Long non-coding RNA NONMMUG014387 promotes Schwann cell proliferation after peripheral nerve injury

Bin Pan, Zhong-ju Shi, Jia-yin Yan, Jia-he Li, Shi-qing Feng
Department of Orthopedics, Tianjin Medical University General Hospital, Tianjin, China

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Abstract
Schwann cells play a critical role in peripheral nerve regeneration through dedifferentiation and proliferation. In a previous study, we performed microarray analysis of the sciatic nerve after injury. Accordingly, we predicted that long non-coding RNA NONMMUG014387 may promote Schwann cell proliferation after peripheral nerve injury, as bioinformatic analysis revealed that the target gene of NONMMUG014387 was collagen triple helix repeat containing 1 (Cthrc1). Cthrc1 may promote cell proliferation in a variety of cells by activating Wnt/PCP signaling. Nonetheless, bioinformatic analysis still needs to be verified by biological experiment. In this study, the candidate long non-coding RNA, NONMMUG014387, was overexpressed in mouse Schwann cells by recombinant adenovirus transfection. Plasmid pHBad-MCMV-GFP-NONMMUG014387 and pHBad-MCMV-GFP were transfected into Schwann cells. Schwann cells were divided into three groups: control (Schwann cells without intervention), Ad-GFP (Schwann cells with GFP overexpression), and Ad-NONMMUG014387 (Schwann cells with GFP and NONMMUG014387 overexpression). Cell Counting Kit-8 assay was used to evaluate proliferative capability of mouse Schwann cells after NONMMUG014387 overexpression. Polymerase chain reaction and western blot assay were performed to investigate target genes and downstream pathways of NONMMUG014387. Cell proliferation was significantly increased in Schwann cells overexpressing lncRNA NONMMUG014387 compared with the other two groups. Further, compared with the control group, mRNA and protein levels of Cthrc1, Wnt5a, ROR2, RhoA, Rac1, JNK, and ROCK were visibly up-regulated in the Ad-NONMMUG014387 group. Our findings confirm that long non-coding RNA NONMMUG014387 can promote proliferation of Schwann cells surrounding the injury site through targeting Cthrc1 and activating the Wnt/PCP pathway.

Key Words: nerve regeneration; peripheral nerve injury; Schwann cells; long non-coding RNAs; proliferation; Wnt/PCP pathway; Cell Counting Kit-8 assay; adenovirus overexpression; sciatic nerve; Cthrc1; neural regeneration
Introduction
Peripheral nerve injury is a common clinical problem that greatly compromises a patient’s quality of life (Kurtzke, 1982; Dubuisson and Kline, 2002; Gordon et al., 2015). Although the peripheral nervous system shows regenerative ability following injury, functional outcome is often unsatisfactory, especially when axons are transected (Dubuisson and Kline, 2002; Bamba et al., 2016; Wang et al., 2016). Thus, understanding the mechanisms involved in peripheral nerve injury and regeneration is important. Schwann cells form the myelin sheath of the peripheral nervous system and are the first cells activated following peripheral nerve injury (Jessen and Mirsky, 2005). Schwann cells modulate their phenotype by rejecting myelin in response to injury (Freidin et al., 2009; Altun and Kurutas, 2016). Subsequently, through proliferation and migration, Schwann cells form Bungner bands to scavenge degenerated myelin and axon debris, and facilitate correct axonal regeneration (Reichert et al., 1994; Wang et al., 2012; Yao et al., 2013; Qian et al., 2016).

