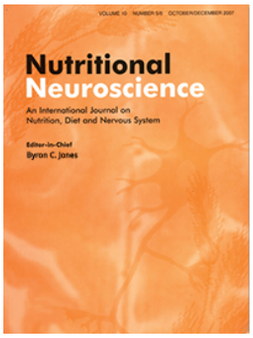

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Neutrophil peptide-1 promotes the repair of sciatic nerve injury through the expression of proteins related to nerve regeneration

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ABSTRACT

Objectives Small-molecule polypeptide neutrophil peptide 1 (NP-1) was reported to promote the regeneration of the sciatic nerve after denervation, but the mechanisms underlying this effect of NP-1 are unclear. Here, we established a Sprague-Dawley rat model of crush injury to study the effect of a single intermuscular injection of NP-1 on the repair of injured peripheral nerves and elucidate the possible underlying mechanism.

Methods 39 rats were randomly selected to join this study and divided into the blank control group (normal group, n=9), experimental group (NP-1 group, n=15), and negative control group (NS group, n=15). The dynamic expression of cytokines in different groups of nerve tissues during Wallerian degeneration was observed using protein chips at different time points after injury. Recovery of injured nerves was determined based on the general condition, local gross morphology of the nerve suture site, sciatic nerve function index, neuroelectrophysiology, and osmic acid staining at 6 weeks after the surgery. The recovery of effector function was determined based on wet weight, hematoxylin-eosin staining, modified Gomori staining, and nicotinamide adenine dinucleotide-tetrazolium reductase staining at 6 weeks after the surgery.

Results It was found that a single topical administration of NP-1 promoted sciatic nerve regeneration after crush injury and affected the expression of proteins related to neurotrophy, inflammation, cell chemotaxis, and cell generation pathways.

KEYWORDS
Peripheral nerve injury repair; NP-1; protein chip; functional study; schwann cells; nerve regeneration; inflammatory factors; peptides

1. Introduction

The peripheral nerve trunk and its branches are often injured when subjected to direct or indirect external forces and their injury manifests as motor, sensory, and autonomic nervous dysfunctions, which are very common symptoms of orthopedic diseases [1,2]. The principle objective of treating peripheral nerve injury is to restore nerve continuity as soon as possible. However, traditional surgical methods do not repair peripheral nerve injury to a satisfactory degree. In recent years, researchers have chosen non-coding RNA [3] and macromolecules [4] as potential therapeutic targets and have achieved some success. As a result, various bioactive substances have attracted attention in the field of peripheral nerve injury repair [5,6]. Administration of neurotrophic substances via injection, oral administration, or gastric lavage maintains their biological activity on injured peripheral nerves to promote regeneration and repair through various pathways [7,8].

Neutrophil peptide 1 (NP-1) is synthesized and secreted by neutrophils in addition to being stored in their azurophilic granules [9]. Upon stimulation of neutrophils, NP-1 is secreted and interacts with other cells or antigens [10]. NP-1 has powerful antiviral and antimicrobial activities [11–13] and is strongly associated with inflammation and cytotoxicity [14,15]. Additionally, it has been confirmed through several studies that NP-1 can increase the regeneration speed and excitatory conductivity of nerve fibers [16]. Our own previous studies have confirmed that continuous intermuscular

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injection of NP-1 can promote the repair and regeneration of the sciatic nerve in rats after sciatic nerve injury [17]. This small-molecule polypeptide has thus attracted increasing attention in the field of peripheral nerve injury repair.

At present, the mechanism through which NP-1 repairs injured peripheral nerves is unknown. Therefore, the current study aimed to elucidate the mechanism of sciatic nerve repair and regeneration by NP-1 after injury using a Sprague–Dawley rat model of crush injury. Particularly, we measured the expression of nerve growth factors, chemokines, pro-inflammatory factors, and monocyte-producing agents in the rat model after treatment with NP-1.

2. Materials and methods

2.1. Experimental animals

Thirty-nine 6-week-old female Sprague–Dawley rats with a definite genetic background (Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) and weighing 140–180 g were randomly selected and housed in specific-pathogen-free cages of the Experimental Animal Center of People's Hospital of Peking University, China. They were kept in individual cages at 24 ± 2°C and 50–55% relative humidity under a 12-h light/dark cycle. Standard pellet feed and clean water were provided *ad libitum*. The experimental procedure and rat treatment followed the regulations of laboratory animal management. This study was approved by the Ethics Committee of People's Hospital of Peking University, China (approval No. 2015-50) on 9 December 2015.

2.2. Animal models

The rats were randomly divided into the blank control group (normal group, $n = 9$), experimental group (NP-1 group, $n = 15$), and negative control group (NS group, $n = 15$). The right sciatic nerve was exposed along the intermuscular space after anesthesia by gas (5% isoflurane, 1.5 ml/min) in the left lateral decubitus position of rats in all three groups. After dissociation, the sciatic nerve was clamped by a hemostatic forceps for 30 s in the NP-1 and NS groups, while the wounds of the rats in the normal group were closed immediately. The muscle was sutured using 4-0 double-needle sutures. Then, 500 µl of 10 µg/ml NP-1 or an equal volume of saline was intermuscularly injected using a 1-ml syringe into the NP-1 and NS groups, respectively. The site was disinfected with iodophor after the incision was closed. Damaged sciatic nerves at the same site from three rats each of the NP-1, NS, and normal groups were harvested at 1, 3, and 5 days after operation for the protein chip experiment. The remaining rats were sampled at the same location 6 weeks after operation.

