

# LncRNA FTX ameliorates neuropathic pain by targeting miR-320a in a rat model of chronic constriction injury

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*Folia Neuropathol* 2023; 61 (3): 291-300

DOI: <https://doi.org/10.5114/fn.2023.126846>

## Abstract

**Introduction:** Long non-coding RNAs (lncRNAs) participate in the process of neuropathic pain (NP). Herein, the goal of this research was to examine the roles of lncRNA five prime to XIST (FTX) in influencing chronic constriction injury (CCI)-induced NP.

**Material and methods:** We have established a rat CCI model to simulate NP in vivo. Reverse transcription-quantitative PCR (RT-qPCR) was used to detect mRNA levels of FTX, microRNA (miR)-320a, and runt-related transcription factor 2 (RUNX2) in the spinal cord. This was followed by subsequent regulation of FTX or miR-320a levels in vivo by intrathecal injection of overexpression FTX or miR-320a mimic lentivirus. The behaviour of rat NP was assessed by the paw withdrawal threshold (PWT) and paw withdrawal latency (PWL). Enzyme-linked immunosorbent assay (ELISA) was used to assess the secretion of pro-inflammatory and anti-inflammatory factors in the spinal cord tissue. A correlation between miR-320a and FTX or RUNX2 was validated by luciferase reporter.

**Results:** FTX levels were reduced in CCI rats ( $p < 0.05$ ), and miR-320a was a direct target of FTX. Overexpression of FTX typically reduced PWL and PWT as well as neuroinflammation thus alleviating NP ( $p < 0.05$ ). However, increasing miR-320a reversed the alleviation of FTX on NP, increased PWL and PWT, and promoted neuroinflammation ( $p < 0.05$ ). Additionally, RUNX2, which is a miR-320a target gene, was significantly repressed in CCI rats and its expression was increased by FTX, however, this increase was attenuated by elevated miR-320a ( $p < 0.05$ ).

**Conclusions:** In the CCI-induced NP rat model, FTX attenuates NP and neuroinflammation by regulating the miR-320a/RUNX2 axis. This provides a new vision for NP treatment.

**Key words:** neuropathic pain, FTX, miR-320a, RUNX2, CCI.

## Introduction

Neuropathic pain (NP) is defined as chronic, disabling pain resulting from lesions or diseases of the somatosensory nervous system [25]. The prevalence of NP is usually 7-10% and occurs after diabetes, spinal cord injury (SCI), stroke, post-herpetic neuropathy infection, and toxic effects of chemotherapy drugs [2], and is characterized by ectopic pain, nociceptive hypersensitivity, and spontaneous pain symptoms such as mood disorders [3]. Although pharmacotherapy, nerve blocks, spinal cord stimulation, and surgical treatment

have been successfully applied in the treatment of neuropathic pain [16], however, significant challenges remain in its treatment. Therefore, more detailed studies of its underlying pathogenesis and the development of new effective management measures are crucial.

Long non-coding RNAs (lncRNAs) are linear RNA molecules that have no protein-coding ability. Aberrant expression of lncRNAs is associated with human diseases, particularly in the nervous system, and has been postulated as a therapeutic biomarker for neurogenic diseases. For instance, lncRNA MALAT1 [26], FIRRE

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[35], PVT1 [42], and CENDES [44] were identified to be involved in the pathological process of NP. Five prime to XIST (FTX) is a highly conserved lncRNA, located on chromosome Xq13.2, containing 2300 nucleotides. FTX is remarkably reduced in hepatitis B virus-associated cirrhosis and is associated with inflammation [22] and is a causative gene in rheumatoid arthritis [8]. FTX was significantly inhibited in hippocampal neuronal cells in both rats suffering from cerebral ischemia-reperfusion injury and *in vitro* oxygen-glucose deprivation/reoxygenation and increasing FTX reduced neuronal cell apoptosis and improved neural injury [34]. Additionally, inflammation and SCI are major contributions to the development of NP [42], and FTX has been reported to be involved in inflammation and SCI repair in microglia [39]. Of more interest is that Su *et al.* explored differentially expressed lncRNAs in the spinal cord after nerve injury, in which FTX was greatly downregulated [31]. Nevertheless, the regulatory mechanism of FTX in NP is unknown.

The current research focused on the role of FTX in NP and its molecular regulatory mechanism by establishing a rat model of chronic constriction injury (CCI), which may serve as novel insights and a broader therapeutic choice for the treatment of NP.

## Material and methods

### Animal research

Permission for the research was received from the First Affiliated Hospital, Zhejiang University School of Medicine Ethics Committee, and to conduct all experiments following the NIH Guide for care and use of laboratory animals. Additionally, this study followed the relevant regulations of the International Association for the Study of Pain (IASP) to reduce discomfort in rats. The Shanghai Laboratory Animal Centre provided 90 female Sprague-Dawley (SD) rats, weighing 290–380 g, housed in a laboratory animal centre at 20–25°C, 50–52% humidity, with a 12-h light/dark cycle, and with free access to food and water.

### Experimental design and grouping

Sprague-Dawley rats were randomly divided into a sham group and a CCI group after one week of adaptation to the environment. Subsequently, to examine the role of FTX in NP rats, the model group was further divided into the CCI + LV-NC group (a negative control lentiviral vector) and the CCI + LV-FTX group (FTX over-expression lentiviral). In another batch of rats, LV-miR-320a (miR-320a mimic lentivirus) and LV-miR-NC (corresponding control miRNA lentivirus) were intrathecally injected into CCI + LV-FTX rats to investigate the potential roles of FTX in NP. Among them, LV-FTX and LV-NC were obtained from GeneChem (Shanghai, China)

LV-miR-320a (miR-320a mimic lentivirus); LV-miR-NC were constructed by RiboBio (Guangzhou, China).

### Chronic constriction injury rat model construction

According to previous studies [19], CCI models were constructed to simulate NP *in vivo*. Briefly, 60 mg/kg pentobarbital was administered intraperitoneally to anesthetize the rats after one week of adaptation to the feeding environment. After a 3 cm incision was made in the skin between the gluteus and biceps femoris muscles on each side, the subcutaneous tissue and muscle membrane were separated to expose the sciatic nerve trunk. Surrounding loose connective tissue was separated, and the sciatic nerve approximately 7 mm in length, was isolated with a glass microneedle. Subsequently, a 4-0 chromic gut suture ligation was performed 2 mm above the trigeminal nerve region and 3 ligations were performed at 1 mm intervals with just enough pressure on the suture to produce a slight thermal scald. Only the isolated sciatic nerve was revealed in the sham group and was not ligated. Rats were euthanized at the end of all experiments using an overdose of pentobarbital sodium (120 mg/kg). And it was confirmed by pupil dilation, muscle relaxation, and complete cardiac arrest. The dorsal spinal cord tissue from L4 to L6 was acquired on days 0, 1, 3, 5, 7, 14, and 21 respectively.

