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Expression and Role of CXCL13 and MiR-186-5p in the Trigeminal Ganglion of a Rat Model of Trigeminal Neuralgia

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ABSTRACT

AIM: To reveal the roles of CXCL13 and miR-186-5p in a rat model (adult male Sprague-Dawley rats, 7–8 weeks old, 180–200 g) of trigeminal neuralgia (TN) established via chronic constriction injury of the infraorbital nerve (ION-CCI).

MATERIAL and METHODS: The results of behavioural tests and the expression levels of miR-186-5p and CXCL13 in the trigeminal ganglion (TG) were compared between the sham and ION-CCI groups, as well as the consequences of the miR-186-5p mimic and inhibitor.

RESULTS: Compared with the sham-operated rats, ION-CCI rats displayed mechanical hypersensitivity in the von Frey hair test. Western blotting revealed the upregulation of CXCL13 and downregulation of miR-186-5p in the TG of ION-CCI rats relative to their expression in sham rats. Furthermore, an miR-186-5p mimic decreased CXCL13 protein levels and increased the mechanical withdrawal thresholds of ION-CCI rats. CXCL13 protein levels also increased after the injection of an miR-186-5p inhibitor. Finally, miR-186-5p was found to be expressed in the TG and was downregulated in ION-CCI rats compared to sham rats.

CONCLUSION: miR-186-5p may negatively regulate CXCL13 to influence the occurrence and development of TN. Collectively, our findings shed new light on novel therapies for the treatment of TN.

KEYWORDS: MiR-186-5p, CXCL13, Trigeminal neuralgia, Trigeminal ganglion, Chronic constriction injury

INTRODUCTION

Neuropathic pain is a serious form of chronic pain caused by nervous system dysfunction or injury. Hyperalgesia and allodynia are typical characteristics of neuropathic pain (7) and are resistant to current treatments (34). Trigeminal neuralgia (TN) is a common type of neuropathic pain in the orofacial region in clinics and is estimated to affect between 12.6 and 28.9 individuals per 100,000 per year (25). TN is currently one of the most difficult pain syndromes to manage. The outcomes of existing treatments are often unsatisfactory (26).

Glial is an activator of neuropathic pain after neurological injury. Cells respond to glia by producing effective neuromodulators, such as pro-inflammatory cytokines and chemokines, as well as growth factors (1,19). Chemokines are important regulators of immune, inflammatory, and neuronal responses in both the peripheral and central nociceptive/pain pathways (4). In the spinal cord, chemokines are involved in regulating nociception, including those influencing neuropathic pain, cancer pain, and inflammatory pain (24). Further, pro-inflammatory cytokines and chemokines are responsible for mediating neuroinflammation.

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C-X-C motif chemokine 13 (CXCL13), also known as B lymphocyte chemoattractant, is a pro-inflammatory chemokine that was originally identified in stromal cells in B-cell follicles. In these cells, CXCL13 regulates the homing of B cells and subsets of T cells (30, 34). Interestingly, CXCL13 is persistently upregulated in spinal neurons in a mouse model of neuropathic pain induced by spinal nerve ligation (SNL) (14, 20, 29). The only CXCL13 receptor expressed in B cells is C-X-C chemokine receptor type 5 (CXCR5) (10). Further, the CXCL13-CXCR5 signalling pathway has been demonstrated to be critical for the induction of neuropathic pain in the SNL model. SNL induces continuous upregulation of CXCL13 expression in spinal neurons with CXCR5-activated spinal astrocytes. In contrast, the expression of CXCL13 is inhibited by miR-186-5p (14).

The CXCL13-CXCR5-mediated astrocytic signalling pathway may play an important role in neuropathic pain (34). For example, intrathecal injection of CXCL13 can activate extracellular signal-regulated kinase (ERK) in the spinal cord. ERK activation can also be induced by SNL; however, this activation was found to be reduced in CXCR5$^{-/-}$ mice. These findings suggest that ERK is a downstream target of the CXCL13-CXCR5 signalling pathway that regulates neuropathic pain (34).