2.3. Post-injury observation

The animals were fed routinely after operation. General conditions, including activity, diet, wound healing, movement of the affected limb, and presence of foot ulcers, were observed and recorded weekly. At 6 weeks after model establishment, the repaired sciatic nerve was exposed, and the local nerve morphology and adhesion of surrounding soft tissue were observed.

2.4. Sciatic functional index (SFI)

The rats walked through a self-made glass channel and their gaits were recorded using a digital camera. The left and right hind foot footprints of each rat were detected when they walked. The following parameters were measured: (1) print length (PL): the longest distance between subsequent footprints; (2) toe spread (TS): the distance between the first toe and the fifth toe; (3) and intermediary toe spread (IT): the distance between the second toe and the fourth toe. Using the right foot as experimental (E) data and the left foot as normal (N) data, three factors were calculated: (1) print length factor (PLF) = (EPL − NPL) / NPL; (2) toe spread factor (TSF) = (ETS − NTS) / NTS; (3) and intermediary toe spread factor (ITF) = (EIT − NIT) / NIT. The values of factors were incorporated into the following Bain-Mackinnon-Hunter (BMH) formula to calculate the SFI: $SFI = -38.3\ (PLF) + 109.5\ (TSF) + 13.3\ (ITF) - 8.8$ [17].

2.5. Electrophysiology

Six weeks after operation, rats were sacrificed via intraperitoneal injection of 1% pentobarbital sodium (30 mg/kg), the right sciatic nerve of the rats was exposed at the clamp, and the nerve conduction velocities of the tibial and common peroneal nerve were measured after repair. Stimulating electrodes were placed at two points far from the clamp, and recording electrodes were inserted into the middle of the corresponding innervating muscles. The motor nerve conduction velocity was measured by the muscle compound action potential method. The following electrical stimulation parameters of the Synergy electrophysiology instrument (Oxford, USA) were set: square-wave stimulation intensity of 0.9 mA, wave width of 0.1 ms, and frequency of 1 Hz.

Compound muscle action potentials were recorded. The latency of compound muscle action potentials obtained through stimulation of the distal and proximal nerve trunks was recorded; the difference (dt) between the two latency periods was calculated; the length of nerve trunks (dl) between the distal and proximal stimulating points was measured; and the motor nerve conduction velocity was calculated as follows: $V = \mathrm{d}l/\mathrm{d}t$ [17].

2.6. Muscle wet weight

The right anterior tibial muscle was harvested, and its innervating nerve was cut off. The deep fascia covering the muscle surface was stripped, and the surface blood was wiped off. The wet weight of the anterior tibial muscle was weighed using an electronic analytical balance and recorded.

2.7. Osmic acid staining

The tibial nerve and common peroneal nerve at the distal end of the sciatic nerve at the clamp site were fixed with 4% paraformaldehyde and washed with running water. After being dipped in 1% osmic acid for 8–12 h, the nerve was washed with running water, dehydrated in a graded alcohol series, and then permeabilized. After paraffin embedding, the sample was serially sliced into 5 μm-thick sections. These sections were permeabilized, mounted, and photographed under an optical microscope (Olympus, Tokyo, Japan). Three clear nerve fibers from each section of the nerves were randomly selected for measuring the thickness of the myelin sheath, axon diameter, and fiber diameter and the average values were calculated.

2.8. Anterior tibial muscle staining

The anterior tibial muscle was harvested, cut, embedded in gum tragacanth, stored at −80°C, and then sliced into 7–8 μm thick frozen sections. The samples were treated with hematoxylin–eosin stain, modified Gomori stain, and nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) stain. For hematoxylin–eosin staining, the sections were fixed with cold acetone solution for 1 min, washed with water, stained with hematoxylin for 2 min, washed with water, differentiated with 1% hydrochloric acid alcohol, washed with water, stained with 1% eosin for 1–2 min, washed with water, permeabilized, and mounted. For modified Gomori staining, the sections were not fixed and stained with hematoxylin for 5 min, washed with running water, differentiated, and then washed with running water. When sections presented blue color, they were washed with running water, dyed with Gomori staining solution for 10 min, differentiated with working fluid (distilled water:Gomori differentiation solution = 4:1 ratio) for 90 s, permeabilized, and then mounted. In case of NADH-TR staining, the sections were not fixed and stained in NADH-TR staining solution directly for 15 min in a 37°C water bath, followed by application of 60% acetone, 90% acetone, 60% acetone, and finally tap water. The sections were mounted with glycerol gelatin. After dyeing, the morphological characteristics of muscle fibers were observed under the optical microscope.

2.9. Protein chip

We divided the functions of target proteins loaded onto the GSR-CYT-3 protein chip (Raybiotech) into four groups: (1) neurotrophy-related protein factors, such as b-nerve growth factor (b-NGF), (2) protein factors directly related to inflammation, such as interleukin (IL)-1β, IL-10, IL-13, TCK-1, and vascular endothelial growth factor (VEGF), (3) chemotaxis-related protein factors, such as B7-2, CINC-2, CINC-3, fractalkine, and LIX, and (4) cell generation-related protein factors, such as tissue inhibitor of matrix metalloproteinase 1 (TIMP-1).

Rat nerve tissue was treated with a Raybiotech kit, according to the manufacturer's instructions. The protein samples were extracted and quantified using the bicinchoninic acid assay. Diluted samples were added and the wells of the chips were sealed one by one. All samples were placed on the horizontal shaker at 4°C and 70 rpm overnight. The samples in each well were removed the following day, washed, shaken, and purified by centrifugation. Cy3-Streptavidin was added to each well. After incubation for 1 h in the dark, the samples were washed and centrifuged. The chip was scanned using a GenePix 4000B chip scanner (Molecular Devices, Palo Alto, CA, USA) [18].