### Intrathecal catheter injection

According to the method of previous studies, pentobarbital was injected intraperitoneally for anaesthesia, a skin incision was made in the midline region of the lumbar spine (L4-S1), and a polyethylene-10 (PE-10, 18 ± 2 cm) catheter was implanted in the rat through the area between L4 and S1 and advanced caudally about 7.8 cm to the subarachnoid space at the lumbar enlargement and in the posterior cervical region and the top of the occipital region. Additionally, intrathecal 10 µl of 5% lidocaine injection and immediate post-anaesthesia bilateral hind limb paralysis proved the successful placement of the tube. The rats were recovered for two days and then used for other experiments. After CCI operations, the lentiviral vectors LV-FTX, LV-NC, LV-miR-320a, and LV-miR-NC were infected by intrathecal injection at  $1 \times 10^7$  TU/0.1 ml for 3 consecutive days.

### Pain threshold assessment

Paw withdrawal threshold (PWT) was applied to examine mechanical pain. Rats were exposed to a clear plastic box (22 × 12 × 22 cm) containing a metal mesh liner at 0, 3, 7, 14, and 21 days postoperatively. After

domestication, a calibrated Von Frey plantar sensory apparatus with a thin wire lead was positioned on the plantar surface of the rat's hind paw and pressure was applied. The pressure applied during contraction was recorded by the instrument when the paw contracted, and the facility cut-off value was 50 g. The average value of pressure after three consecutive stimuli was taken.

Paw withdrawal latency (PWL) was performed via the BEM-410 automated thermal pain stimulator device to assess thermal hyperalgesia. The rats were placed in a clear glass box with raised glass, and the surface of the left hind paw was exposed to a radiant heat source separated by a glass plate for the time between stimulation and paw retraction and the mean of three consecutive test results was taken. A 30 s cut-off time was performed to prevent tissue burns in rats during the experiment.

### Reverse transcription-quantitative PCR

Rat L4-L5 spinal cord tissues were obtained separately, and total RNA was extracted and isolated by TRIzol reagent. A Nanodrop spectrophotometer checked the purity and concentration of RNA. Reverse transcription of 500 ng RNA complementary DNA by FastKing gDNA Dispelling RT SuperMix kit or miRcute Plus miRNA First-Strand cDNA kit. Reverse transcription-quantitative PCR (RT-qPCR) amplification reactions were undertaken on a LightCycler 480 machine using cDNA as a template and mixing primers and reagents from the SuperReal PreMix Plus (SYBR) and miRcute Plus miRNA qPCR kit (SYBR Green). GAPDH and U6 for normalizing lncRNA, mRNA, and miRNA expression were used as internal controls. And they were calculated in  $2^{-\Delta\Delta Ct}$  and performed in triplicate. The RT-qPCR was conducted using the following primer sequences: FTX: 5'-GAATGCCTTGTGAGGCAGTTG-3' (forward) and 5'-TGTCCTCATCATGGATGATCTG-3' (reverse); miR-320a: 5'-GGGCTAAAAGCTGGGTTGA-3' (forward) and 5'-CAGTGCGTGTGCTGGAGT-3' (reverse); RUNX2 5'-CATGGCCGGGAATGATGAG-3' (forward) and 5'-TGTGAAGACCGTTATGGTCAAAGTG-3' (reverse); GAPDH: 5'-GGA-TGCTGGAGGTCTGCGAGGAAC-3' (forward) and 5'-GAGA-GGAAGCGTGTGAGGCGGTAG-3' (reverse); U6: 5'-GCAG-GAGGTCTTACAGAGT-3' (forward) and 5'-TCTAGAGGA-GAAGCTGGGGT-3' (reverse). RT-qPCR analysis was carried out as follows: 95°C for 15 min, followed by 40 cycles of 95°C for 15 s, 55°C for 30 s and 70°C for 30 s.

### Enzyme-linked immunosorbent assay

Inflammation factors, interleukin (IL)-6, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$ , IL-4 and IL-10 in rats' spinal cord tissues were measured by enzyme-linked immunosorbent assay (ELISA) kit. Rat IL-6 ELISA kit

(ab234570), rat TNF- $\alpha$  ELISA kit (ab234712), rat IL-1 $\beta$  ELISA kit (ab255730), rat IL-10 ELISA kit (ab214566), and rat IL-4 ELISA kit (ab100770) were obtained from Abcam (Cambridge, MA, USA). Briefly, postoperative (14 days') rat spinal cord tissue was spiked with the protein lysate RIPA. 3 h after lysis, the supernatant was collected by centrifugation at 10,000 rpm for 10 min. We set up standard wells and sample wells on the ELISA plate, and diluted samples and protein standards added and incubated at 37°C for 90 min, washed and secondary antibody was added, and incubated at room temperature for 2 h, measured absorbance at 405 nm after incubation at room temperature with nitrobenzene phosphate, and protein concentration was calculated.

### Dual-luciferase reporter analysis

The binding sites of FTX and runt-related transcription factor 2 (RUNX2) were combined with miR-320a by the online software StarBase 3.0 and miRWalk. Wild-type (WT) and mutant (MUT) containing miR-320a binding sites were constructed and subcloned onto the luciferase reporter plasmid pmirGLO to form the FTX-WT, RUNX2-WT, FTX-MUT, and RUNX2-MUT plasmids, respectively. 293T were inoculated in 48-well plates and miR-320a mimic or inhibitor was mixed with 200 ng of FTX-WT, RUNX2-WT, FTX-MUT, and RUNX2-MUT plasmids, and supplemented with Lipofectamine 2000 at room temperature for 20 min and then dropped the mixture into 293T. And the cells were lysed after 48 h, and the activity was evaluated by Dual-luciferase reporter assay (Promega).