Long non-coding RNAs (lncRNAs) contain more than 200 nucleotides (Rinn and Chang, 2012). According to accumulating evidence, researchers have shown that lncRNAs regulate protein-coding gene expression at epigenetic, transcriptional, and post-transcriptional levels. Consequently, the role of lncRNAs in regulating diverse biological and pathological processes has gradually been recognized (Gupta et al., 2010; Min et al., 2016). Sense lncRNA loci are on the same DNA strand as the corresponding gene, but unlike other subgroups of lncRNAs, studies of sense lncRNAs are sparse (Wright, 2014). The sense lncRNA, Oct4P4, regulates Oct4P4 gene expression at the epigenetic level to impede mouse embryonic stem cell renewal (Scarola et al., 2015; Wongtrakoon-gate et al., 2015). Additionally, Wongtrakoon-gate et al. (2015) identified a sense lncRNA steroid receptor RNA activator that regulated gene expression at the transcriptional level. These findings indicate important roles of sense lncRNAs in regulation of gene expression. In a previous study, we performed microarray analysis of the sciatic nerve after injury. We identified a sense lncRNA, NONMMUG014387, that was upregulated after peripheral nerve injury. Bioinformatic analysis revealed that the target gene of NONMMUG014387 was collagen triple helix repeat containing 1 (Cthrc1) (Pan et al., 2017). Interestingly, we found that NONMMUG014387 may promote Schwann cell proliferation in a Schwann cell line. LncRNA NONMMUG014387 is located at chr15:39,076,900-39,079,473, and expressed in multiple tissues such as peripheral nerve, hippocampus, lung, spleen, and thymus, although its role is still unknown.

Cthrc1 is a highly conserved glycoprotein that was first identified by Pyagay et al. (2005) after comparing impaired and normal arteries. Cthrc1 promotes cell proliferation in a variety of cells by activating Wnt/planar cell polarity (PCP) signaling (Yang et al., 2015). Cthrc1 is also involved in selective activation of Wnt pathways (Sang et al., 2016). Wnt signaling pathways are categorized into two types: the canonical Wnt/β-catenin pathway, and non-canonical pathways, which consist of Wnt/PCP and Wnt/Ca2+ pathways (Yamamoto et al., 2008). Wnt pathway activation is the result of the interaction between Wnt proteins and Frizzled (Fzd) receptors and pathway-specific coreceptors (Habas and Dawid, 2005). The canonical pathway coreceptors include low-density lipoprotein receptor-related protein (LRP)-5 and LRP6 (Takada et al., 2005), while the non-canonical coreceptor is receptor tyrosine kinase like orphan receptor 2 (RO2) (Tami et al., 2000). Cthrc1 is a Wnt cofactor protein that selectively activates the Wnt/PCP pathway by forming the Cthrc1-Wnt-Fzd/Ror2 complex (Yamamoto et al., 2008).

To further examine the role of the lncRNA, NONMMUG014387, we isolated and cultured Schwann cells from C57BL6 mice. Next, we performed experiments to examine the effect of NONMMUG014387 on Schwann cell proliferation, and determine whether NONMMUG014387 regulates Cthrc1 expression and participates in the Wnt/PCP pathway.

Materials and Methods

Animals
A total of 12 male specific-pathogen-free C57BL6 mice (aged 2 months old and weighing 18–22 g) were obtained from the Animal Lab Center of Academy of Military Medical Sciences of China (production license number: SCXK (Jun) 2012-0004; application license number: SYXK (Jin) 2014-0002). All mice were housed and maintained on 12-hour light/dark cycles, and allowed free access to food and water. Mice were kept in standard cages, with six animals per cage. Schwann cells were isolated from the mice. Mice with Schwann cells without intervention were used as the control group. The Ad-GFP and Ad-NONMMUG0148387 groups were transfected with Ad-GFP and NONMMUG0148387 overexpression vectors.

The study protocol was approved by the Animal Ethics Committee of Tianjin Medical University of China (approval number: TMuAEC 20170008). The experimental procedure followed the United States National Institutes of Health Guide for the Care and Use of Laboratory Animal (NIH Publication No. 85-23, revised 1985).