The trigeminal ganglion (TG) plays an important role in nociceptive transmission and regulation from the peripheral oral and maxillofacial regions to the central nervous system (23). Specifically, TG neurons become hyperactive after injury and generate and release various molecules that activate satellite glial cells and macrophages. These cells then release cytokines, such as interleukin (IL)-1β, tumour necrosis factor (TNF)-α, and IL-6, which are involved in trigeminal nerve injury and ectopic orofacial pain. Trigeminal nerve injury activates astrocytes and microglia to generate and release various molecules. These molecules then activate second-order neurons in the trigeminal spinal subnucleus caudalis and upper cervical spinal cord (C1/C2) (22). Increased CXCL13 and CXCR5 levels after partial infraorbital nerve ligation (pIONL) have been observed in mouse models, which is followed by neuropathic pain in the maxillofacial region through the ERK-mediated induction of pro-inflammatory cytokines (32). Therefore, the CXCL13/CXCR5/ERK/TNF-α and IL-1β pathways may represent important targets for the treatment of neuropathic pain, such as TN in the orofacial region (32).

ERK, p38, and c-Jun N-terminal kinase (JNK) belong to the mitogen-activated protein kinase (MAPK) family and are activated after peripheral nerve injury in the dorsal root ganglia and within the spinal cord (12). The pIONL mouse model has been utilised to study neuropathic pain in the maxillofacial region to verify whether MAPK family proteins are downstream targets of the CXCL13-CXCR5 pathway in the TG. Prior findings have shown that p38, but not JNK, is activated in the TG of the pIONL mouse model, whereas pIONL-induced neuropathic pain in the maxillofacial region is relieved in CXCR5$^{-/-}$ mice without activation of p38 (33). These findings indicate that the CXCL13-CXCR5-p38 signalling pathway may play an important role in neuropathic pain in the maxillofacial region (33).

Specific microRNAs (miRNAs) may trigger neuropathic pain by altering gene transcription and protein expression (6). One miRNA, miR-186-5p, negatively regulates CXCL13 expression in the spinal cord of the SNL mouse model, thereby attenuating SNL-induced pain hypersensitivity (14). Studies investigating the relationship between miR-186-5p and CXCL13 have primarily focused on SNL-induced neuropathic pain. Therefore, this study aimed to establish a mouse model of TN via chronic constriction injury of the infraorbital nerve (ION-CCI) to determine whether miR-186-5p expression affects mechanical withdrawal thresholds by regulating the expression of CXCL13 in the TG.

MATERIAL and METHODS

Animals

All animal breeding and experiments were approved by the Biomedical Ethics Committee of Anhui Medical University (No. 20190236). Clean adult male Sprague-Dawley rats (7–8 weeks old, 180–200 g) were housed in sawdust-lined cages at 25°C under a 12-h light-dark cycle. The relative humidity of the feeding room was maintained between 40% and 50%. Fewer than five rats were housed per cage and provided food and water *ad libitum* at the Animal Lab Center of Anhui Medical University (China).

All rats underwent adaptive training one week before surgery and were stimulated five consecutive times (at an interval of 30 s each) in the left whisker pad area with von Frey hairs. Rats used in the experiments were calm and tolerated the hair stimulation on the whisker pad area after one week of training with fibrillary stimulation using the Von Frey brush. Rats in different groups were housed in separate cages.

Experimental design

In the present study, rats were divided into the following three groups: ION-CCI group, sham group, and control group. The sham and ION-CCI groups were compared in terms of the results of behavioural tests and the expression levels of miR-186-5p and CXCL13 in the TG. Thereafter, rats in the ION-CCI group were randomly divided into two subgroups; subgroup one was treated with an miR-186-5p mimic while subgroup two was treated with saline to investigate the impact of an miR-186-5p mimic on the results of the behavioural tests. Finally, rats in the control group were divided into two subgroups; subgroup one was treated with an miR-186-5p inhibitor while subgroup two was treated with saline, and then subjected to behavioural testing and western blotting analysis to determine the impact of miR-186-5p.

Surgeries

Rats in the ION-CCI group (n = 33) and sham group (n=21) were anaesthetised with 3 mg/mL of sodium pentobarbital (30 mg/kg weight, i.p.). The left surgical area was sterilised with 1% iodophor. Thereafter, the skull and nasal bone were exposed via a scalp incision 1 cm away from the left alar. The IONs on the left side of the face were isolated, and the proximal and distal ends of the left suborbital nerve were separated by ligation with a 5-0 silk thread, with a ligation interval of

approximately 2 mm. To achieve the effects of constriction, we adhered to the criteria formulated by Bennett and Xie (3). The skin wound was then routinely sutured using a 3-0 silk suture. The operation for the sham group was the same as that for the ION-CCI group, except that the sham group only had exposure to the ION, without ligation. The control group did not undergo any operative procedure.