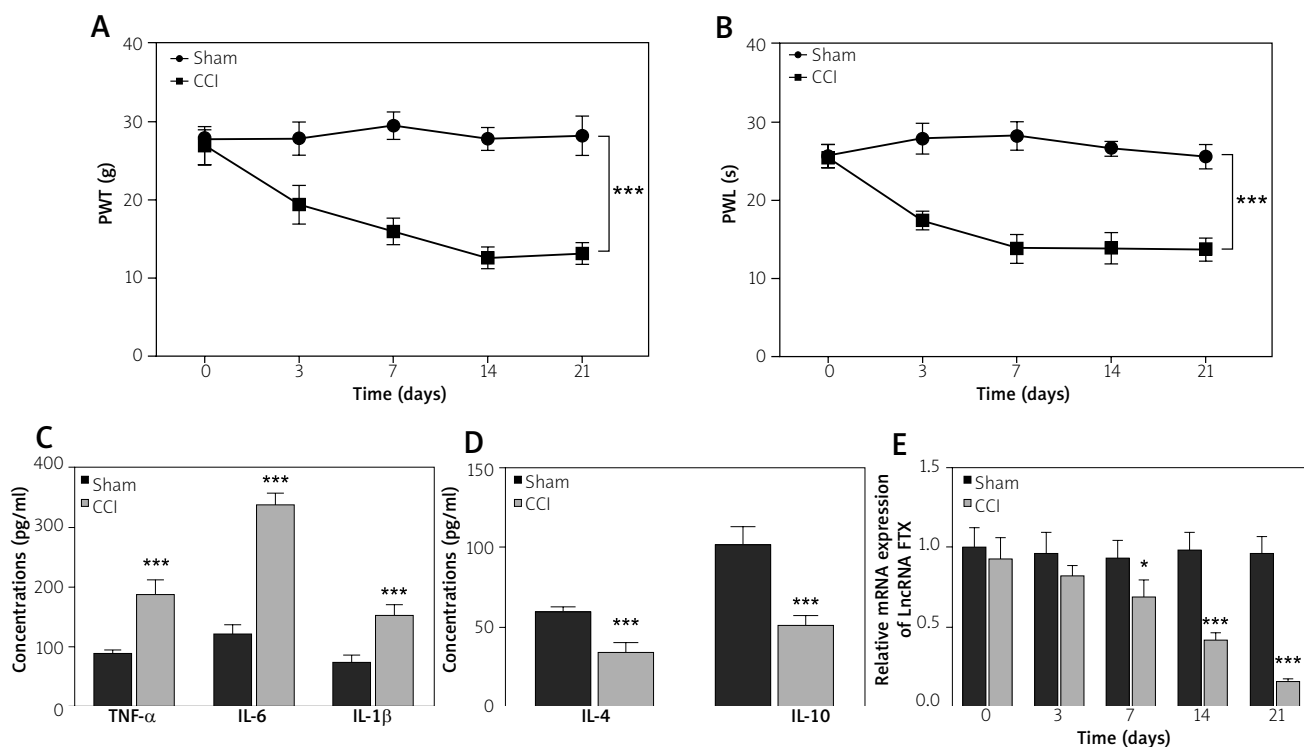
### Statistical analysis

GraphPad Prism 6 software was applied for data processing. At least triplicate analyses were performed with measurements expressed as mean  $\pm$ SD. Student's *t*-test and one way ANOVA by Tukey's multiple comparison post hoc test was conducted to evaluate the statistical significance between two or multiple groups. Statistical significance was established at  $p < 0.05$ .

## Results

### FTX was downgauged in rats undergoing CCI

After the CCI rat model was constructed, the PWT and PWL were first measured. As shown in Figure 1A, B, both PWT and PWL were significantly lower in the CCI group ( $p < 0.05$ ). The results verified that the CCI models were successfully constructed. ELISA indicated that the CCI caused the release of pro-inflammatory factors TNF- $\alpha$ , IL-6, and IL-1 $\beta$  ( $p < 0.05$ , Fig. 1C) and suppressed the anti-inflammatory factors IL-4 and IL-10 ( $p < 0.05$ , Fig. 1D).



**Fig. 1.** FTX was decreased in rats undergoing chronic constriction injury (CCI). Levels of PWT (A) and PWL (B) in CCI rats. Levels of pro-inflammatory cytokines TNF- $\alpha$ , IL-6, and IL-1 $\beta$  (C) and anti-inflammatory factors IL-4 and IL-10 (D) in rats' spinal cord were measured by ELISA. E) RT-qPCR was performed to investigate the mRNA levels of FTX in CCI and sham rats at days 0, 3, 7, 14, and 21.  $n = 10$ ; \* $p < 0.05$ , \*\*\* $p < 0.001$  vs. sham group.

Critically, the mRNA levels of FTX were time-dependently reduced in the CCI group ( $p < 0.05$ , Fig. 1E). Therefore, we speculate whether the down-regulated FTX regulates neuro-inflammation and is involved in NP progression.

### Elevated FTX alleviates NP in CCI rats

To examine the potential functions of FTX in CCI-induced NP progression, the intrathecal LV-FTX virus was injected to overexpress FTX for loss-of-function experiments. CCI + LV-FTX dramatically increased FTX mRNA expression in the rat spinal cord compared with CCI + LV-NC ( $p < 0.05$ , Fig. 2A). Additionally, the CCI model-induced PWT was significantly restored due to the increase in FTX ( $p < 0.05$ , Fig. 2B). Consistently, PWL was markedly increased in the FTX overexpressed group of LV-FTX compared to CCI + LV-NC ( $p < 0.05$ , Fig. 2C). Based on the above study, it was confirmed that FTX increase alleviated NP behaviour in CCI rats.

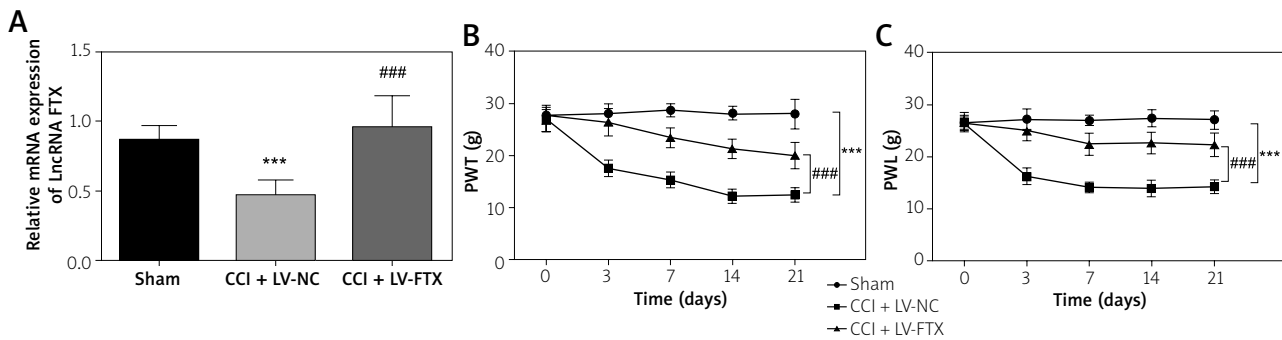
### Increased FTX attenuates the neuroinflammation *in vivo*

Subsequently, we further examined the regulation of neuroinflammatory factor secretion by increased FTX

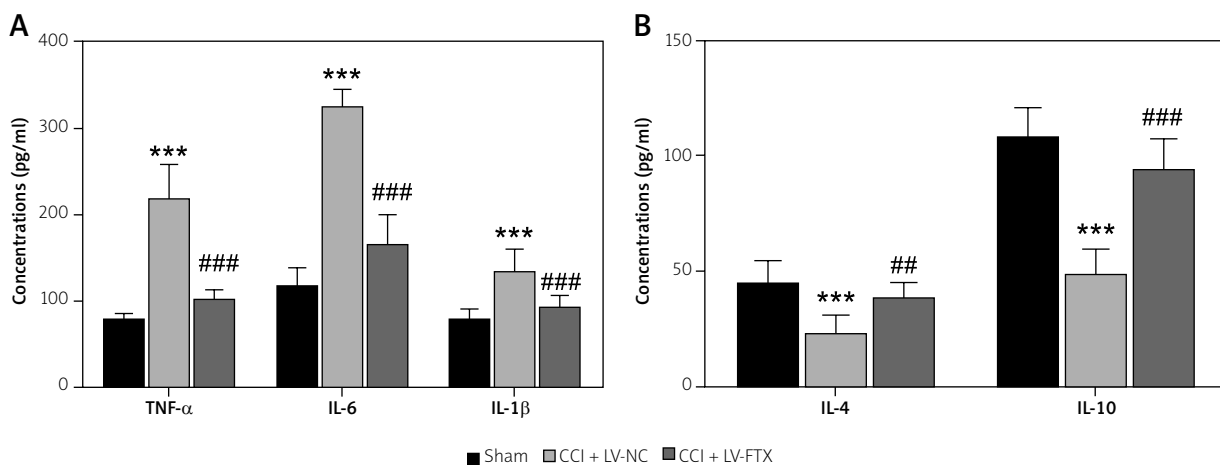
in a CCI rat model. ELISA results showed that CCI-induced secretion of pro-inflammatory factors (TNF- $\alpha$ , IL-6, and IL-1 $\beta$ ) were markedly inhibited by overexpression of FTX ( $p < 0.05$ , Fig. 3A). What is more, compared to the CCI + LV-NC group, the secretion of anti-inflammatory factors (IL-4 and IL-10) was increased in the elevated FTX group ( $p < 0.05$ , Fig. 3B). Thus, the data support that reduced FTX may exacerbate the progression of NP by promoting neuroinflammation.