2.10. Statistical analysis

SPSS 20.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. SFI, neurohistological analysis, electrophysiological analysis, and b-NGF expression results were compared between the two groups by one-way analysis of variance. IL-1β, IL-10, IL-13, TCK-1, VEGF, B7-2, CINC-2, CINC-3, fractalkine, LIX, and TIMP-1 expression levels were compared between the two groups by independent sample t-tests. Measurement data were expressed as mean ± standard deviation (SD). Results were considered statistically significant at $P < 0.05$. In the protein chip experiment, the data from the NS and NP-1 groups were compared simultaneously. When the

ratio at a given time point for the NS and NP-1 groups increased or decreased by 15% or more, there was considered to be a significant difference between the two groups at this time point [19].

3. Results

3.1. General conditions

All rats survived and were in good condition. The rats ate and drank water normally post the surgery; there was no fever or inflammation, such as redness or swelling around the incision site, no infection was detected at the incision site or anywhere else in the body, and there were no other complications during the study. The crush injury rat model was successfully established. At 6 weeks after model establishment, compared with the normal group, varying degrees of muscle atrophy were observed upon the innervation of the model rats in the NP-1 and NS groups before sacrifice. Although the repaired nerve tissues were evenly connected, nerve adhesions appeared at the incision site.

3.2. SFI

The absolute value of SFI was higher in both the NP-1 and NS groups than in the normal group, and the SFI was significantly lower in the NP-1 group than in the NS group ($P = 0.046$; Table 1).

3.3. Nerve conduction velocity

The conduction velocities of the right tibial nerve and common peroneal nerve in the NP-1 and normal groups were higher than those in the NS group. The conduction velocity of the tibial nerve in the NP-1 group was significantly higher than that in the NS group ($P = 0.011$). However, the conduction velocity of the common peroneal nerve in the NP-1 group was not significantly different from that in the NS group ($P = 0.057$; Table 1).

Table 1. Measures of functional indicators in different groups.

Indicator	NP-1 group	NS group	Normal group
Sciatic functional index	−9.33 ± 4.27	−20.96 ± 5.60*	−4.68 ± 4.70
Tibial nerve conduction velocity (m/s)	25.93 ± 3.42	17.70 ± 1.56*	69.63 ± 5.08
Common peroneal nerve conduction velocity (m/s)	26.28 ± 2.06	19.63 ± 4.42	66.57 ± 5.80
Myelin sheath thickness of tibial nerve (μm)	1.26 ± 0.07	0.96 ± 0.16*	2.86 ± 0.14
Myelin sheath thickness of common peroneal nerve (μm)	1.45 ± 0.07	1.13 ± 0.13*	2.39 ± 0.16
Axon diameter of tibial nerve (μm)	3.11 ± 0.21	2.83 ± 0.31	5.22 ± 0.25
Axon diameter of common peroneal nerve (μm)	3.43 ± 0.94	2.96 ± 0.12	4.69 ± 0.25
Tibial nerve fiber diameter (μm)	5.63 ± 0.08	4.74 ± 0.62	10.93 ± 0.16
Common peroneal nerve fiber diameter (μm)	6.33 ± 1.08	5.22 ± 0.38	9.48 ± 0.08
Anterior tibial muscle wet weight (g)	0.47 ± 0.07	0.40 ± 0.04*	0.46 ± 0.02

*$P < 0.05$, NS group vs. NP-1 group.

3.4. Neurohistological observation

The myelin sheath thickness of medullated nerve fibers, axon diameter, and nerve fiber diameter of the two groups of rats that underwent surgery were smaller than those of the normal group. There was no significant difference in axon diameter and nerve fiber diameter between the NP-1 and NS groups, although the thickness of the myelin sheath in the NP-1 group was significantly greater than that in the NS group ($P = 0.038$ and $P = 0.019$ of myelin sheath thickness of tibial nerve and common peroneal nerve, respectively; Table 1, Figure 1).

3.5. Muscle wet weight and histological observations

The anterior tibial muscle was significantly heavier in the NP-1 group than in the NS group ($P = 0.042$), but there was no significant difference between the weights of the anterior tibial muscles of the NP-1 group and the normal group ($P = 0.714$) (Table 1, Figure 2). The results of muscle fiber staining showed that the cross-sectional area of muscle fibers in the NP-1 group was similar to that in the NS group and that there were no significant differences in staining among the NP-1, NS, and normal groups (Figure 2).

3.6. Cytokine expression

The expression of the neurotrophy-related protein factor b-NGF peaked on the first day post-surgery and then decreased (Table 2, Figure 3).

The expression levels of the inflammation related protein factors IL-1β, IL-13, and TCK-1 were highest on the third day and then decreased in the NP-1 and NS groups. The expression of IL-10 in the NS group decreased after the third day, while that in the NP-1 group continued to increase. The expression of VEGF in the NS group began to decrease after the third day, while that in the NP-1 group began to decrease after the first day (Table 2, Figure 4).

