ACTA ABP BIOCHIMICA POLONICA

Vol. 69, No 4/2022
819–824
https://doi.org/10.18388/abp.2020_6185

Regular paper

Demethylase FTO promotes neuropathic pain development *via* regulating the m6A methylation levels of CXCR3

Song Fei Wu, Yang Wang and Qiu Cheng Zhao✉

Department Anesthesiol, The Second Hospital of Dalian Medical University, Dalian, Liaoning116023, China

Objective: Neuropathic pain (NPP) is an indirect or direct pain caused by somatic sensory nervous system dysfunction or primary injury, which is considered to be one of the most serious public health problems. This study aimed to investigate the role of adiposity-associated protein (FTO) in NPP. Materials and Methods: Sciatic nerve injury (SNI) treatment was performed to establish an NPP model *in vivo*. The qRT-PCR and western blot assays were conducted to measure the relative mRNA and protein expressions. Additionally, the paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) of the mice were measured on days 0, 1, 3, 5, 7, and 14. The m6A level of CXCR3 was determined with Methylated RNA immunoprecipitation (MeRIP) assay and the inflammatory factor expressions were determined with Elisa kits. Results and Discussion: The FTO and CXCR3 expressions were up-regulated and the METTL14 expression was down-regulated in SNI mice. FTO-silenced increased the m6A and decreased the mRNA levels of CXCR3 in SNI mice. Furthermore, FTO-silenced decreased the mRNA stability of the CXCR3. Besides, in the SNI mice, FTO-silenced increased the PWL and PWT, and decreased the TNF-α, IL-1β, and IL-6 levels. While overexpressed CXCR3 inverted the FTO-silenced effects. Conclusions: Knockdown of FTO relieved the NPP progression *via* triggering the demethylation of CXCR3, thereby down-regulating the CXCR3 expression.

Keywords: neuropathic pain, FTO, CXCR3, chronic constriction injury, m6A

Received: 28 January, 2022; revised: 31 March, 2022; accepted: 11 October, 2022; available on-line: 02 December, 2022

✉e-mail: zhaoqiucheng@hotmail.com

Abbreviations: FTO, adiposity-associated protein; IL-1β, Interleukin 1β; m6A, 6-methyladenine; NPP, Neuropathic pain; SNI, ciatic nerve injury; TNF-α, tumor necrosis factor α; PWL, paw withdrawal latency; PWT, paw withdrawal threshold

INTRODUCTION

Neuropathic pain (NPP) is an indirect or direct pain caused by somatic sensory nervous system dysfunction or primary injury, which is considered to be one of the most serious public health problems (Saffarpour & Nasirinezhad, 2017; Vincenzetti *et al.*, 2019). NPP is secondary to various types of clinical diseases such as trauma, stroke, infection, diabetes, multiple sclerosis and cancer (Alles *et al.*, 2018; Jensen *et al.*, 2011). At present, most treatments for NPP can only alleviate symptoms, but can not cure them completely (Wang *et al.*, 2017; Xie *et al.*, 2017). The main reason is that the molecular mechanism of the development of NPP is still unclear. Although there are therapeutic drugs for different mechanisms in clinic, the efficacy is limited and the side effects are obvious (Finnerup *et al.*, 2020). In recent years, neuroinflammation has been shown to be closely related to the development and progression of NPP (Ellis & Bennett, 2013; Yadav & Weng, 2017; Zhou *et al.*, 2019). Therefore, an in-depth understanding of the basic molecular mechanism of neuroinflammation is of great significance to explore new and more effective treatments to alleviate NPP.