Drug injection

The miR-186-5p mimic and miR-186-5p inhibitor were synthesised by RiboBio (14). In a preliminary experiment, the mechanical withdrawal thresholds were found to significantly change during the first and second weeks. Rats in the ION-CCI group (12 rats) were randomly separated into the following two subgroups (six rats in each group) on day 11 after surgery: an ION-CCI + miR-186-5p mimic group and an ION-CCI + saline group. Rats in the ION-CCI + miR-186-5p mimic group were injected once daily for three days with 15 mL of saline containing the miR-186-5p mimic (5 mg). The injections were performed as described by Neubert et al. (21). After performing anaesthesia and disinfection, the maxillary bone was palpated to identify the most anterior rostral portion of the zygomatic process. The microinjector was inserted through the infraorbital foramen into the palpated portion at a depth of approximately 1–2 mm. The needle was positioned at 10° relative to the middle of the head, the tip of the needle was advanced 15 mm through the foramen rotundum, and the miR-186-5p mimic was injected into the TG. Rats in the ION-CCI + saline group served as controls and were injected with 15 mL of saline only.

A control group (12 rats) that did not undergo any anaesthesia or surgery was randomly divided into a control + miR-186-5p inhibitor group and a control + saline group (six rats in each group). Rats in the control + miR-186-5p inhibitor group were injected with 15 mL of saline containing the miR-186-5p inhibitor (5 mg) in the TG area through the left infraorbital foramen once daily for three days. Rats in the control + saline group served as a control for the control + miR-186-5p inhibitor group and received injections of 15 mL of saline only.

Behavioural tests

Behavioural tests were performed by an experimenter blinded to the experimental conditions of each rat. Mechanical withdrawal thresholds were tested by stimulating the left whisker pad area with von Frey hairs on days 3, 5, 7, 14, and 21 after surgery. The different diameters and lengths of von Frey hairs represented different stimulating intensities, with different strengths of 0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 0.6, 1.0, 1.4, and 2.0 g. Each rat was placed in a new cage and allowed to freely roam during measurements of withdrawal thresholds. The stimulations were administered once rats were acclimated, relaxed, and quiet in this new cage. The test was repeated 10 times at each intensity level. The mechanical withdrawal threshold in the whisker pad area of the surgical side of rats was recorded as the minimum stimulation intensity, with at least six positive mechanical stimulation reactions. Positive reactions included one or more of the following: (1) withdrawal in the form of a rapid contraction when the von Frey hair filaments contacted the ipsilateral whiskers of the rat; (2) escape or aggressive behaviour, including escapes, contracture, burial, and/or biting or grasping of the filament; (3) asymmetric facial grooming consisting of an uninterrupted series of at least three face-wash strokes directed at the stimulated facial area. Three measurements were recorded at intervals of 10 min to obtain the average of the measured values.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Rats in each group were euthanized by an overdose of sodium pentobarbital. The TG of each rat was then removed, the bilateral TG was exposed, and the TG on the operative side was dissected. Total RNA from the TG was extracted using TRIzol reagent (Thermo Fisher Life Technologies, USA). The iQ5 real-time PCR detection system and SYBR Green were used for qRT-PCR. The qRT-PCR procedure was designed by Sangon Biotech (Shanghai, China). The following primers were used: Cxcl13 forward, 5′-AGA GCT GAA GGG TGA ACT CC-3′; Cxcl13 reverse, 5′-AGG CAG ATG GCC AGT AGA AG-3′; miR-186-5p forward, 5′-TCA AAG AAT TCT CCT TTT GGG CT-3′; and miR-186-5p-3′ reverse, 5′-CGC TTC ACG AAT TTG CGT GTC AT-3′. The following cycling conditions were employed for PCR: 95°C for 60 s, followed by 40 cycles of thermal cycling at 95°C for 20 s and 60°C for 60 s. Quantification was performed by normalising the cycle threshold (Ct) values with those of β-actin and analysing the data using the $2^{-\Delta\Delta CT}$ method.