### MiR-320a functions as a target of FTX

We attempted to understand the mechanisms by which FTX regulates NP. StarBase database found that FTX has bound to miR-320a (Fig. 4A). And the dual-luciferase reporter displayed that the luciferase activity of FTX-WT was reduced upon con-transfection with miR-320a mimic ( $p < 0.05$ , Fig. 4B), while no statistical difference was observed for FTX-MUT. What is more, miR-320a levels were higher in CCI rats as the post-operative time increased ( $p < 0.05$ , Fig. 4C). Overexpression of FTX significantly suppressed the increase of miR-320a caused by CCI induction ( $p < 0.05$ , Fig. 4D).



**Fig. 2.** Elevated FTX could facilitate the attenuation of neuropathic pain (NP) in chronic constriction injury (CCI) rats. **A)** RT-qPCR was applied to investigate the levels of FTX in the rat spinal cord after intrathecal injection of LV-FTX. NP-related behaviours: PWT (**B)**) and PWL (**C)**) were measured in CCI rats after increasing FTX.  $n = 10$ ; \*\*\* $p < 0.001$  vs. sham group, ### $p < 0.001$  vs. CCI + LV-NC.



**Fig. 3.** Elevated FTX alleviated the neuroinflammation in chronic constriction injury (CCI) rats. ELISA for the determination of pro-inflammatory factors (TNF- $\alpha$ , IL-6, IL-1 $\beta$ ) (**A**) and anti-inflammatory factors (IL-4 and IL-10) (**B**) in rat spinal cord tissue.  $n = 10$ ; \*\*\* $p < 0.001$  vs. sham group, ### $p < 0.001$  vs. CCI + LV-NC.

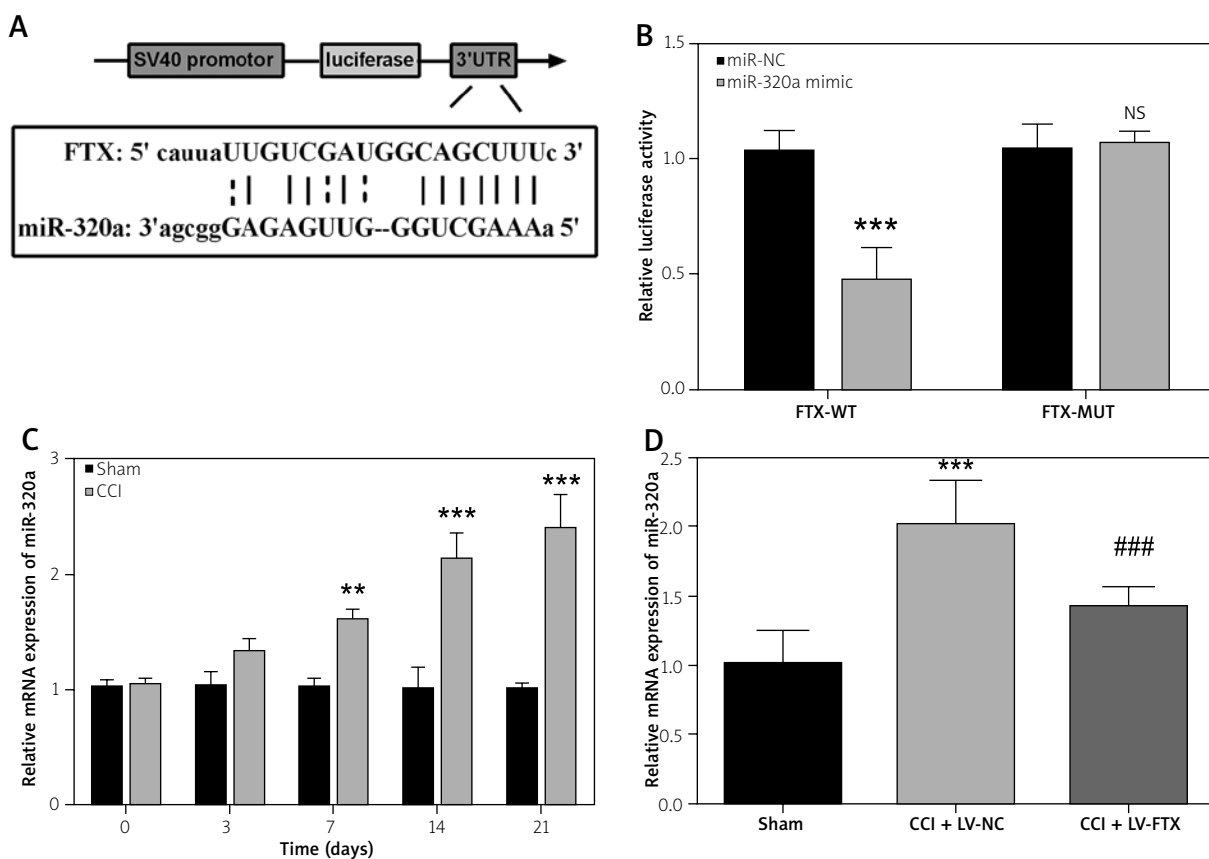
### Elevated miR-320a reversed the alleviation of NP behaviour and neuro-inflammation by overexpression of FTX

The effects of miR-320a on the NP behaviour and neuro-inflammation were then analysed. There was a significant elevation of miR-320a in CCI + LV-FTX rats by intrathecal injection of LV-miR-320a ( $p < 0.05$ , Fig. 5A), which reversed the inhibition of miR-320a by FTX. Additionally, elevated miR-320a also suppressed the increase in PWL and PWT compared to CCI + LV-NC + LV-miR-NC, reversing the alleviation of FTX on NP behaviour in CCI rats ( $p < 0.05$ , Fig. 5B, C). In terms of neuroinflammation, increased miR-320a reversed the suppression of pro-inflammatory factors and promotion of anti-inflammatory factors by FTX, increasing the neuroinflammatory response ( $p < 0.05$ , Fig. 5D, E). In conclusion, FTX may regulate neuroinflammation

through adsorption of miR-320a and thus participate in NP progression.