Accumulating evidence showed that inflammatory mediators, including proinflammatory cytokines, chemokines and growth factors, play an important role in the sensitization of peripheral and central neurons (Ji *et al.*, 2016; Jiang *et al.*, 2017). Chemokines are a special class of cytokines, which are composed of more than 50 members. According to the structure, chemokines are divided into CC, CXC, CX3C and XC. Chemokine receptors are divided into CCR, CXCR, CX3CR and XCR, with about 20 members (Miller & Mayo, 2017; Vilgelm & Richmond, 2019). The classic function of chemokines is to regulate leukocyte infiltration in inflammatory and immune diseases by forming a concentration gradient soluble or fixed in the matrix (Legler & Thelen, 2016). CXCR3 is associated with a variety of human diseases, including chronic inflammation, immune dysfunction, cancer metastasis and pruritus (Szentes *et al.*, 2018, Tokunaga *et al.*, 2018). In dorsal root ganglion, CXCR3 was expressed in neurons and continuously enhanced after sciatic nerve injury (SNI) (Chen *et al.*, 2019). Furthermore, CXCR3 is also up-regulated in neurons after SNI of the spinal cord, spinal nerve ligation or sciatic nerve (Jiang *et al.*, 2017). However, the specific mechanism of CXCR3 in NPP remains unclear.

Epigenetics refers to the regulation of gene expression and stable inheritance through the interaction between genome and environment without changing the DNA sequence, mainly including DNA methylation, histone modification, chromatin remodeling, RNA modification and so on (Cabej, 2019). 6-methyladenine (m6A) refers to the methylation of the sixth nitrogen atom of adenine. It is a common apparent modification in mammalian mRNA (He *et al.*, 2019), accounting for 0.1% ~ 0.4% of all adenosine modifications (Fu *et al.*, 2014). Previous research suggested NPP may be partly due to the maladjustment of RNA m6A modification of pain-related genes induced by methyltransferase/demethylase (Albik & Tao, 2021; Li *et al.*, 2020). Adiposity-associated protein (FTO) is the first identified m6A demethylase, which can eliminate m6A modification of mRNA. Ma *et al.* (Ma *et al.*, 2021) demonstrated spinal nerve ligation promoted the FTO binding to the matrix metallopeptidase 24 mRNA,

which ultimately led to the NPP genesis. Therefore, in the current study, we suspected that FTO may play an important role in the progress of NPP via regulating the m6A methylation levels of CXCR3 in NPP. Our research provided a novel therapeutic target for the treatment of NPP.

MATERIALS AND METHODS

SNI model preparation and grouping

The C57BL/6 mice (22±2.23 g, 6-week-old) were obtained from the Experimental Animal Center of Guangzhou University of Traditional Chinese Medicine. Then the mice were divided into Sham group and SNI group. The SNI model establishment was performed according to a previous study (Wang & Liu, 2021), the mice were fixed after deep anesthesia (intraperitoneally injecting 50 mg/kg sodium pentobarbital), and the sciatic nerve trunk was fully exposed. Four ligations were performed with 4-0 silk thread, with an average interval of 1~2 mm. The degree of ligation should be mild nerve collapse. The mice in the Sham group only exposed the sciatic nerve without ligation.

Lentiviral vector production

The recombinant lentivirus containing FTO shRNA (LV-sh-FTO), CXCR3 overexpressed vector (LV-CXCR3) or their negative controls (LV-sh-nc and LV-nc) was packaged with pGCSIL-GFP vector provided by GeneChem (Shanghai, China). All vectors were injected into the mice through the intrathecal catheter.

Behavioral determination

The paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) of the mice were measured on days 0, 1, 3, 5, 7, and 14. The mice prepared for the test were placed in plexiglass cages with metal grids and the environment was kept quiet. After adapting to the environment for 30 minutes, the left plantar of the mice were stimulated with a series of von Frey filament with logarithmical incremental stiffness (0.016~1g). Then the maximum vertical pressure before foot retraction was recorded, which is the PWT of mice. The stimulation was repeated for 3 times with an interval of more than 10 s, and the mean value was taken as PWT. Additionally, the left plantar of mice was irradiated with BME-410A thermal radiation stimulator. Then the time from the beginning of irradiation to the occurrence of the foot contraction reaction was recorded, which is the PWL of rats. The stimulation was repeated 3 times with an interval of 5 min, and the mean value was taken as PWL. In addition, the number of times the mice shook, lifted, or licked their paws in 1 minute was recorded, which is the frequency of rats. After the behavioral determination, the mice in each group were sacrificed after intraperitoneally injected with 10% chloral hydrate, and the dorsal spinal samples were removed rapidly and stored at –80°C for further experiments.