Western blotting

Rats in each group were euthanized by an overdose of sodium pentobarbital. Thereafter, the ipsilateral TGs were dissected and homogenised in lysis buffer containing protease and phosphatase inhibitors (Beyotime, Shanghai, China). Protein concentrations were determined using a BCA protein assay (Pierce, Rockford, IL, USA). A 20-mg sample of each TG protein preparation was loaded onto 4–12% sodium dodecyl sulphate polyacrylamide gels. The proteins were then separated by electrophoresis at 100 V for 90 min and transferred to a polyvinylidene difluoride membrane (Merck Millipore, Darmstadt, Germany) at 220 mA for 1 h. The protein band of CXCL13 was 13 kDa, and that of β-actin was 43 kDa. The membrane was then blocked with 5% milk and incubated overnight at 4°C with antibodies against CXCL13 (rabbit, 1:500, Abcam, ab112521) and β-actin (mouse, 1:1000, Zs-BIO, TA-09). The western blots were then incubated with IRDye 800 CW secondary antibodies for 2 h at room temperature. The intensities of the selected protein bands were analysed using ImageJ software (NIH, Bethesda, MD, USA).

Statistical Analysis

SPSS version 19.0 was used to perform statistical analysis of all experimental data. The results of the behavioural experiments and qPCR across groups were compared using repeated-measures analysis of variance. Independent-sample t-tests were used to compare two groups of data in the western blotting experiments. Statistical significance was set at $p<0.05$.

RESULTS

Mechanical withdrawal thresholds are significantly decreased in ION-CCI rats.

In the present study, a rat model of TN was established via ION-CCI. In the sham group, no significant difference in mechanical withdrawal thresholds was found before surgery compared to those found at three weeks after surgery ($p>0.05$) (Figure 1). On the third day after surgery, mechanical withdrawal thresholds were 0.87 ± 0.19 g in the sham group and 0.12 ± 0.06 g in the ION-CCI group ($n=6$, $p<0.001$). Mechanical withdrawal thresholds were significantly lower in the ION-CCI group than in the sham group at least three weeks after surgery. Such findings confirmed the successful establishment of our ION-CCI rat model of TN.

miR-186-5p mRNA expression is downregulated while CXCL13 mRNA expression is upregulated in the TGs of ION-CCI rats.

To determine possible similarities between our rat model of TN and those of a previously established mouse model of SNL, we measured the mRNA levels of miR-186-5p and CXCL13 in the TG via qRT-PCR at the first and second weeks after surgery. No significant difference in the mRNA levels of miR-186-5p or CXCL3 was found in the sham group at the first or second week after surgery compared with those before surgery ($p>0.05$) (Figure 2). The mRNA levels of miR-186-5p were significantly decreased in the ION-CCI group at the first and second weeks after surgery compared with those in the sham group ($p<0.001$) (Figure 2A). In contrast, the mRNA levels of CXCL13 were significantly upregulated at the first and second weeks after surgery compared with those in the sham group ($p<0.001$) (Figure 2B). The significant changes in miR-186-5p and CXCL13 mRNA levels in the second week after surgery corresponded with the observed changes in mechanical withdrawal thresholds. Collectively, these results indicate that changes in the mRNA levels of miR-186-5p and CXCL13 may be associated with TN.

Protein expression of CXCL13 is significantly upregulated in the TGs of ION-CCI rats.

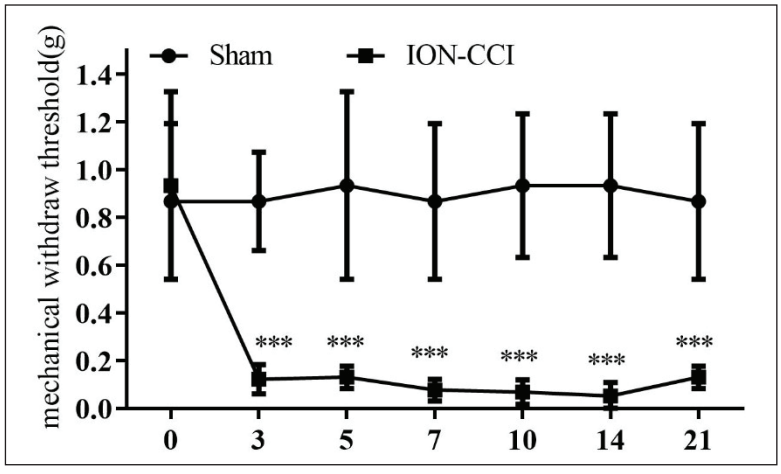

Figure 1: Mechanical withdrawal thresholds to a vibrissa pad in the sham and ION-CCI groups (compared with the sham group, ***$p<0.001$, $n=6$ in each group).