The expression levels of the chemotaxis-related protein factors B7-2 and fractalkine increased continuously from the first day in the NP-1 group, while they began to decrease in the NS group on the third day. The expression levels of CINC-3 and LIX in the NS

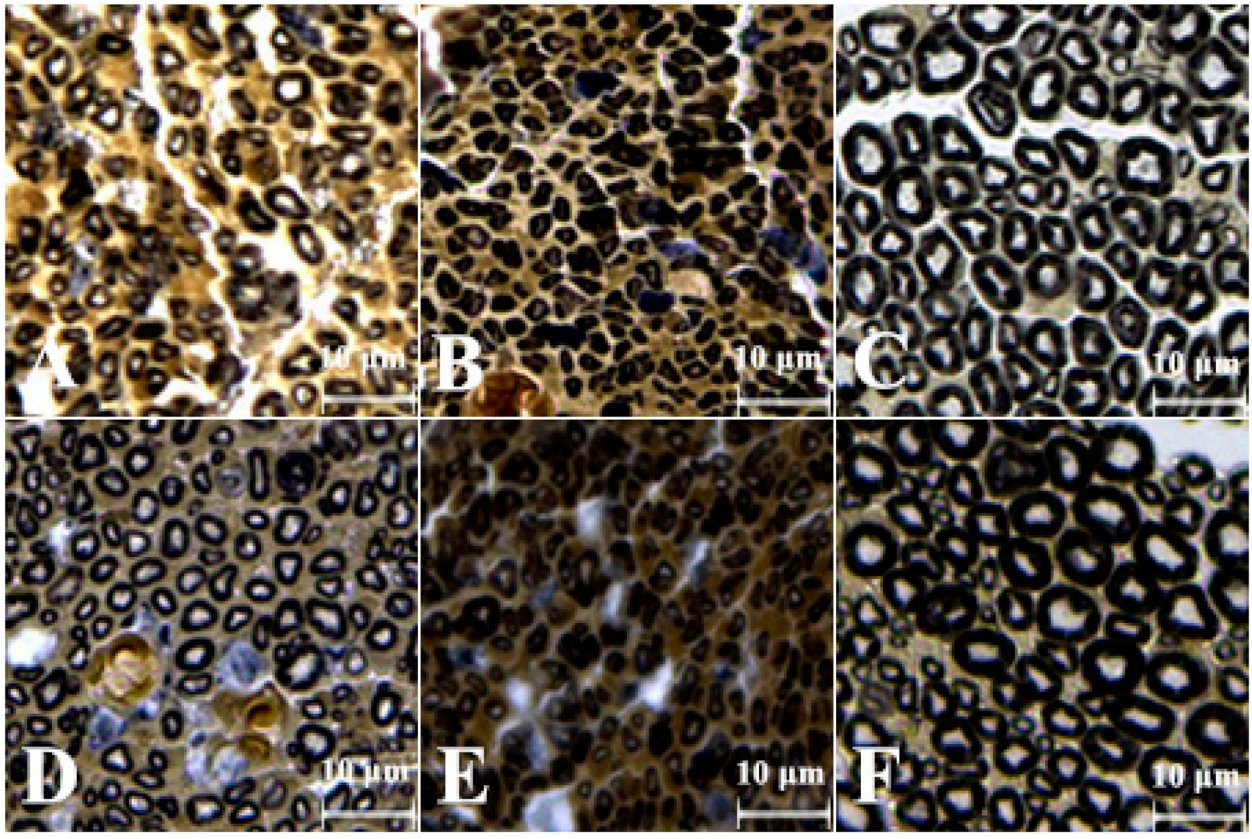

Figure 1. Neurohistological observations. Tibial nerve and common peroneal nerve in the (A, D) NP-1-injected group, (B, E) NS group (saline-injected negative control group), and (C, F) normal group.

group peaked on the third day, and then decreased. The expression of CINC-3 in the NP-1 group showed the same trend as that in the NS group, but the expression of LIX peaked on the first day and then decreased. Lastly, CINC2 expression peaked on the first day and declined over the course of the experiment in both groups (Table 2, Figure 5). Expression of the cell generation-related protein factor TIMP-1 increased continuously in both groups (Table 2, Figure 6).

4. Discussion

Peripheral nerve injury is common in the field of trauma, and the current treatment outcomes for it are not favorable. Peripheral nerve injury often causes limb dysfunction or disability, placing a heavy burden on patients and their families [20]. In order to improve outcomes for patients, it is crucial to restore the continuity of injured nerves as soon as possible. Traditional drug therapies have had some success in achieving this. However, with the continued development of new technology, researchers have discovered additional therapies that can better promote the repair of injured peripheral nerves. Defensins, which are small bioactive molecular peptides, have been widely used for the treatment of peripheral nerve injury. NP-1, an alpha-defensin, is of particular interest to researchers in the field of nerve injury, as our previous study [17] showed that continuous injection of NP-1 into the gluteus maximus muscle can promote the repair and regeneration of injured sciatic nerves. Researchers have also found that NP-1 can improve the regeneration rate and conductivity of nerve fibers during early stages of sciatic nerve regeneration [16]. To further study the effect of a single topical administration of NP-1 on the repair of peripheral nerve injury, we established a crush injury model of sciatic nerve injury in rats and observed the effect of a single intermuscular injection of NP-1 on the functional recovery of injured nerves.