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

The RNA was separated from the dorsal spinal samples with TRIzol (Invitrogen, CA, USA). The reverse transcription was performed with Hifair® V Reverse Transcriptase Kit (YEASEN, Shanghai, China). Then the qRT-PCR was performed on the QuantStudio 6 Flex Realtime PCR 163 System with a 2×Hieff®PCR Master Mix kits provided by YEASEN Biotechnology Co., Ltd. The reaction system was as follows: 21 μL cDNA, 2 μL Upstream primer, 2 μL downstream primer and 25 μL H_2O. The reaction conditions: 94°C for 5 min; 94°C for 30s, 50°C for 30 s, 72°C for 30 s, 35 cycles; 72°C for 10 min; stored at 4°C. The primers were synthesized by Tsingke Biotechnology Co., Ltd. (Beijing, China). The results were calculated with $2^{-\Delta\Delta Ct}$ method using GAPDH as a control.

Western blot

The dorsal spinal samples were lysed with RIPA buffer (Beyotime, Nantong, China) to separate the total protein. All proteins were measured with BCA kits (Beyotime) to calculate the concentrations. Next, the 10% SDS-PAGE was carried out and the proteins were transferred onto the polyvinylidene fluoride (PVDF) membrane at 300 mA. After blocking with 5% fetal bovine serum for 2 h, the membranes were immersed in the antibodies for 12 h (anti-FTO, dilution: 1:600; anti-METTL3, dilution: 1:800; anti-METTL14, dilution: 1:1200; anti-ALKBH5, dilution: 1:1000; anti-WTAP, dilution: 1:1000; anti-YTHDF2, dilution: 1:800; CXCR3, dilution: 1:1200; Abcam, USA). The next day, the members were washed and incubated with the second antibody (horseradish peroxidase-conjugated, dilution: 1:6000). The protein expression was detected using ECL chemiluminescent substrate system (Beyotime). We measured the band intensity using Image J software and then calculated the protein expressions normalized to the loading control GAPDH.

Enzyme-linked immunosorbent assay (ELISA)

The levels of Interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), IL-6 and IL-10 in the dorsal spinal samples were determined by ELISA kits (SenBeiJia Biological Technology Co., Ltd., Nanjing, China). All operations shall strictly comply with the instructions of the kits. The absorbance values were measured at 450 nm.

Methylated RNA immunoprecipitation (MeRIP) assay

The m6A levels of CXCR3 were measured with a Magna MeRIP™ m6A kit (Cloud-seq Biotechnology Co., Ltd, Shanghai, China). Briefly, 30 μg RNA was incubated with the MeRIP reagent. Magna ChIP protein A/G Magnetic Beads were resuspended and incubated with anti-m6A as well as anti-IgG antibodies for 12 h at 4°C. Finally, the magnetic beads were collected, and the RNA level was analyzed by qRT-PCR.

Statistics

SPSS 22.0 (IBM, SPSS, USA) was selected for statistical analysis. Data in this study were expressed as mean ±S.D. (n=6). The significance analysis between the two groups was analyzed with Student's *t*-test and one-way ANOVA was performed for multiple groups. $P<0.05$ indicated a significant difference.