### RUNX2 was a direct target of miR-320a

To illustrate the specific mechanism of NP repression by FTX/miR-320a, we searched for the target of miR-320a. miR-320a has a binding sequence with RUNX2 (Fig. 6A). And dual-luciferase reporter displayed that miR-320a mimic suppressed the luciferase activity of RUNX2-WT ( $p < 0.05$ , Fig. 6B) and had no effect on RUNX2-MUT. Additionally, RUNX2 was typically reduced in the spinal cord of CCI rats with increasing duration of surgery ( $p < 0.05$ , Fig. 6C). Finally, elevated FTX increased the expression of RUNX2 in CCI rats, while overexpression of miR-320a diminished this facilitation ( $p < 0.05$ , Fig. 6D). These results confirmed that miR-320a directly acts on RUNX2 involved in NP progression.



**Fig. 4.** miR-320a functions as a target of FTX. **A)** Binding site sequences of miR-320a and FTX predicted by StarBase database. **B)** Dual-luciferase reporter to check the targeting between FTX and miR-320a. **C)** RT-qPCR was applied to explore the mRNA levels of miR-320a in the rat spinal cord. **D)** mRNA levels of miR-320a in rat spinal cord tissue after intrathecal injection of LV-FTX.  $n = 10$ ;  $***p < 0.001$  vs. sham group or miR-NC,  $###p < 0.001$  vs. CCI + LV-NC.

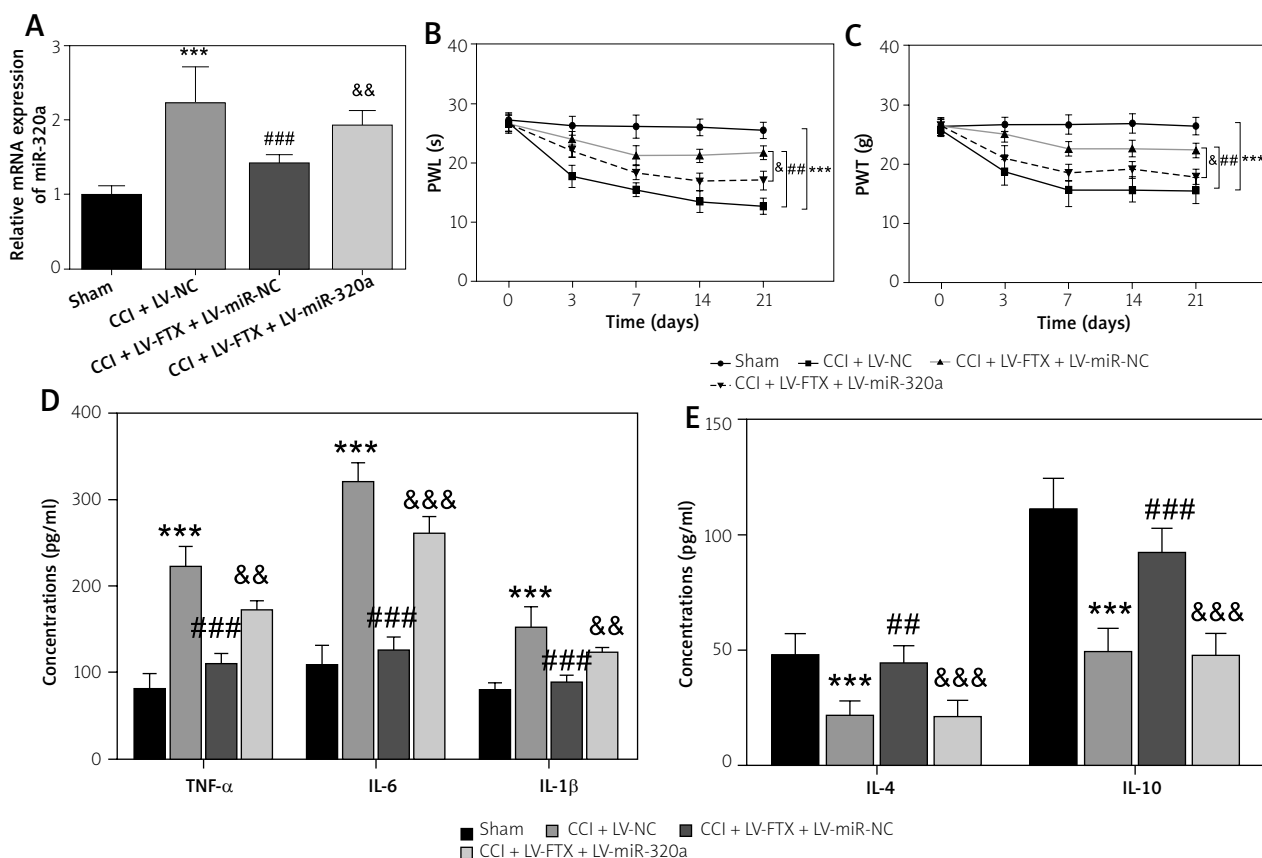
## Discussion

Neuropathic pain is a complex chronic pain, caused by disease or trauma that disrupts the neuroplasticity of the somatosensory system and induces neuroinflammation resulting in neuropathy [21]. Common drugs such as anticonvulsants acting on calcium channels, tricyclic antidepressants (amitriptyline), and 5-hydroxytryptamine noradrenaline reuptake inhibitors (duloxetine) only moderately alleviate NP but do not fully address the underlying pathophysiology, making it particularly critical to developing new targets for the treatment of NP [20,38].

The bilateral sciatic nerve chronic CCI model is considered to be a representative model for humans suffering from NP [10]. To better understand the molecular mechanism of NP, we used the CCI model to simulate the occurrence of NP. Although the death of 1 rat existed at the time of model construction, we took into account

the expected mortality rate before the experiment and thus we constructed the model in more rats than planned. Herein, we observed that both PWT and PWL, indicators of NP severity, were significantly suppressed in the model group with increasing time, which is consistent with previous findings [43]. The results suggest that we have successfully constructed an *in vivo* NP model in rats. Furthermore, chronic inflammation leads to neurological dysfunction, which is the main cause of NP, and therefore the treatment of NP usually focuses on suppressing the inflammation [43]. Similar to previous studies, we found that pro-inflammatory factors were significantly increased, while anti-inflammatory factors were significantly suppressed in the spinal cord tissue of CCI model rats [27,28].