We intermuscularly injected 10 μg/mL NP-1 in rats with sciatic nerve injury, which allowed for significant contact between NP-1 and the injured sciatic nerve. This method is simple, avoids multiple trauma caused by continuous injection, and is easy to realize clinically. At 6 weeks after NP-1 injection, we found that, compared with the NS group, the conduction velocities of the tibial nerve and common peroneal nerve increased after distal bifurcation of the injured sciatic nerve. Moreover, the myelin sheaths of both the tibial and common peroneal nerves were thicker and the axon

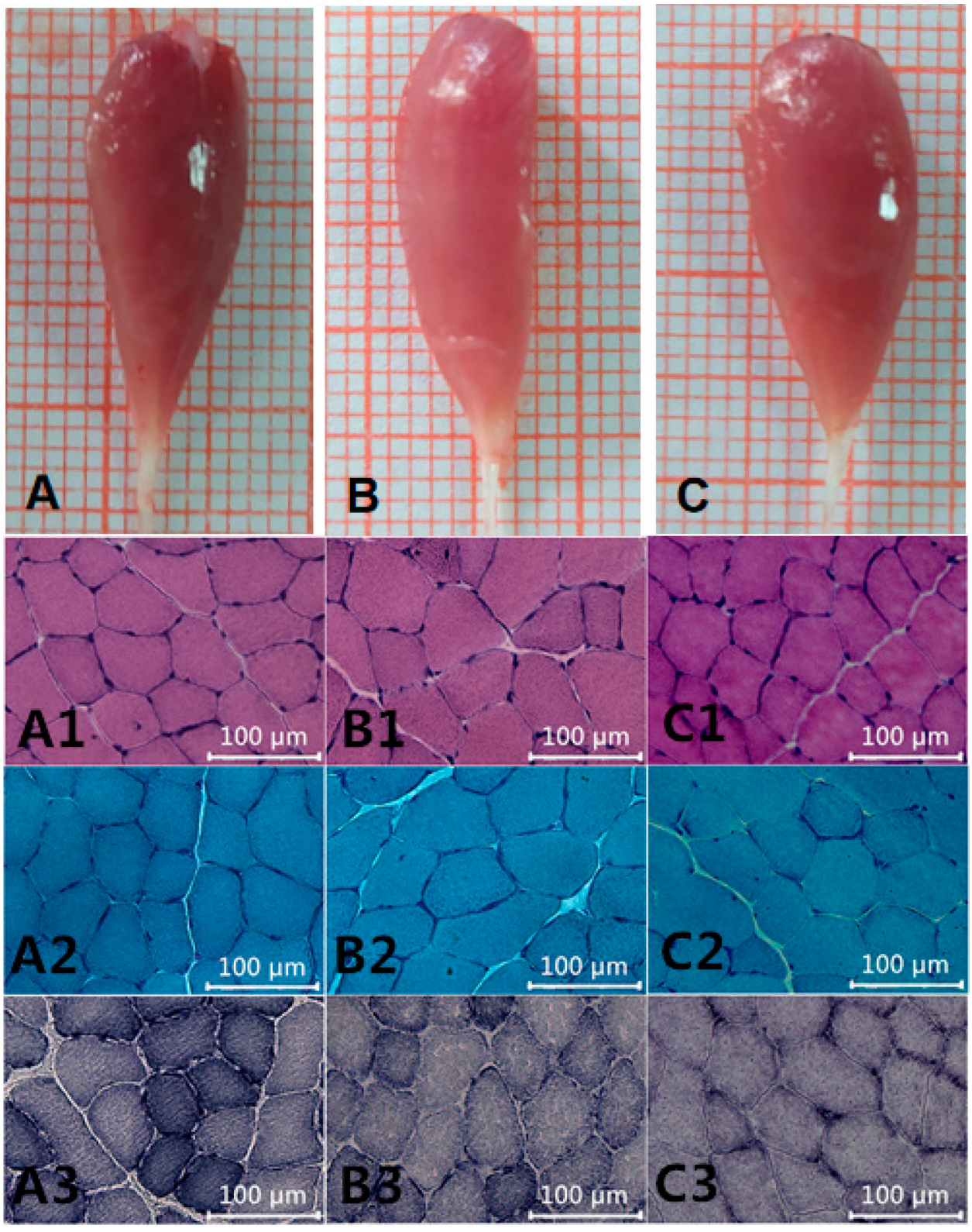

Figure 2. Histological analysis of the anterior tibial muscle. Anterior tibial muscle in the (A) NP-1 group, (B) NS group (saline-injected negative control group), and (C) normal group. A1-C1: HE staining of the muscle fibers. A2-C2: Gomori staining of the muscle fibers. A3-C3: NADH-TR staining of the muscle fibers.

and fiber diameters of the tibial and common peroneal nerves were restored. SFI was closer to normal in the group treated with NP-1 than in the NS group. Although the anterior tibial muscle in the NP-1-injected group did not demonstrate any difference from that in the normal or NS-1 group in stained

Table 2. Changes in the expression of neural repair -related protein factors at different time point.

Protein	1 d	3 d	5 d
b-NGF	15.80%*	32.17%*	48.38%*
IL-1β	8.08%	16.10%*	−0.28%
IL-10	2.48%	−8.51%	33.40%*
IL-13	−1.90%	−17.03%#	27.69%*
TCK-1	35.73%*	−6.47%	2.00%
VEGF	−12.89%	−66.59%#	−69.57%#
B7-2	−4.55%	−0.50%	30.42%*
CINC-2	−20.34%#	−31.45%#	−63.49%#
CINC-3	−37.06%#	−27.99%#	7.83%
Fractalkine	−8.26%	−13.25%	47.32%*
LIX	14.54%	3.13%	16.45%*
TIMP-1	55.71%*	7.55%	−21.12%#

Notes: The value in Table 2 means the ratio of the difference between the NP-1 group and the NS group to the NP-1 group.
*Increased by more than 15%.
#Reduced by more than 15%.

sections, the wet weight of the anterior tibial muscle in the NP-1-injected group was greater in the NP-1 injected group. Thus, after sciatic nerve injury, a single intermuscular injection of 10 μg/mL NP-1 in the muscle gap could promote the regeneration of the sciatic nerve in 6 weeks and was beneficial to the recovery of nerve and effector muscle function.