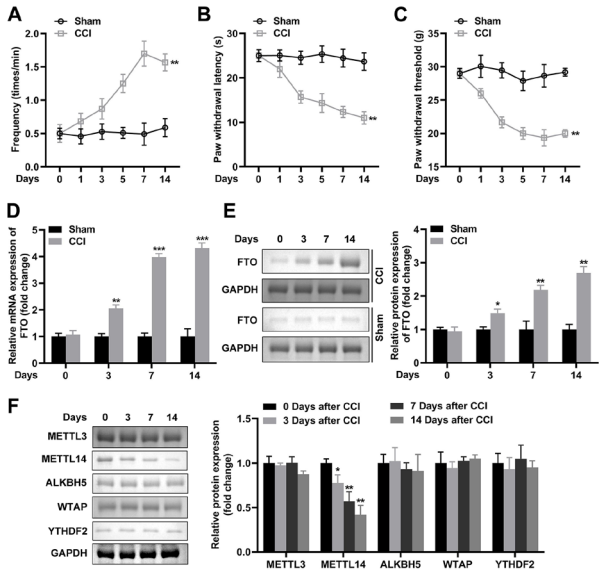

Figure 1. SNI induced the pain hypersensitivity of the mice and modulated the FTO and METTL14 expressions.
(**A–C**) SNI treatment increased the frequency and decreased the PWL and PWT. (**D–E**) The expressions of FTO were measured at the mRNA and protein levels by qRT-PCR and western blot assays. (**F**) The protein expressions of METTL3, METTL15, ALKBH5, WTAP and YTHDF2 were measured by western blot. *P<0.05, **P<0.01.

RESULTS

SNI induced the pain hypersensitivity of the mice and modulated the FTO and METTL14 expressions

After SNI stimulation, the frequency of the mice shaking, lifting, or licking their paws in 1 minute was dramatically increased, while the PWL and PWT were dramatically decreased in the SNI group on days 1, 3, 5, 7, and 14 (Fig. 1A–C). The qRT-PCR and western blot results indicated that the FTO was remarkably up-regulated on days 3, 7, and 14 at both mRNA and protein levels (Fig. 1D–E). Additionally, the protein expressions

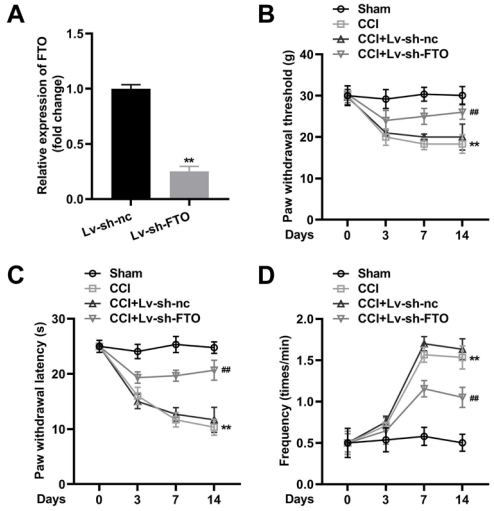

Figure 2. FTO-silenced relieved the pain hypersensitivity of the mice.
(**A**) The FTO expression was determined after Lv-sh-FTO transfection. (**B–D**) FTO-silenced decreased the frequency and increased the PWL and PWT of the SNI mice. **P<0.01 *vs* Sham group. ##P<0.01 *vs* SNI+Lv-sh-nc group.

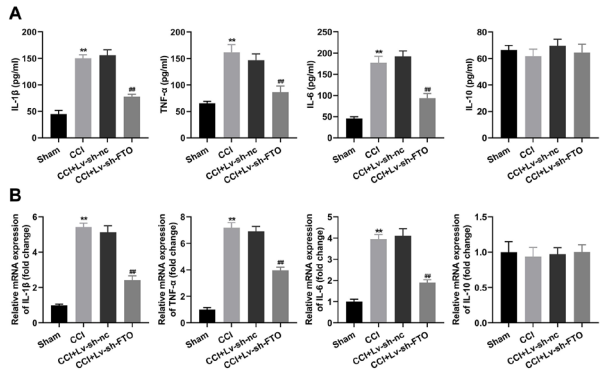

Figure 3. FTO-silenced decreased the IL-1β, TNF-α and IL-6 levels.
The levels of IL-1β, TNF-α, IL-6 and IL-10 were determined with Elisa (**A**) and qRT-PCR (**B**) assays. **P<0.01 *vs* Sham group. ##P<0.01 *vs* SNI+Lv-sh-nc group.

of METTL14 were remarkably down-regulated on days 3, 7, and 14 (Fig. 1F).