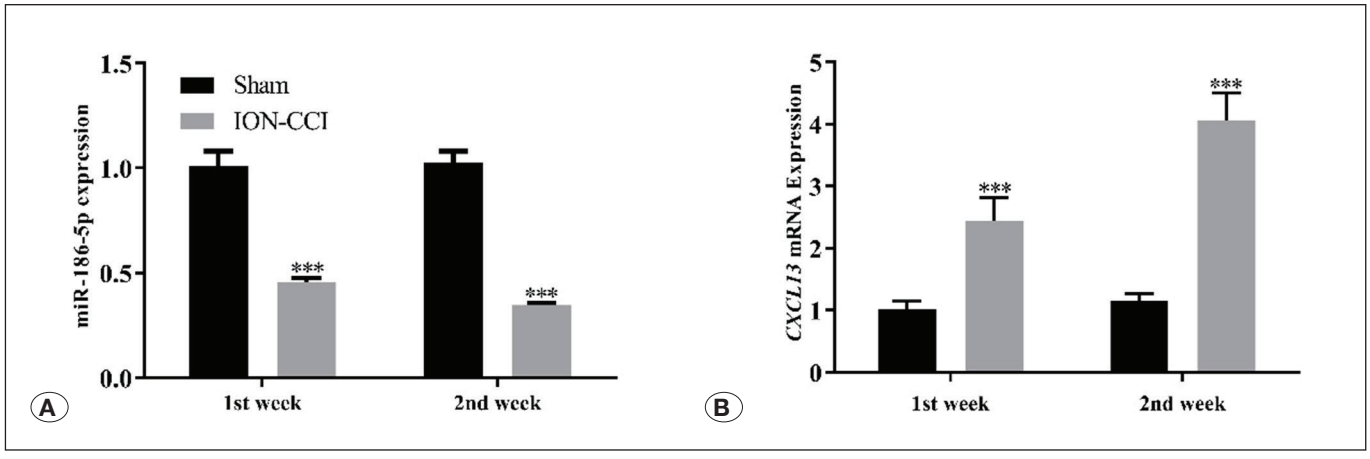

Figure 2: Expression of miR-186-5p and *CXCL13* mRNA in the TGs of sham and ION-CCI groups. **A)** Quantitative data analysis of miR-186-5p mRNA levels in the TGs of the sham and ION-CCI groups. **B)** Quantitative data analysis of *CXCL13* mRNA levels in the TGs of sham and ION-CCI groups (compared with the sham group, ***$p<0.001$ at the same postoperative time, $n=6$ in each group).

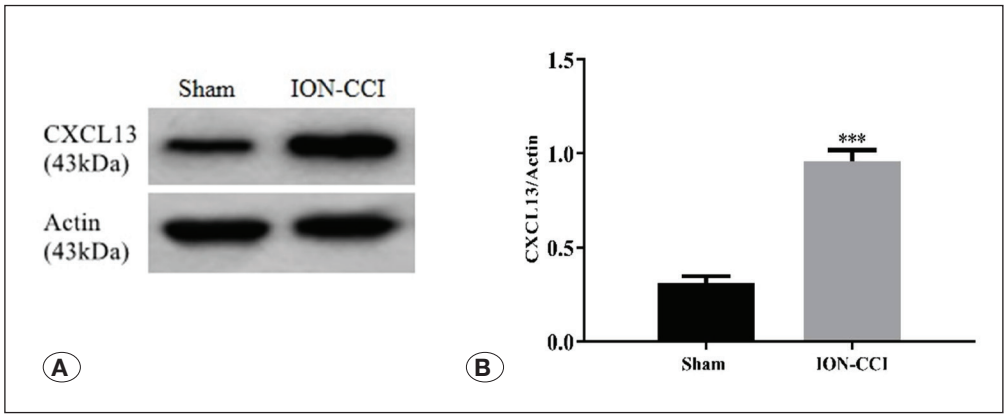

Figure 3: Protein expression of CXCL13 in the TG.
A) Representative immunoblotting bands of CXCL13 protein in the TGs of the sham group and the ION-CCI group at the second week after surgeries.
B) Quantitative data analysis of CXCL13 protein expression in the TGs between the sham group and the ION-CCI group at the second week after surgeries (compared with the sham group, ***p<0.001, n=3 in each group).