The NP-1 used in this study was isolated from the polymorphonuclear neutrophils of rabbits, which has a sequence similar to that of human NP-1-3 [21,22]. NP-1 is important for resisting pathogenic microorganisms and regulating the inflammatory process, and thereby plays an important role in nerve-related diseases. After peripheral nerve injury, the expression of pro-inflammatory factors, such as IL-1β and TNF, rises rapidly. NP-1 regulates the expression of pro-inflammatory factors, such as TNF-α and IFN [23]. Sciatic nerve function is impaired when pro-inflammatory factors are increased, which may be associated with the inhibition of

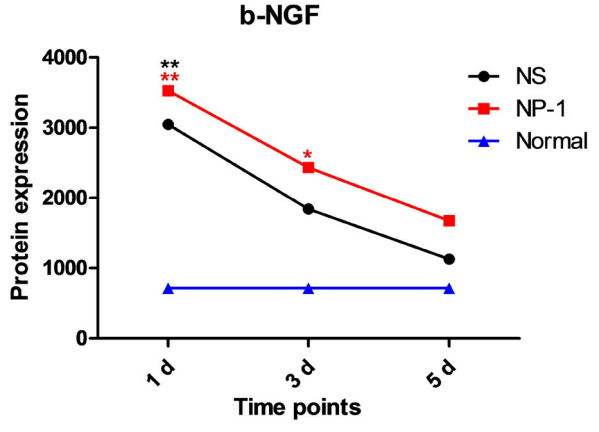

Figure 3. Expression of the neurotrophy-related protein factor. Changes in the expression of neurotrophy-related protein factor at different time point should refer to Table 2. Asterisk (*) indicates comparison with the normal group. *$P < 0.05$; **$P < 0.01$.

neutrophil infiltration [24]. At the initial stage of Waller's degeneration, pro-inflammatory factors can promote the recruitment of macrophages, after which the expression of the related pro-inflammatory factors is down-regulated. In this way, pro-inflammatory factors coordinate with each other to complete the regeneration of injured axons [25]. Siqueira et al. confirmed that proper regulation of the inflammatory response after peripheral nerve injury is essential for axonal regeneration and functional recovery [26]. In this process, the recruitment of macrophages can remove the myelin tissue at the lesion site [27] to relieve adverse effects, which inhibit the regeneration of the myelin sheath [28]. The regulation of pro-inflammatory factors and immune processes by NP-1 may be responsible for causing these downstream effects and promoting the repair of peripheral nerve injury.

In this study, b-NGF as well as inflammation-related factors such as IL-1β, IL-10, IL-13, TCK-1 and VEGF were increased after the sciatic nerve injury. The protein b-NGF is an important neurotrophic factor that plays an important role in regulating nerve growth [29]. IL-1β, IL-10, and IL-13 belong to the interleukin family of proteins, which is one of the families most widely studied for its role in stimulating inflammation or anti- or pro-inflammation responses to many diseases [30–32]. TCK-1, also known as CXCL7, is associated with the inflammation of neurons in the brain [33]. VEGF is also strongly associated with inflammation. Inflammatory reactions induced by hypoxia can increase the secretion of VEGF [34]. Inflammation induced by IL-1β can also lead to changes in the levels of pro-inflammatory factors, such as VEGF and IL-6. The sciatic nerve injury also led to increased expression of chemotaxis-related proteins such as B7-2, CINC-2/3, fractalkine and LIX. B7-2 (CD86) is an important costimulatory molecule that participates in the immune response and signal transduction of primary B lymphocytes [35]. Increased expression of B7-2 may participate in the secretion of pro-inflammatory factors, such as TCP-1 and TNF-α, through the presenting function of macrophages [31]. Both CINC-2 and CINC-3 are neutrophil chemokines that can promote the migration and adhesion of neutrophils. Expression at the site of injury may be the main factor affecting the infiltration of neutrophils [36], which are important effectors of acute inflammation [37]. Fractalkine is also an important chemokine [38] that participates in the migration and activation of lymphocytes and phagocytes and regulates pro-inflammatory factors in gram-negative bacterial infection-related diseases [39]. LIX is a chemokine, also known as CXCL5, that plays a regulatory role in inflammation and cancer cell growth caused by cancer