Sciatic nerve injury model and Schwann cell culture
Twelve 2-month-old mice were anesthetized before bilateral ligation of the sciatic nerves. Mice were intraperitoneally anesthetized using 2% chloral hydrate (0.2 mL/10 g). The sciatic nerve was exposed, and a 10-0 suture used to tie a knot 1 cm distal to the ischial tuberosity to completely constrict the nerve for 60 seconds. This elicited a reflex response (Gobianchi et al., 2017), which allowed complete transection of neural fibers without breaking the epineurium. The suture was then carefully released and the lesion site marked with a 10-0 Ethilon suture (Boivin et al., 2007). After the muscles and skin were sutured, the mice were maintained for one week. Afterwards, mice were sacrificed by an overdose of intraperitoneal injection of 10% chloral hydrate (0.5 mL/10 g), and 10-mm long sciatic nerve sections distal to the ligations (1 cm distal to the ischial tuberosity) were collected. Schwann cells were isolated after digestion and filtration of sciatic nerve fragments.
Schwann cell culture medium was prepared by supplementing DMEM with 10% fetal bovine serum, 2 μM forskolin, 10 ng/mL heregulin-β-1, and 50 ng/mL basic fibroblast growth factor according to a previous method (Wang et al., 2013). DMEM and fetal bovine serum were purchased from Hyclone (Shanghai, China). Forskolin was obtained from Sigma (St. Louis, MO, USA). Heregulin-β-1 and basic fibroblast growth factor were from Peprotech, Inc. (Rocky Hill, NJ, USA). Schwann cells were stained with an anti-S100 antibody for identification (Yu et al., 2017).

**Table 1 Primer sequence**

| Gene              | Sequence (5’ to 3’) | Product size (bp) |
|-------------------|---------------------|-------------------|
| NONMMUG014387     | Forward: AAA GGG ATT ACA GGC ACA CG | 2,539 |
|                   | Reverse: CCA GGC CAT TTA CTC AGC AT | 1,164 |
| Chthrc1           | Forward: CGA AAT AAA GGC TCT GAC GA | |
|                   | Reverse: TTA ACT TTG CTT TTT CAT TCA GC | |
| Wnt5a             | Forward: GAA TCC CAT TTG CAA CCC CTC ACC | 5,683 |
|                   | Reverse: GCT CCT CGT GTA CAT TTG CTC CCC | 2,835 |
| ROR2              | Forward: GTC CAA CGC ACA GCC CAA ATC |  |
|                   | Reverse: CCG GTT GCC AAT GAA GCG TG |  |
| RhoA              | Forward: AGC CTC ATG CGG TTA ATT TG | 524 |
|                   | Reverse: TCT TTG AAT TAG CGC CTC GT | |
| Rac1              | Forward: ATG CAG GCC ATC AAG TGT | 2,398 |
|                   | Reverse: ATT TAC AAC AGC AGG CAT TT | 1,311 |
| JNK               | Forward: AGC AGA AGA AAC GTG ACA AC | |
|                   | Reverse: GCT GCA CAC ACT ATT CCT TGA G | |
| ROCK              | Forward: TTC ATG TCC GAC GTG TAA CC | 5,293 |
|                   | Reverse: TTG ACA GCG TTC GAG GAG AG | |
| Beta-catenin      | Forward: TCTT GCC CCT TGT CCC GCA AAT CA | 3,386 |
|                   | Reverse: TCC ACA AAT TGC TGC GTC CCA | |
| GAPDH             | Forward: CGT GTT CCT ACC CCC AAT GT | 1,329 |
|                   | Reverse: TGT CAT CAT ACT TGG CAG GTT TCT | |

Cthrc1: Collagen triple helix repeat containing 1; Wnt5a: Wnt family member 5A; ROR2: receptor tyrosine kinase like orphan receptor 2; RhoA: ras homolog family member A; Rac1: RAS-related GTPase family member 1; JNK: Jun N-terminal kinase; ROCK: Rho-associated coiled-coil containing protein kinase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Schwann cell culture medium was prepared by supplementing DMEM with 10% fetal bovine serum, 2 μM forskolin, 10 ng/mL heregulin-β-1, and 50 ng/mL basic fibroblast growth factor according to a previous method (Wang et al., 2013). DMEM and fetal bovine serum were purchased from Hyclone (Shanghai, China). Forskolin was obtained from Sigma (St. Louis, MO, USA). Heregulin-β-1 and basic fibroblast growth factor were from Peprotech, Inc. (Rocky Hill, NJ, USA). Schwann cells were stained with an anti-S100 antibody for identification (Yu et al., 2017).