FTO-silenced relieved the pain hypersensitivity of the mice

Next, we verified the Lv-sh-FTO effect and found that Lv-sh-FTO dramatically decreased the FTO expressions (Fig. 2A). Furthermore, Lv-sh-FTO dramatically decreased the frequency of the mice shaking, lifting, or

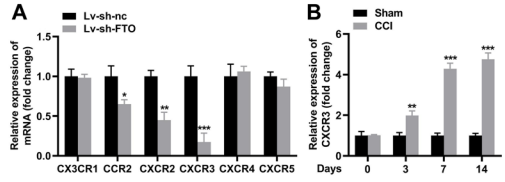

Figure 4. CXCR3 exhibited a low level in SNI mice.
(**A**) The mRNA expressions of CX3CR1, CCR2, CXCR2, CXCR3, CXCR4 and CXCR5 were detected by qRT-PCR after Lv-sh-FTO transfection. (**B**) The CXCR3 levels in the SNI mice were determined by qRT-PCR. *P<0.05. **P<0.01. ***P<0.001.

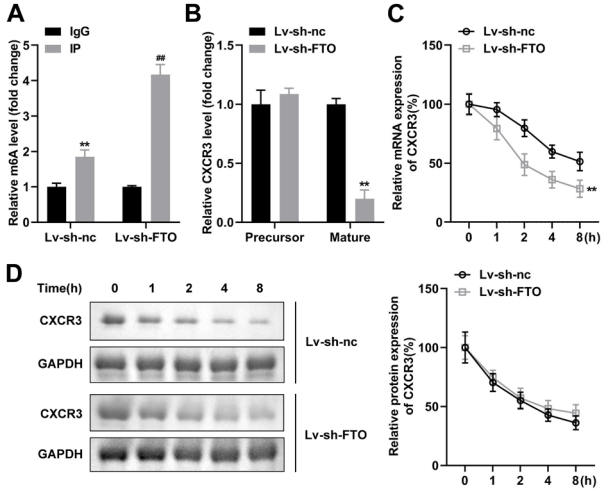

Figure 5. FTO modulated the mRNA stability of CXCR3.
(**A**) The relative m6A levels of CXCR3 was measured after Lv-sh-FTO transfection by MeRIP. (**B**) The expression of mature or precursor mRNA of CXCR3 after Lv-sh-FTO transfection by qRT-PCR. (**C–D**) The mRNA and protein stability of the CXCR3 were analyzed after Lv-sh-FTO transfection by qRT-PCR and western blot.

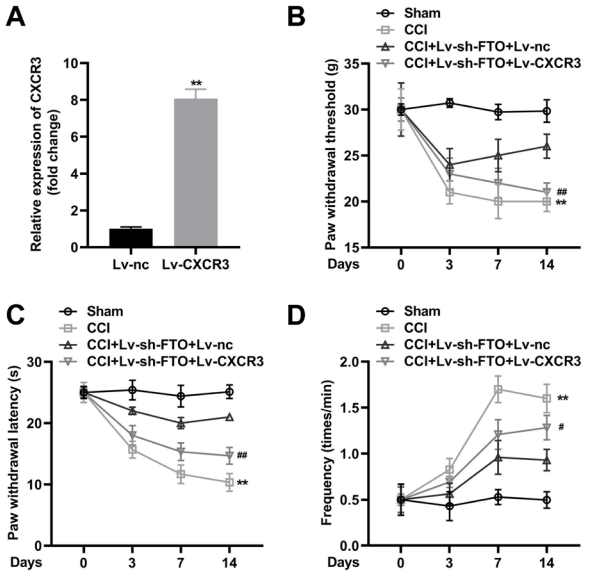

Figure 6. Overexpressed CXCR3 inverted the role of FTO-silenced in SNI mice.
(**A**) The CXCR3 expression was detected after Lv-CXCR3 transfection. (**B–D**) Over-expressed CXCR3 inverted the effects of Lv-sh-FTO on the frequency and the PWL and PWT of the SNI mice. $^{**}P<0.01$ *vs* Sham group. $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* SNI+Lv-sh-FTO+Lv-nc group.