Our qRT-PCR results indicated that the mRNA levels of both miR-186-5p and CXCL13 were changed, but in different directions, following ION-CCI. Therefore, we assessed the changes in the protein expression of CXCL13 following ION-CCI. The protein level of CXCL13 in the second week after surgery was significantly higher in the ION-CCI group than in the sham group, indicating that ION-CCI surgery-induced an increase in CXCL13 expression (p<0.001) (Figure 3). The injury caused by ION-CCI may therefore induce increased expression of the CXCL13 protein and have a possible association with TN.

miR-186-5p mimic ameliorates ION-CCI-induced decreases in mechanical withdrawal thresholds and reduces CXCL13 expression in the TG.

We used an miR-186-5p mimic to increase the expression of miR-186-5p to determine the possible interaction between miR-186-5p and CXCL13, and the occurrence and development of TN. Mechanical withdrawal thresholds were significantly higher in the ION-CCI + miR-186-5p mimic group than in the ION-CCI + saline group on day 13 after surgery; the miR-186-5p mimic had already been injected each day for two consecutive days (p<0.01) (Figure 4A). The miR-186-5p inhibitor significantly reduced the mechanical withdrawal thresholds in the control + miR-186-5p inhibitor group compared to those in the control + saline group (second day, p<0.05; third day, p p<0.01) (Figure 4B).

We proceeded to determine whether the effects of the miR-186-5p mimic and miR-186-5p inhibitor on mechanical withdrawal thresholds were related to the protein levels of CXCL13 in the TG. Western blotting revealed that CXCL13 protein levels were significantly lower on the third day after drug injection in the ION-CCI + saline group (p<0.001) (Figure 4C, D), but significantly higher in the miR-186-5p inhibitor group on the third day after drug injection (p<0.001) (Figure 4E, F). These results suggest that miR-186-5p negatively regulates CXCL13 expression to influence the occurrence and development of TN.

DISCUSSION

TN is considered more severe and harder to treat than any other type of chronic pain (2). Although many scholars have attempted to explain TN pain using theories of peripheral aetiology and central aetiology, the specific pathogenesis of TN remains unknown. Various surgical treatments and drugs have been employed to treat TN; however, these approaches do not provide satisfactory pain relief (31). Therefore, novel animal models of TN are needed to further elucidate the aetiology and pathogenesis of TN and identify and develop new TN treatments.

Damage to peripheral nerves or tissues triggers inflammatory responses in local tissues but also increases the expression of inflammatory factors, such as TNF-α, IL-1β, and various chemokines (8,9,11,13). CXCL13 is a particularly important chemokine that plays a key role in the establishment of adaptive immune response by attracting B cells and promoting antibody production and local inflammation. Previous studies revealed that spinal neurons upregulate CXCL13 expression (15) after SNL, similar to TG neurons (32). Furthermore, CXCL13 and CXCR5 have been demonstrated to be involved in neuroinflammation, as detected in both neurons and glia (14). CXCL13 expression is also associated with chronic pain, as it is persistently upregulated in mouse spinal neurons after SNL and triggers spinal astrocytic activation via CXCR5, which in turn maintains neuropathic pain (14). CXCL13 also acts as a marker that reflects the local inflammatory activity of disease (27), and the CXCR5-CXCL13 pathway is involved in the initial generation of immune memory (28).

miRNAs have been demonstrated to play essential roles in the development and maintenance of inflammatory pain. Furthermore, miRNAs serve as pain regulators in various chronic pain models, such as those induced by sciatic nerve ligation (16), diabetic neuropathy (5), and CCI (17,18). A previous study found that miR-186-5p, miR-325-3p, and miR-1264-3p targeted CXCL13 mRNA; however, only the expression level of miR-186-5p was decreased on days 1, 3, and 10 post-SNL. Further, miR-186-5p was found to co-localise with NeuN and CXCL13. These results suggest that miR-186-5p is negatively associated with CXCL13 during the development of neuropathic pain (14).