**Construction and transfection of recombinant adenovirus vectors**

For ectopic expression, plasmid pHBAd-MCMV-GFP-NONMMUG014387 and pHBAd-MCMV-GFP were transfected into Schwann cells. Full-length NONMMUG014387 (Hanbio Biotechnology Co., Shanghai, China) was subcloned into pHBAd/MCMV/GFP vector (Hanbio Biotechnology Co.) and regulated by the murine cytomegalovirus (MCMV) promoter. Green fluorescent protein (GFP) was regulated by the CMV promoter. After sequencing, adenoviral vector DNAs and packaging vectors were transfected into 293T cells (Hanbio Biotechnology Co., Shanghai, China). Lipofiter™ (Hanbio Biotechnology Co.) was used for transfection. Forty-eight hours after cotransfection, adenovirus in supernatants was collected and filtered through 0.45 μm filters. Finally, adenovirus was purified using ultracentrifugation and titer determination. The final concentration of recombinant Ad-GFP was 2 × 10^{10} PFU/mL, and recombinant Ad-NONMMUG014387 was 1 × 10^{10} PFU/mL. Schwann cells were then transfected with Ad-GFP and Ad-NONMMUG014387 for 36 hours, and subsequently reseeded for total RNA extraction. Polymerase chain reaction (PCR) was used to detect expression of NONMMUG014387.

**Cell proliferation assay**

After Schwann cells were overexpressed with GFP and NONMMUG014387, cell proliferation experiments were performed using a cell counting kit-8 (CCK-8) to determine Schwann cell proliferation. Cells in each group were inoculated in 96-well plates with 3 × 10^{4} cells per well and three repeated wells for each group. Each group was incubated for 0, 1, 2, 3, and 4 days at 37°C. Then, 10 μL CCK-8 solution was added to each well, and incubation terminated after 3 hours. The optical density (OD) value of each well was measured at 450 nm with a microplate reader (Hanbio Biotechnology Co.). The corresponding OD value represents cell proliferation.

**Quantitative real-time PCR**

After overexpression of Schwann cells with GFP and NONMMUG014387, real-time PCR was performed. Total RNA was extracted using TRIzol, according to the manufacturer’s instructions. RNA was reverse transcribed into cDNA using a kit (Hanbio Biotechnology Co.). Quantitative PCR was performed using 20 μL reactions, which contained 1 μL cDNA template (1:100 dilution), 10 μL 2x Real-time PCR Master Mix, 0.8 μL forward primer (10 μM), 0.8 μL reverse primer (10 μM), and ddH_{2}O. The reaction conditions were 95°C for 3 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds, and 72°C for 20 seconds. Glyceralde-
Hyde-3-phosphate-dehydrogenase (GAPDH) was used as the internal control. Relative changes in gene expression levels were analyzed using the \(2^{-\Delta\Delta Ct}\) method, as described previously (Schmittgen and Livak, 2008). Reverse transcriptase (Toyobo, Osaka, Japan), QPCR mix (Toyobo), BioTek NDS5000 (Toyobo), and LightCycler 96 (Roche, Basel, Switzerland) were used for real-time PCR. Gene expression in the control group was normalized to 1, while gene expression in the Ad-NONMMUG014387 group was normalized to the control group (optical density ratio). All assays were performed in triplicate. The primer sequences are shown in Table 1.

**Western blot assay**

After Schwann cells were overexpressed with GFP and NONMMUG014387, western blot assays were performed. Protein extracts were separated from Schwann cells using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 10% and 4% SDS polyacrylamide gels. Proteins were transferred to polyvinylidene fluoride membranes for 90 minutes. Membranes were then blocked using 5% non-fat dry milk for 1 hour at 37°C and incubated overnight at 4°C with a rabbit polyclonal antibody recognizing Chc1 (1:2,000; Abcam, Cambridge, UK), rabbit polyclonal antibody recognizing β-actin (1:2,000; Abcam), rabbit polyclonal antibody recognizing β-catenin (1:2,000; Abcam), rabbit polyclonal antibody recognizing Wnt family member 5A (Wnt5A) (1:2,000; Abcam), rabbit polyclonal antibody recognizing Ras homolog family member A (RhoA) (1:2,000; Abcam), rabbit polyclonal antibody recognizing RhoA-associated coiled-coil containing protein kinase (ROCK) (1:2,000; Abcam). After washing with Tris-buffered saline with Tween, blots were incubated with horseradish peroxidase-labeled anti-rabbit secondary antibody (1:5,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 hours at room temperature. Densitometric analysis of resultant protein bands was performed using Quantity One software (Bio-Rad, Hercules, CA, USA). All grayscale values were normalized to β-actin (Wang et al., 2015). All assays were performed in triplicate.