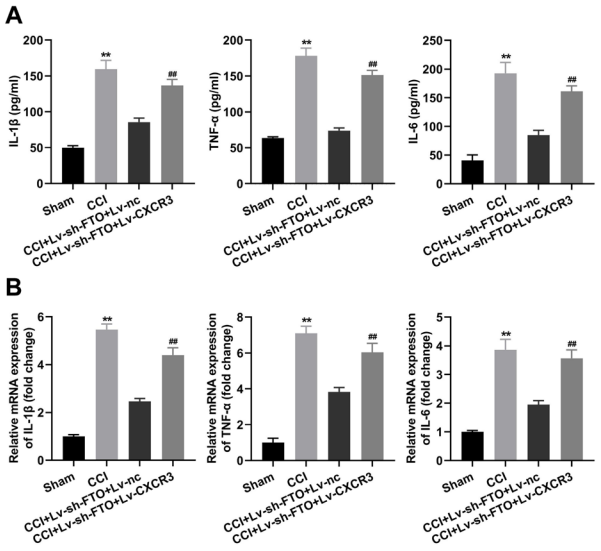

Figure 7. Overexpressed CXCR3 increased the IL-1β, TNF-α and IL-6 levels.
The levels of IL-1β, TNF-α and IL-6 were determined with Elisa (**A**) and qRT-PCR (**B**) assays. $^{**}P<0.01$ *vs* Sham group. $^{\#\#}P<0.01$ *vs* SNI+Lv-sh-FTO+Lv-nc group.

licking their paws in 1 minute, and increased the PWL and PWT of the SNI mice on days 3, 7, 14 (Fig. 2B–D).

FTO-silenced decreased the IL-1β, TNF-α and IL-6 levels

SNI treatment dramatically increased the IL-1β, TNF-α and IL-6 levels of the mice. While FTO-silenced dramatically decreased the IL-1β, TNF-α and IL-6 levels of the SNI mice (Fig. 3A). Besides, the results of the qRT-PCR were consistent with it (Fig. 3B).

CXCR3 exhibited a low level in SNI mice

After Lv-sh-FTO treatment, the CCR2, CSCR2 and CXCR3 expressions were remarkably decreased, among which the CXCR3 expression was the lowest (Fig. 4A). Then, we further found that CXCR3 expression was dramatically up-regulated in the SNI mice on days 3, 7, and 14 (Fig. 4B).

FTO modulated the mRNA stability of CXCR3

The results of m6A RNA-RIP-qPCR showed that Lv-nc-FTO dramatically increased the m6A levels of CXCR3 (Fig. 5A). Furthermore, FTO-silenced also significantly decreased the mRNA expression of CXCR3 and exhibited no effect on the expression of precursor mRNA of CXCR3 (Fig. 5B). Besides, the mRNA stability of CXCR3 in the Lv-sh-FTO group was greater than that in the Lv-sh-nc group (Fig. 5C), while FTO-silenced exhibited no effect on the protein stability of CXCR3 (Fig. 5D).

Overexpressed CXCR3 inverted the role of FTO-silenced in SNI mice

Subsequently, we verified the Lv-CXCR3 effect and found that Lv-CXCR3 dramatically increased the CXCR3 expressions (Fig. 6A). What is more, we confirmed that overexpressed CXCR3 dramatically increased the frequency of the mice shaking, lifting, or licking their paws in 1 minute, and decreased the PWL and PWT of the SNI mice on days 3, 7, and 14 (Fig. 6B-D).

Overexpressed CXCR3 increased the IL-1β, TNF-α and IL-6 levels

Finally, we found that overexpressed CXCR3 dramatically increased the IL-1β, TNF-α and IL-6 levels compared with the SNI+Lv-sh-FTO+Lv-nc group (Fig. 7A), which was consistent with the results of qRT-PCR (Fig. 7B).