Most studies that have investigated the role of miR-186-5p in pain only focused on SNL-induced neuropathic pain. Thus, whether miR-186-5p plays a similar role in TN remains unknown. Our findings indicate that the expression of miR-186-5p is

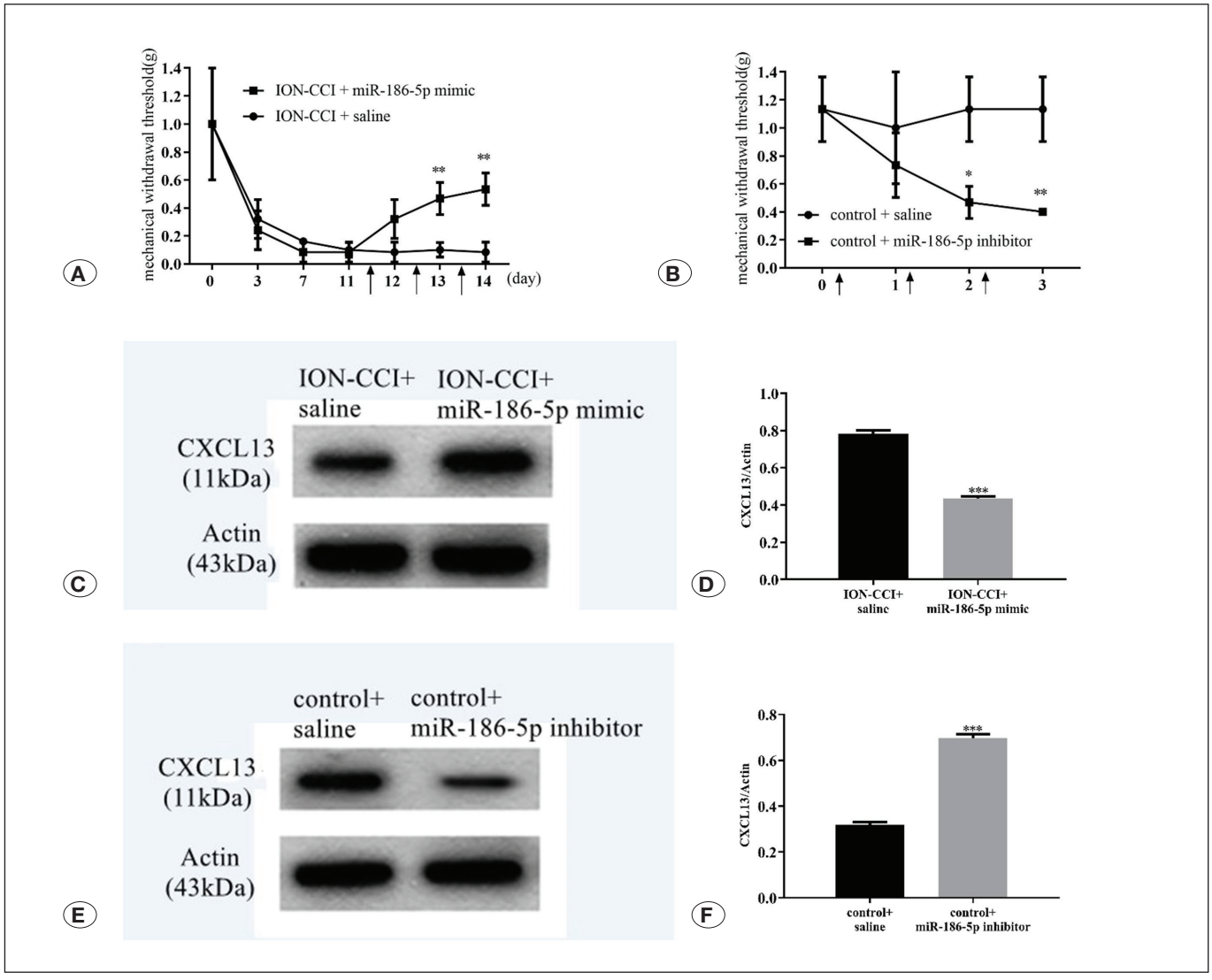

Figure 4: Effects of an miR-186-5p mimic or inhibitor on mechanical withdrawal thresholds and CXCL13 protein expression in the TG. **A)** Statistical analysis of mechanical withdrawal thresholds between the ION-CCI + saline group and the ION-CCI + miR-186-5p mimic group (compared with the ION-CCI + saline group, **p<0.01, n=3). **B)** Statistical analysis of mechanical withdrawal thresholds in the control + saline group and the control + miR-186-5p inhibitor group (compared to the saline group, *p<0.05, **p<0.01, n=3). **C)** Representative immunoblotting bands of CXCL13 protein from the TGs of the ION-CCI + saline group and the ION-CCI + miR-186-5p mimic groups. **D)** Quantitative data analysis of protein levels of CXCL13 in the TGs of the ION-CCI + saline group and the ION-CCI + miR-186-5p mimic group (compared with the ION-CCI + saline group, ***p<0.001, n=3). **E)** Representative immunoblotting bands of CXCL13 protein from the TGs of the control + saline group and the control + miR-186-5p inhibitor group. **F)** Quantitative data analysis of CXCL13 protein expression in the TGs of the control + saline group and the control + miR-186-5p inhibitor group (compared with the control + saline group, ***p<0.001, n=3). Note that the arrows in panels A and B denote the administration times.

downregulated in the TG after ION-CCI and overexpression of miR186-5p via an miR186-5p mimic reduces the expression of CXCL13. Such change alleviates the pain caused by ION-CCI, as indicated by higher mechanical withdrawal thresholds in the control + miR-186-5p inhibitor group compared to the control + saline group, and suggests a relationship between miR-186-5p and mechanical withdrawal thresholds in rats. CXCL13 protein levels were found to decrease after treatment with an miR-186-5p mimic in ION-CCI rats and increase via an miR-186-5p inhibitor. Collectively, these results suggest that miR-186-5p negatively regulates CXCL13 levels to influence the occurrence and development of TN.