**Statistical analysis**

All data are represented as the mean ± SD. SPSS 18.0 software (IBM, Armonk, IL, USA) was used for statistical analysis. Student's t-test was used to compare samples from two different groups. One-way analysis of variance and Tukey's post hoc test were used to compare samples from three different groups, and values of P < 0.05 considered statistically significant.

**Results**

**Successful construction of a lncRNA NONMMUG014387 overexpression model**

To further investigate the function and role of the lncRNA NONMMUG014387 in nerve regeneration, we cultured Schwann cells from injured sciatic nerves. After digestion and filtration, the major cell components of sciatic nerve fragments included Schwann cells and fibroblasts. Schwann cells exhibited a spindle-like morphology and had oval nuclei with 2–3 projections from the cell body, while fibroblasts exhibited an irregular morphology and were larger than Schwann cells. Although Schwann cells were the major components, many fibroblasts remained after the first purification, whereas after the second purification, nearly all the cells were Schwann cells.

Subsequently, we overexpressed lncRNA NONMMUG014387 in Schwann cells using an adeno virus. The pHAd-MCMV-GFP overexpression carrier profile is shown in Figure 1A. The target gene (i.e., NONMMUG014387) was inserted into the multiple cloning site (MCS) domain and regulated by the MCMV promoter. The carrier induced GFP expression, which was activated by the CMV promoter. After transfection, Schwann cells were divided into three groups: NONMMUG014387 overexpression group (Ad-NONMMUG014387-GFP), GFP group (Ad-GFP), and control group. As shown in Figure 1B, NONMMUG014387 expression in the Ad-NONMMUG014387-GFP group was markedly increased compared with the Ad-GFP and control groups, which demonstrates successful construction of the lncRNA NONMMUG014387 overexpression model.

**NONMMUG014387 overexpression promoted Schwann cell proliferation**

To determine the effect of lncRNA NONMMUG014387 overexpression on Schwann cell proliferation, we performed CCK-8 proliferation assays to determine the proliferation capacity of Schwann cells. As shown in Figure 1C, the proliferation ability of Schwann cells in the Ad-NONMMUG014387-GFP, Ad-GFP, and control groups were all significantly increased with increasing culture time. Indeed, no differences were observed among the three groups of Schwann cells at 1 day. However, after 1 day, cell proliferation was significantly increased in Schwann cells overexpressing lncRNA NONMMUG014387 compared with cells in the other two groups. These data indicate that lncRNA NONMMUG014387 plays a role in promoting Schwann cell proliferation.

**LncRNA NONMMUG014387 amplified Chc1 expression and activated the Wnt/PCP pathway**

To further determine the mechanism of lncRNA NONMMUG014387-induced Schwann cell proliferation, we examined expression of Chc1 in Schwann cells overexpressing lncRNA NONMMUG014387. As shown in Figure 2A–C, mRNA and protein levels of Chc1 were up-regulated in Schwann cells overexpressing lncRNA NONMMUG014387, indicating that lncRNA NONMMUG014387 promotes Schwann cell proliferation by amplifying Chc1 expression.