Dysregulated lncRNAs assume critical function in neuronal injury and neuroinflammation, and most are involved in NP progression. lncRNA p21 is implicated in glial cell apoptosis and inflammatory response through



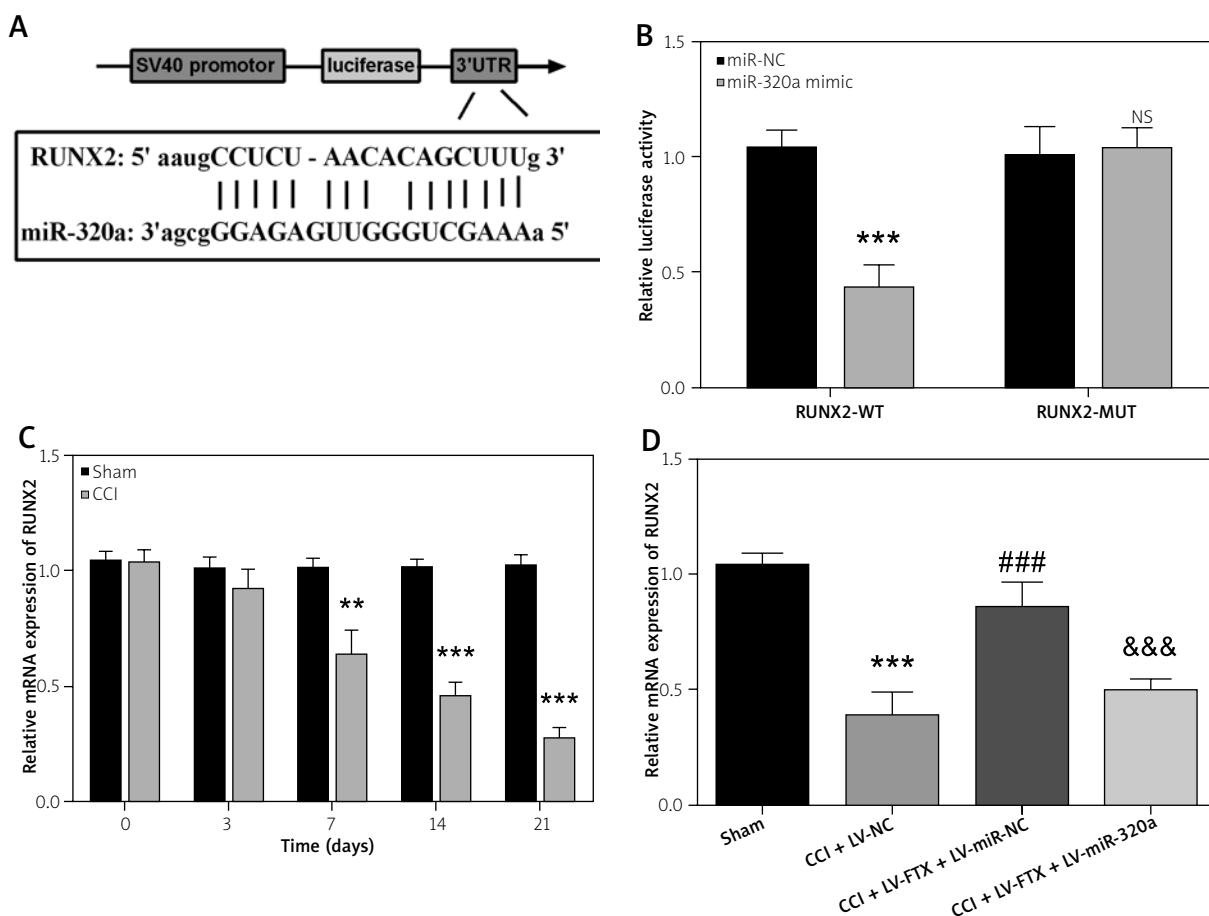
**Fig. 5.** Elevated miR-320a reversed the alleviation of neuropathic pain (NP) behaviour and neuro-inflammation by overexpression of FTX. **A**) mRNA levels of miR-320a after intrathecal injection of LV-miR-320a. PWT (**B**) and PWL (**C**) assessed the regulation of miR-320a overexpression on mechanical ectopic and hyperalgesia. ELISA was employed to assess the effect of elevated miR-320a in chronic constriction injury (CCI) rats on the secretion of pro-inflammation (**D**) and anti-inflammation factors (**E**).  $n = 10$ ; \*\*\* $p < 0.001$  vs. sham group, ## $p < 0.01$ , ### $p < 0.001$  vs. CCI + LV-NC, && $p < 0.01$ , &&& $p < 0.001$  vs. CCI + LV-FTX + LV-miR-NC.

the regulation of miR-181b/TNFAIP 1 thus participating in the progression of NP [24]. Upregulated MEG3 exacerbates NP in CCI rats as well as LPS-induced astrocyte inflammatory release and expression [9].

The potential role of dysregulated FTX has been noted in asthma [29], NAFLD [36], and colorectal cancer [45]. Previously, it has been shown that FTX covalently attenuates epilepsy-induced apoptosis in hippocampal neurons and exerts neuroprotective effects [18]. Neurite growth is closely associated with neuronal degeneration after central nervous system (CNS) injury, and FTX overexpression eliminated the Nogo-66-induced reduction in neurite length [47]. Previous studies have confirmed that the rat's hind paw is susceptible to NP after prolonged ischemia-reperfusion [14], while FTX is significantly reduced in SD rats with cerebral ischemia-reperfusion [34]. Spinal cord injury is one of the major causes of NP, FTX is abnormally decreased after spinal cord injury, and its knockdown promotes the

induction of inflammatory response by LPS [42]. Herein, we discovered that FTX mRNA levels declined significantly with increasing time after CCI surgery. And when FTX was overexpressed in CCI rats, it significantly attenuated NP-related behaviours, PWL and PWT, and suppressed the inflammatory response induced by CCI.

LncRNAs are known to adsorb miRNAs, thereby diminishing the regulation of mRNA by miRNAs. Numerous miRNAs have been reported to be involved in reporting various diseases such as neuropathy, cardiovascular diseases, cancer, as well as NP. For example, miR-138, miR-140, and miR-223 are involved in the progression of NP [13,17,46]. One study analysed differential miRNA expression in plasma from neuropathic chronic musculoskeletal pain patients and found that miR-320a was significantly reduced [6]. Ischemic preconditioning is involved in neuroprotection in diabetic rats through modulation of miR-320a [1]. Additionally, miR-320a was identified as an inflammatory regulator



**Fig. 6.** RUNX2 was a direct target of miR-320a. **A)** The binding sequence between miR-320a and RUNX2. **B)** Dual-luciferase reporter analysis was applied to verify the relationship between miR-320a and RUNX2. **C)** mRNA levels of RUNX2 in rat spinal cord after chronic constriction injury (CCI) surgery. **D)** mRNA levels of RUNX2 in rat spinal cord tissue after intrathecal injection of LV-FTX + LV-miR-320a.  $n = 10$ ;  $**p < 0.01$ ,  $***p < 0.001$  vs. sham group or miR-NC,  $###p < 0.001$  vs. CCI + LV-NC,  $\&\&p < 0.001$  vs. CCI + LV-FTX + LV-miR-320a.

and was reported to be associated with inflammatory bowel disease [5], atherosclerosis [40], and acute lung injury [15]. The targeting relationship between miR-320a and FTX was confirmed in osteosarcoma [12]. Our study identified potential binding sites for FTX and miR-320a, and the dual-luciferase reporter confirmed them. And miR-320a was typically increased in CCI rats and FTX inhibited the level increase, but this inhibition was typically attenuated when miR-320a was overexpressed. Additionally, elevated miR-320a significantly attenuated the mitigating effect of FTX on NP behaviour and neuro-inflammation.