In a study of CXCL13 and CXCR5, expression of CXCL13 and CXCR5 was found in TG neurons and colocalised with neurofilament 200 (NF200), IB4, and CGRP (32). CXCR5 is expressed in neurons in the TG and ACC, and astrocytes in the spinal cord, compared to the expression of CXCL13 in different areas of the body (14,29,32). Such findings indicate that CXCL13/CXCR5 mediates neuron-astrocyte interaction in the spinal cord and neuron-neuron interactions in the TG

and ACC. Studies using animal models of TN indicated that CXCL13 could mediate neuropathic pain in the maxillofacial region via the CXCR5/ERK pathway.

ERK is activated by injection of CXCL13, and ERK activation induced by SNL was found to be reduced in $Cxcr5^{-/-}$ mice (14). Thus, ERK may be downstream of CXCL13/ CXCR5 signalling. Furthermore, CXCL13/CXCR5 signalling was found to play a role in orofacial neuropathic pain by producing pro-inflammatory cytokines, such as ERK-dependent TNF-α and IL-1β, in the TG (32).

Currently, some pieces are still missing in the XXX problem. For example, CXCR5 is the only receptor of CXCL13, and pain hypersensitivity is attenuated in CXCR5 knockout mice (14). However, whether the effects of miR-186-5p on CXCL13 influence the expression of CXCR5 remain unknown, as the expression of pro-inflammatory cytokines was not determined in our study. Furthermore, CXCL13/CXCR5 increases Nav1.8 current density via p38, and CXCL13-induced pain hypersensitivity is inhibited by intrathecal injection of the p38 inhibitor, SB203580, or Nav1.8 sodium channel blocker, A-803467 (29). Therefore, future research should focus on following the pathway of miR-186-5p-CXCL13 to determine the possible involvement of ERK and other MAPK family proteins, such as p38 and JNK, in TN.

CONCLUSION

Collectively, our findings indicate that miR-186-5p and CXCL13 are expressed in rat TG. Further, miR-186-5p was found to be downregulated while CXCL13 caused an increase in the mechanical withdrawal thresholds in our ION-CCI rat model of TN. miR-186-5p also negatively regulated CXCL13 to influence the occurrence and development of TN in our ION-CCI rat model.

AUTHORSHIP CONTRIBUTION

Study conception and design: LF

Data collection: LY

Analysis and interpretation of results: XS

Draft manuscript preparation: LF

Critical revision of the article: LW

Other (study supervision, fundings, materials, etc...): YW

All authors (LF, LY, XS, LW, YW) reviewed the results and approved the final version of the manuscript.

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