Because Chc1 is involved in selective activation of the Wnt pathway, we determined whether Wnt signaling is activated by Chc1 amplification in Schwann cells overexpressing NONMMUG014387. To examine activation of the Wnt/β-catenin pathway, we measured β-catenin expression. As
shown in Figure 2D–F, compared with the control group, β-catenin protein was unchanged. To examine activation of Wnt/PCP signaling, we measured expression of critical proteins of the Wnt signaling pathway. The Wnt5a, ROR2, RhoA, Rac1, JNK, and ROCK proteins were significantly up-regulated in Schwann cells in the Ad-NONMMUG014387 group (Figures 3 and 4). This suggests that the Wnt/PCP pathway is induced by altered Cthrc1 expression in Schwann cells when IncRNA NONMMUG014387 is overexpressed.

Discussion
Peripheral nerve regeneration after injury requires dedifferentiation, proliferation, and migration of Schwann cells (Reichert et al., 1994; Rotshenker, 2011; Wang et al., 2012; Al-Zer and Kalbouneh, 2015). Our previous microarray results show that NONMMUG014387 is up-regulated following peripheral nerve injury, while bioinformatic predictions show that IncRNA NONMMUG014387 plays a role in Schwann cells. Therefore, we performed experiments to examine the effect of IncRNA NONMMUG014387 on Schwann cells. Through adenoviral overexpression of IncRNA NONMMUG014387 in Schwann cells, we show that Schwann cell proliferation is significantly enhanced compared with control cells, indicating that IncRNA NONMMUG014387 promotes Schwann cell proliferation during nerve regeneration.

We further found that Cthrc1 expression levels are enhanced in Schwann cells overexpressing IncRNA NON-
Figure 3 NONMMUG014387 lncRNA overexpression increased Wnt5a and Ror2 expression.
(A) Representative western blot assay for Wnt5a and Ror2 in control and Ad-NONMMUG014387-GFP Schwann cells. (B, D) Detection of Wnt5a and ROR2 mRNA expression. (C, E) Detection of Wnt5a and Ror2 protein expression. Data are expressed as the mean ± SD (n = 3; Student’s t test). *P < 0.05, vs. control group. Wnt5a: Wnt family member 5A; Ror2: receptor tyrosine kinase like orphan receptor 2; lncRNAs: long non-coding RNAs.

Figure 4 NONMMUG014387 overexpression increased protein RhoA, Rac1, p-JNK, and ROCK expression.
(A) Representative western blot assay for RhoA, Rac1, p-JNK, and ROCK in control and Ad-NONMMUG014387-GFP Schwann cells. (B, D, F, H) Detection of RhoA, Rac1, p-JNK, and ROCK mRNA expression. (C, E, G, I) Detection of RHOA, RAC1, p-JNK, and ROCK protein expression. Data are expressed as the mean ± SD (n = 3; Student’s t test). *P < 0.05, vs. control group. RhoA: ras homolog family member A; Rac1: RAS-related C3 botulinum substrate 1; JNK: Jun N-terminal kinase; ROCK: Rho-associated coiled-coil containing protein kinase.
MMUGO14387. Cthrc1 is a multifunctional signaling molecule involved in a variety of cellular behavioral alterations. For example, Cthrc1 facilitates matrix extracellular signal-regulated kinase through extracellular signal-regulated kinase, which increases colon cancer cell invasiveness (Oishi et al., 2003). In addition, Cthrc1 activates angiopoietin-2 secretion to alter endothelial cell motility and facilitate angiogenesis during tumorigenesis (Kim et al., 2014). Studies have reported that Schwann cells exhibit active migration abilities upon Cthrc1 silencing (Lee et al., 2016). Cthrc1 plays a positive role in Schwann cell proliferation because Cthrc1 overexpression promotes Schwann cell proliferation, whereas Cthrc1 knockdown compromises Schwann cell proliferation (Zhou et al., 2014). In our experiments here, Schwann cells exhibit increased proliferation with increased Cthrc1 mRNA and protein levels, which is consistent with these findings. The mechanism of Cthrc1 regulation in Schwann cells is unknown, which led us to investigate the signaling pathways by which Cthrc1 promotes Schwann cell proliferation.