RUNX2 has been described as a transcription factor for osteoblast differentiation, with the potential role in inflammation as well as neurological damage in addition to bone-related diseases. Wang *et al.* discovered that RUNX2 was upregulated in sciatic nerve injury and was involved in the palliative effect of curcumin [33]. It was also involved in nerve protrusion growth and

migration after sciatic nerve compression [7]. RUNX2 is involved in the inflammatory response in a variety of diseases, such as cardiac aortic valve calcification [30], vascular injury repair [37], osteoarthritis [23], and atherosclerosis [4]. More importantly, the targeting relationship of RUNX2 with miR-320a was confirmed in polycystic soft nest syndrome [41], osteoarthritis [32], and MSC adipocyte differentiation [11]. RUNX2 was found to target binding to miR-320a in our study and RUNX2 was reduced in CCI rats. There are undeniable limitations of this study. The role of the FTX/miR-320a/RUNX2 axis in pain needs to be validated in more rat samples and clinical samples. Overall, the association of the FTX/miR-320a/RUNX2 axis in the progression of NP was demonstrated for the first time in this study. We showed that FTX attenuates NP-related behaviours and neuroinflammation in CCI-induced NP rat models by inhibiting miR-320a/RUNX2.



## Funding

This study was funded by the Natural Science Foundation of Zhejiang Province (LQ22H090022).

## Disclosure

The authors report no conflict of interest.

## References

- Altintas O, Ozgen Altintas M, Kumas M, Asil T. Neuroprotective effect of ischemic preconditioning via modulating the expression of cerebral miRNAs against transient cerebral ischemia in diabetic rats. *Neurol Res* 2016; 38: 1003-1011.
- Boakye PA, Tang SJ, Smith PA. Mediators of neuropathic pain; focus on spinal microglia, CSF-1, BDNF, CCL21, TNF-alpha, Wnt ligands, and interleukin 1beta. *Front Pain Res (Lausanne)* 2021; 2: 698157.
- Chen P, Wang C, Lin D, Li B, Ye S, Qu J, Wang W. Identification of Slc6a19os and SOX11 as two novel essential genes in neuropathic pain using integrated bioinformatic analysis and experimental verification. *Front Neurosci* 2021; 15: 627945.
- Chen Y, Zhao X, Wu H. Transcriptional programming in arteriosclerotic disease: a multifaceted function of the Runx2 (Runt-related transcription factor 2). *Arterioscler Thromb Vasc Biol* 2021; 41: 20-34.
- Cordes F, Demmig C, Bokemeyer A, Bruckner M, Lenze F, Lenz P, Nowacki T, Tepaspe P, Schmidt HH, Schmidt MA, Cichon C, Bettenworth D. MicroRNA-320a monitors intestinal disease activity in patients with inflammatory bowel disease. *Clin Transl Gastroenterol* 2020; 11: e00134.
- Dayer CF, Luthi F, Le Carre J, Vuistiner P, Terrier P, Benaim C, Giacobino JP, Leger B. Differences in the miRNA signatures of chronic musculoskeletal pain patients from neuropathic or nociceptive origins. *PLoS One* 2019; 14: e0219311.
- Ding D, Zhang P, Liu Y, Wang Y, Sun W, Yu Z, Cheng Z, Wang Y. Runx2 was correlated with neurite outgrowth and schwann cell differentiation, migration after sciatic nerve crush. *Neurochem Res* 2018; 43: 2423-2434.
- Dolcino M, Tinazzi E, Puccetti A, Lunardi C. Long non-coding RNAs target pathogenetically relevant genes and pathways in rheumatoid arthritis. *Cells* 2019; 8: 816.
- Dong J, Xia R, Zhang Z, Xu C. lncRNA MEG3 aggravated neuropathic pain and astrocyte overaction through mediating miR-130a-5p/CXCL12/CXCR4 axis. *Aging (Albany NY)* 2021; 13: 23004-23019.
- Dou L, Lin H, Wang K, Zhu G, Zou X, Chang E, Zhu Y. Long non-coding RNA CCAT1 modulates neuropathic pain progression through sponging miR-155. *Oncotarget* 2017; 8: 89949-89957.
- Hamam D, Ali D, Vishnubalaji R, Hamam R, Al-Nbaheen M, Chen L, Kassem M, Aldahmash A, Alajez NM. microRNA-320/RUNX2 axis regulates adipocytic differentiation of human mesenchymal (skeletal) stem cells. *Cell Death Dis* 2014; 5: e1499.
- Huang S, Zhu X, Ke Y, Xiao D, Liang C, Chen J, Chang Y. lncRNA FTX inhibition restrains osteosarcoma proliferation and migration via modulating miR-320a/TXNRD1. *Cancer Biol Ther* 2020; 21: 379-387.
- Jin Y, Xu L, Xu Y. Effect of intrathecal injection of miRNA-138 on neuropathic pain in rats undergoing partial sciatic nerve ligation and its underlying mechanism. *Ann Palliat Med* 2021; 10: 6873-6882.
- Kim KW, Ha MJ, Jung KY, Kwak KH, Park SS, Lim DG. Reactive oxygen species and N-methyl-D-aspartate receptor-mediated central sensitization in hindlimb ischemia/reperfusion injury-induced neuropathic pain rats. *Korean J Anesthesiol* 2009; 56: 186-194.
- Lee H, Zhang D, Zhu Z, Dela Cruz CS, Jin Y. Epithelial cell-derived microvesicles activate macrophages and promote inflammation via microvesicle-containing microRNAs. *Sci Rep* 2016; 6: 35250.
- Li H, Fan L, Zhang Y, Cao Y, Liu X. SNHG16 aggravates chronic constriction injury-induced neuropathic pain in rats via binding with miR-124-3p and miR-141-3p to upregulate JAG1. *Brain Res Bull* 2020; 165: 228-237.
- Li J, Zhu Y, Ma Z, Liu Y, Sun Z, Wu Y. miR-140 ameliorates neuropathic pain in CCI rats by targeting S1PR1. *J Recept Signal Transduct Res* 2021; 41: 401-407.
- Li X, Giri V, Cui Y, Yin M, Xian Z, Li J. lncRNA FTX inhibits hippocampal neuron apoptosis by regulating miR-21-5p/SOX7 axis in a rat model of temporal lobe epilepsy. *Biochem Biophys Res Commun* 2019; 512: 79-86.
- Li Z, Li A, Yan L, Yang T, Xu W, Fan P. Downregulation of long noncoding RNA DLEU1 attenuates hypersensitivity in chronic constriction injury-induced neuropathic pain in rats by targeting miR-133a-3p/SRPK1 axis. *Mol Med* 2020; 26: 104.
- Li Z, Li X, Chen X, Li S, Ho IHT, Liu X, Chan MTV, Wu WKK. Emerging roles of long non-coding RNAs in neuropathic pain. *Cell Prolif* 2019; 52: e12528.
- Lim EY, Lee C, Kim YT. The antinociceptive potential of Camellia japonica leaf extract, (-)-epicatechin, and rutin against chronic constriction injury-induced neuropathic pain in rats. *Antioxidants (Basel)* 2022; 11: 410.
- Liu X, Li C, Zhu J, Li W, Zhu Q. Dysregulation of FTX/miR-545 signaling pathway downregulates Tim-3 and is responsible for the abnormal activation of macrophage in cirrhosis. *J Cell Biochem* 2018; 120: 2336-2346.
- Liu X, Li X, Hua B, Yang X, Zheng J, Liu S. WNT16 is upregulated early in mouse TMJ osteoarthritis and protects fibrochondrocytes against IL-1beta induced inflammatory response by regulation of RUNX2/MMP13 cascade. *Bone* 2021; 143: 115793.
- Liu Y, Sun H, Sun Y. lncRNA p21, downregulating miR-181b, aggravates neuropathic pain by upregulating Tnfrsf1 and inhibit the AKT/CREB axis. *Brain Res Bull* 2021; 171: 150-161.
- Luo D, Li X, Tang S, Song F, Li W, Xie G, Liang J, Zhou J. Epigenetic modifications in neuropathic pain. *Mol Pain* 2021; 17: 17448069211056767.
- Ma X, Wang H, Song T, Wang W, Zhang Z. lncRNA MALAT1 contributes to neuropathic pain development through regulating miR-129-5p/HMGB1 axis in a rat model of chronic constriction injury. *Int J Neurosci* 2020; 130: 1215-1224.
- Murray I, Bhanot G, Bhargava A. Neuron-glia-immune triad and cortico-limbic system in pathology of pain. *Cells* 2021; 10: 1553.
- Raimondo TM, Mooney DJ. Anti-inflammatory nanoparticles significantly improve muscle function in a murine model of advanced muscular dystrophy. *Sci Adv* 2021; 7: eabh3693.
- Shen Y, Yang G, Zhuo S, Zhuang H, Chen S. lncRNA FTX promotes asthma progression by sponging miR-590-5p and upregulating JAK2. *Am J Transl Res* 2021; 13: 8833-8846.
- Sikura KÉ, Combi Z, Potor L, Szerafin T, Hendrik Z, Méhes G, Gergely P, Whiteman M, Beke L, Fürtös I, Balla G, Balla J. Hydrogen sulfide inhibits aortic valve calcification in heart via regu-