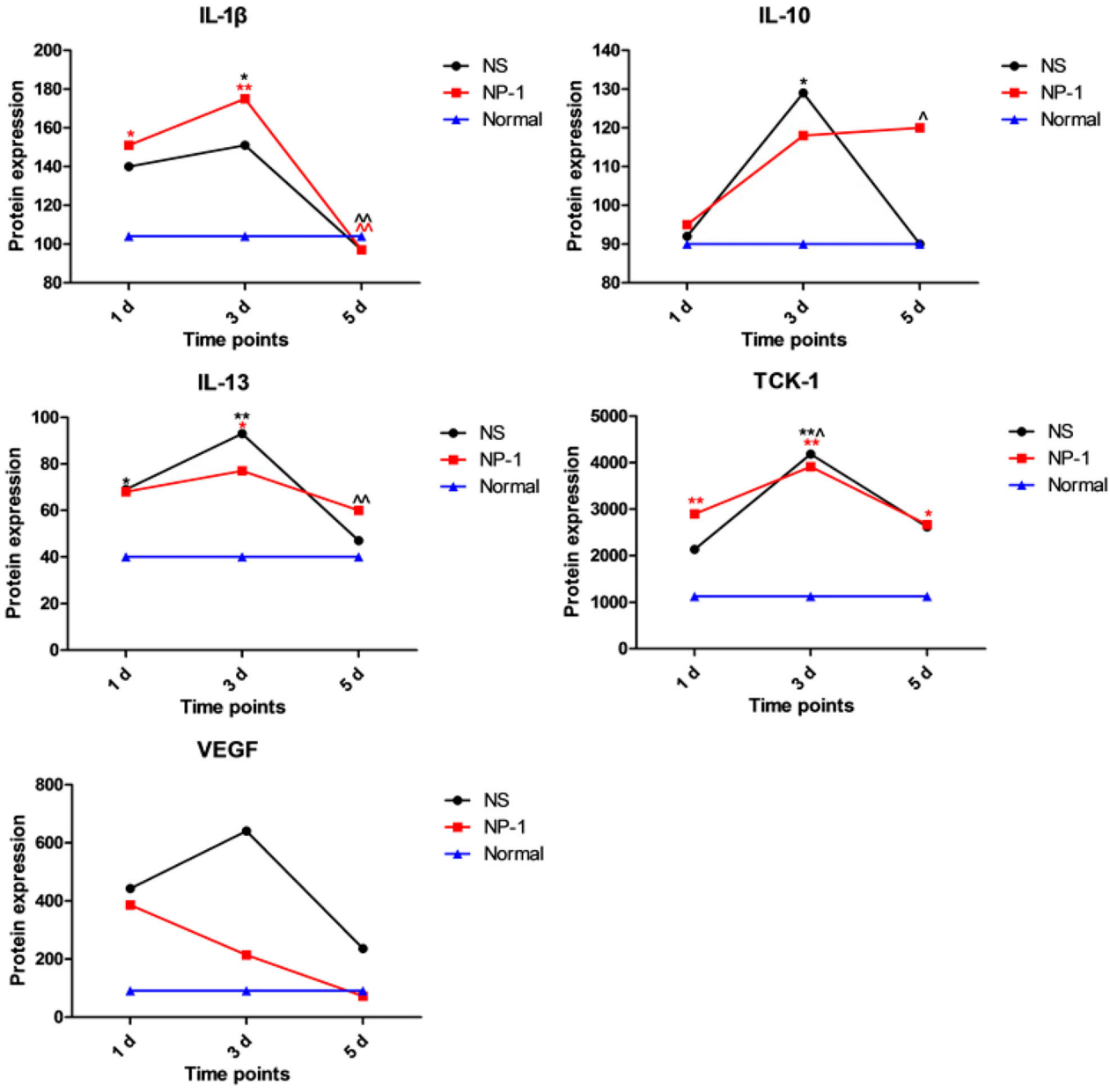

Figure 4. Expression of proteins directly related to inflammation. Changes in the expression of proteins directly related to inflammation at different time point should refer to Table 2. Asterisk (*) indicates comparison with the normal group, while number sign (^) indicates comparison between results at 3 d and 1 d or 5 d and 3. *,^ $P < 0.05$; **,^^ $P < 0.01$.

metastasis [40]. TIMP-1, which was also increase in the NP-1 and NS groups, is a matrix metalloproteinase inhibitor that plays an important role in the formation of neutrophils. An increase in TIMP-1 can induce an increase in neutrophil levels in mice [41]. Our results showed that a single intermuscular injection of saline did not affect the repair of injured sciatic nerves. Our study suggests that, days after the sciatic nerve crushing injuries in rats, protein factors, such as CINC-2 and LIX, may have caused neutrophils to aggregate at the injured site and induce inflammation. B7-2, TIMP-1, and other proteins could have induced the differentiation of macrophages and granulocytes, leading to further aggregation of macrophages and neutrophils and promotion of inflammation at the injured site. Although both pro- and anti-inflammatory factors were up-regulated, the pro-inflammatory effect of NP-1 may outweigh the anti-inflammatory effect. The secretion of TCK-1 increased, which could have further strengthened the inflammatory response. This subsequently increased the secretion of b-NGF to promote injury repair. VEGF, CINC-3, and fractalkine were not significantly increased over the course of the experiment. Our results indicate that the inflammatory response played a role in