Cthrc1 selectively induces Wnt/PCP signaling by interacting with the extracellular Wnt-Fzd/Ror2 complex (Yamamoto et al., 2008; Sang et al., 2016). Selective activation of Wnt/PCP signaling by Cthrc1 has also been demonstrated in gastrointestinal stromal tumor cells (Apra et al., 2012). We further determined whether IncRNA NONMMUGO14387-mediated Cthrc1 enhancement activates the Wnt/PCP pathway in Schwann cells. We found that Ror2, the coreceptor for the Wnt/PCP pathway, was enhanced with NONMMUGO14387 overexpression in Schwann cells, indicating that the Wnt/PCP pathway is activated by Cthrc1 in Schwann cells. Cthrc1 inhibits the canonical Wnt/β-catenin pathway by simultaneously suppressing Dishevelled2, without further modification of the downstream component, β-catenin (Yamamoto et al., 2008; Sang et al., 2016). In our experiment, no difference in β-catenin expression was observed between Schwann cells overexpressing IncRNA NONMMUGO14387 and the control group, which further reflects the reliability of our experiments.

The small GTPases, Rac and RhoA, are two parallel downstream components of Wnt/PCP signaling (Ma et al., 2014). These small GTPases regulate cytoskeletal dynamics, and are involved in cell division and modulating morphology (Habas et al., 2003; Nodari et al., 2007; Jung et al., 2011). JNK is an effector of Rac, while ROCK is an effector of RhoA (Muraskowski et al., 2011; Riou et al., 2013). ROCK protein is essential for epithelial cell phenotype alterations (Rosso et al., 2005). Researchers have shown that RhoA/ROCK and Rac1/JNK regulate epithelial cell morphogenesis in an antagonistic manner (Kalaji et al., 2012). In our experiments, elevated RhoA, Rac1, JNK, and ROCK protein levels were detected in Schwann cells overexpressing IncRNA NONMMUGO14387, indicating that the downstream components of Wnt/PCP are activated by enhanced Cthrc1 expression to promote Schwann cell proliferation. However, whether RhoA/ROCK and Rac1/JNK antagonize protein function in Schwann cells requires further study.

It is intriguing that miR-9 inhibits Cthrc1 expression by direct binding to its 3'-UTR to alter Schwann cell behavior (Zhou et al., 2014; Lee et al., 2016). Moreover, we show that miR-9 expression is down-regulated in Schwann cells overexpressing IncRNA NONMMUGO14387. These findings are consistent with the competing endogenous RNA (ceRNA) hypothesis. According to the ceRNA hypothesis, lncRNA and mRNA share the same microRNA response element to regulate each other by competitively binding to microRNA (Salmena et al., 2011; Martin et al., 2016). However, whether IncRNA NONMMUGO14387 regulates Cthrc1 in a miR-9-dependent manner requires further investigation.

Our study provides new understanding of nerve regeneration after peripheral nerve injury. However, all our conclusions rely on IncRNA overexpression, with no loss-of-function assays. Accordingly, the detailed mechanism of IncRNA NONMMUGO14387-mediated Cthrc1 expression remains to be determined. More experiments should be performed to validate our results.

Taken together, our study first reveals a critical interaction between IncRNA NONMMUGO14387, Cthrc1, and the Wnt/PCP pathway in Schwann cells. After sciatic nerve injury, NONMMUGO14387 expression was increased, and consequently, expression of Cthrc1 was increased. Increased Cthrc1 expression activated the Wnt/PCP pathway, which promoted Schwann cell proliferation. Our study provides new understanding of nerve regeneration and a potential mechanism for repairing peripheral nerve injury.

Author contributions: BP, JYY and SQF designed this study. BP, JYY and ZJS performed this study. BP, JYY and ZJS analyzed data. BP, JYY and ZHL wrote the paper. BP and SQF provided critical revision of the paper. SQF and JYY provided the funding and supervision. All authors approved the final version of the paper.

Conflicts of interest: None declared.

Research ethics: The study protocol was approved by the Animal Ethics Committee of Tianjin Medical University of China (approval number: TMUaMEC 2017008). The experimental procedure followed the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).

Declaration of patient consent: Datasets analyzed during the current study are available from the corresponding author on reasonable request.

Data sharing statement: Datasets analyzed during the current study are available from the corresponding author on reasonable request.

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