- lating RUNX2 by NF- $\kappa$ B, a link between inflammation and mineralization. *J Adv Res* 2021; 27: 165-176.
31. Su S, Li M, Wu D, Cao J, Ren X, Tao YX, Zang W. Gene transcript alterations in the spinal cord, anterior cingulate cortex, and amygdala in mice following peripheral nerve injury. *Front Cell Dev Biol* 2021; 9: 634810.
  32. Wang CG, Hu YH, Su SL, Zhong D. LncRNA DANCR and miR-320a suppressed osteogenic differentiation in osteoporosis by directly inhibiting the Wnt/ $\beta$ -catenin signaling pathway. *Exp Mol Med* 2020; 52: 1310-1325.
  33. Wang G, Wang Z, Gao S, Wang Y, Li Q. Curcumin enhances the proliferation and myelination of Schwann cells through Runx2 to repair sciatic nerve injury. *Neurosci Lett* 2022; 770: 136391.
  34. Wang W, Hu Y, Zhang Y. FTX Attenuates cerebral ischemia-reperfusion injury by inhibiting apoptosis and oxidative stress via miR-186-5p/MDM4 pathway. *Neurotox Res* 2022; 40: 542-552.
  35. Wen Y, Fan X, Bu H, Ma L, Kong C, Huang C, Xu Y. Downregulation of lncRNA FIRRE relieved the neuropathic pain of female mice by suppressing HMGB1 expression. *Mol Cell Biochem* 2021; 476: 841-852.
  36. Wu H, Zhong Z, Wang A, Yuan C, Ning K, Hu H, Wang C, Yin X. LncRNA FTX represses the progression of non-alcoholic fatty liver disease to hepatocellular carcinoma via regulating the M1/M2 polarization of Kupffer cells. *Cancer Cell Int* 2020; 20: 266.
  37. Wu K, Cai Z, Liu B, Hu Y, Yang P. RUNX2 promotes vascular injury repair by activating miR-23a and inhibiting TGFBR2. *Ann Transl Med* 2021; 9: 363.
  38. Xian S, Ding R, Li M, Chen F. LncRNA NEAT1/miR-128-3p/AQP4 axis regulating spinal cord injury-induced neuropathic pain progression. *J Neuroimmunol* 2021; 351: 577457.
  39. Xiang W, Jiang L, Zhou Y, Li Z, Zhao Q, Wu T, Cao Y, Zhou J. The lncRNA Ftx/miR-382-5p/Nrg1 axis improves the inflammation response of microglia and spinal cord injury repair. *Neurochem Int* 2021; 143: 104929.
  40. Zhang C, Wang X. miR-320a targeting RGS5 aggravates atherosclerosis by promoting migration and proliferation of ox-LDL-stimulated vascular smooth muscle cells. *J Cardiovasc Pharmacol* 2022; 80: 110-117.
  41. Zhang CL, Wang H, Yan CY, Gao XF, Ling XJ. Deregulation of RUNX2 by miR-320a deficiency impairs steroidogenesis in cumulus granulosa cells from polycystic ovary syndrome (PCOS) patients. *Biochem Biophys Res Commun* 2017; 482: 1469-1476.
  42. Zhang P, Sun H, Ji Z. Downregulating lncRNA PVT1 relieves astrocyte overactivation induced neuropathic pain through targeting miR-186-5p/CXCL13/CXCR5 axis. *Neurochem Res* 2021; 46: 1457-1469.
  43. Zhang Q, Zhou L, Xie H, Zhang H, Gao X. HAGLR aggravates neuropathic pain and promotes inflammatory response and apoptosis of lipopolysaccharide-treated SH-SY5Y cells by sequestering miR-182-5p from ATAT1 and activating NLRP3 inflammasome. *Neurochem Int* 2021; 145: 105001.
  44. Zhang Q, Zhu D, Li Q. LncRNA CRNDE exacerbates neuropathic pain in chronic constriction injury-induced (CCI) rats through regulating miR-146a-5p/WNT5A pathway. *Bioengineered* 2021; 12: 7348-7359.
  45. Zhao K, Ye Z, Li Y, Li C, Yang X, Chen Q, Xing C. LncRNA FTX contributes to the progression of colorectal cancer through regulating miR-192-5p/EIF5A2 axis. *Onco Targets Ther* 2020; 13: 2677-2688.
  46. Zhu J, Yang J, Xu J. miR-223 Inhibits the polarization and recruitment of macrophages via NLRP3/IL-1 $\beta$  pathway to meliorate neuropathic pain. *Pain Res Manag* 2021; 2021: 6674028.
  47. Zuo Y, Sun H, Song L, Hu Y, Guo F. LncRNA FTX involves in the Nogo-66-induced inhibition of neurite outgrowth through regulating PDK1/PKB/GSK-3 $\beta$  pathway. *Cell Mol Neurobiol* 2020; 40: 1143-1153.