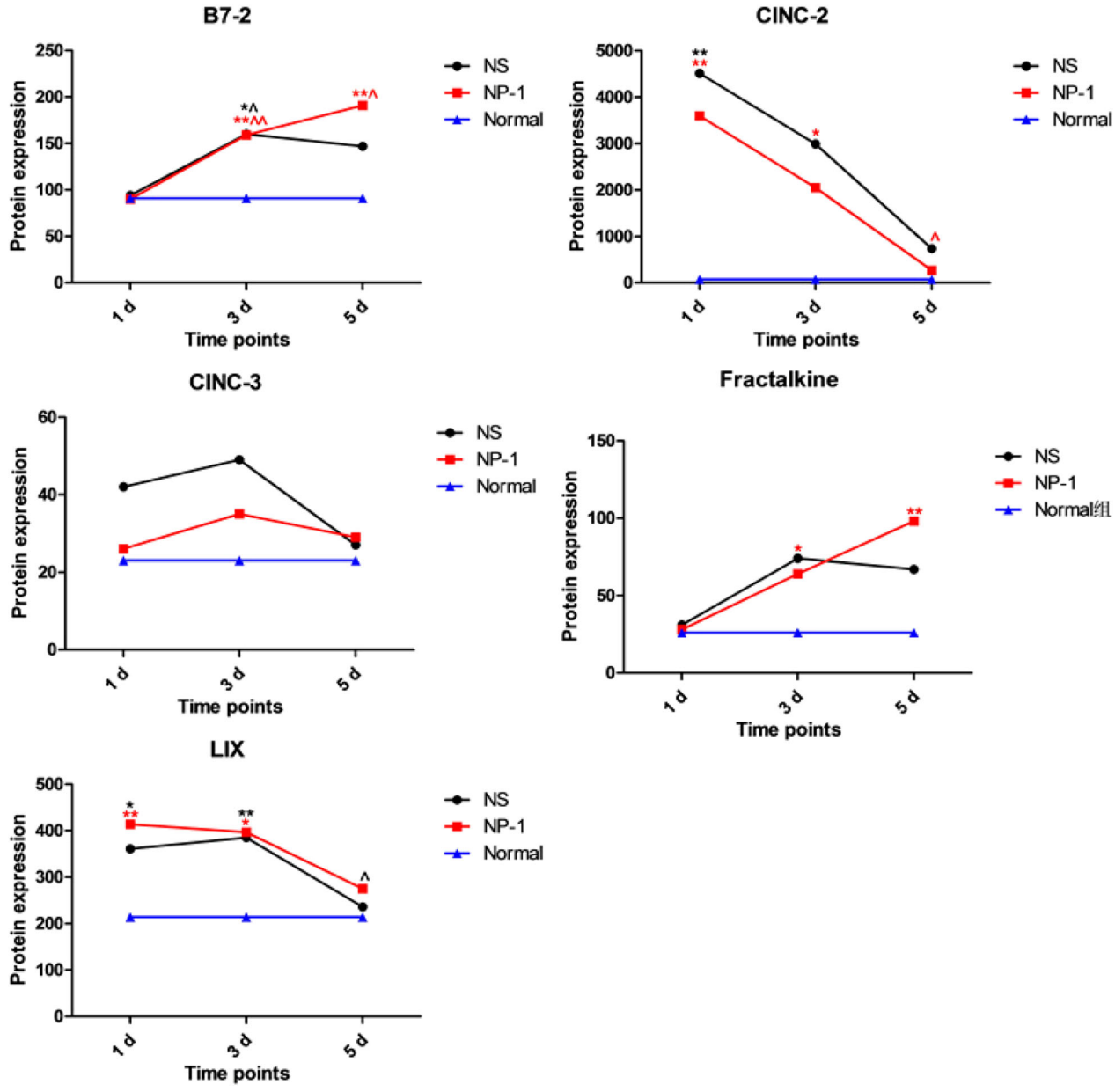

Figure 5 Expression of chemotaxis-related protein factors. Changes in the expression of chemotaxis-related protein factors at different time point should refer to Table 2. Asterisk (*) indicates comparison with the normal group, while number sign (^) indicates comparison between results at 3 and 1 d or 5 d and 3. *,^ $P < 0.05$; **,^^ $P < 0.01$.

the post-injury repair of the sciatic nerve, likely via an increase in b-NGF.

A single intermuscular injection of NP-1 significantly affected the secretion of factors involved in sciatic nerve-injury repair when compared with injection of physiological saline. According to our previous analysis, at different time points after NP-1 action, CINC-2, fractalkine, LIX, B7-2, and TIMP-1 play different roles, possibly through the differentiation of macrophages and neutrophils and their aggregation at the injured site. Secretion of the pro-inflammatory factors IL-1β and TCK-1 increased and was stronger than that of the anti-inflammatory factor IL-13. Therefore, this response would eventually lead to increased inflammation due to the synergistic effects of IL-10, VEGF, and CINC-3, which would then accelerate the repair of sciatic nerve injury. NP-1 promotes inflammation more strongly than it inhibits it, thus accelerating the secretion of b-NGF. This improves the integrity and function of the sciatic nerve, while healing the injury more quickly. This, in turn, restores the function of the effector muscle post-injury.

In conclusion, we believe that a single topical administration of 10 μg/mL NP-1 can promote sciatic nerve

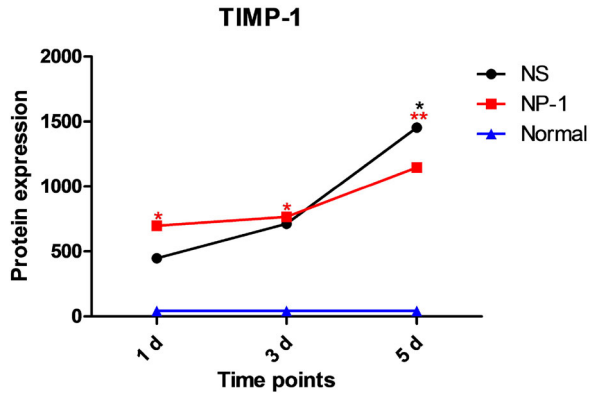

Figure 6. Expression of the cell generation-related protein factor. Changes in the expression of the cell generation-related protein factor at different time point should refer to Table 2. Asterisk (*) indicates comparison with the normal group. $^{*}P < 0.05$; $^{**}P < 0.01$.

regeneration after crush injury in rats due to the effects of NP-1 on the expression of proteins related to neurotrophy, inflammation, cell chemotaxis, and cell generation, which may lead to the promotion of nerve regeneration. We have elaborated on the effect of NP-1 on peripheral nerve regeneration and elucidated the potential mechanism by which NP-1 influences the repair of sciatic nerve injury in rats. Our study provides potential targets and possible related factors for the treatment and study of peripheral nerve injury.

Disclosure statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request. The authors declare no competing financial interests exist.

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