DISCUSSION

Our research demonstrated that demethylase FTO was up-regulated and methylase METTL14 was down-regulated in the SNI mice. Meanwhile, CXCR3 was also up-regulated. Knockdown of FTO hindered the mRNA expression and m6A levels of CXCR3, and over-expressed CXCR3 antagonized the Lv-sh-FTO effects in SNI mice.

NPP caused by SNI of the sciatic nerve is related to an excessive inflammatory response of the nervous system. The production of a large number of inflammatory factors may lead to the occurrence and maintenance of persistent pain (Ellis & Bennett, 2013; Zheng *et al.*, 2019). The release of proinflammatory cytokines (IL-6, TNF-α and IL-1β) is closely related to the pathogenesis of NPP (Lu *et al.*, 2014). A previous study showed that the overexpression of IL-6 in dorsal root ganglion neurons induced by calpain-2 can lead to NPP after motor nerve injury (Zang *et al.*, 2015). IL-1β, as an endogenous ligand of the IL-1 receptor, is a powerful proinflammatory cytokine, which contributes to the development of hyperalgesia and peripheral and central sensitization (Liu *et al.*, 2017). In addition, the nuclear factor κB (NF-κB) signal pathway was activated by IL-1β, leading to the mobilization of neuronal calcium, stimulation of TNF-α as well as other pro-inflammatory mediators (Agalave *et al.*, 2014). Many studies demonstrated that after SNI treatment, a severe inflammatory reaction occurred in mice (Cai *et al.*, 2020; Zhang *et al.*, 2020). Additionally,

aberrantly expressed FTO was confirmed to induce inflammation in various diseases (Hu *et al.*, 2019; Yu *et al.*, 2021). In the current study, we found FTO was up-regulated in SNI mice, and FTO-silenced relieved the pain hypersensitivity and inflammation of the SNI mice. Our results were similar to those reported by Li and others (Li *et al.*, 2020), who indicated that inhibiting the increase of FTO induced by nerve injury relieved the NPP progressions. All these findings implied that FTO might play a key role in NPP.

Subsequently, we explored the specific mechanism of FTO in SNI mice. As the first identified m6A demethylase, FTO was reported to be involved in various cancers by regulating the m6A methylation level of a series of genes. For instance, Li and others (Li *et al.*, 2019) confirmed that abnormally expressed FTO promoted the malignant behavior of hepatocellular carcinoma cells via triggering the demethylation of PKM2. In lung cancer, FTO induced the proliferation of non-small cell lung cancer cells by decreasing the m6A methylation level and increasing the mRNA expression of ubiquitin-specific protease (Li *et al.*, 2019). Except for cancers, existing studies have shown that FTO may be closely related to the lesions of neurodegenerative diseases (Li *et al.*, 2018) and cardiovascular disease (Mathiyalagan *et al.*, 2019). However, the reports on the specific mechanism of FTO in NPP progression were limited. In this study, we demonstrated that FTO regulated NPP progression by decreasing the m6A methylation level and increasing the mRNA expression of CXCR3 in SNI mice. The results of molecular biology clinical research showed that CXCR3 belongs to the G protein-coupled receptor superfamily. It is a snake-shaped transmembrane glycoprotein with only one peptide chain structure, and located on the human Xp13 chromosome (Koper *et al.*, 2018; Tokunaga *et al.*, 2018). In the NPP model, the expression level of CXCR3 in the spinal cord usually increased and CXCR3 is a key receptor involved in the mechanism of NPP. Study have exhibited that CXCR3 knockout mice had a high tolerance to SNI-induced NPP. And the occurrence time of mechanical pain sensitivity will be delayed (Li *et al.*, 2021). In addition, there is increasing evidence that proinflammatory factors and chemokines can be synthesized and secreted by spinal cord neurons and dorsal root ganglion (He *et al.*, 2020). In the current study, after SNI treatment, the CXCR3 levels were dramatically up-regulated. Knockdown of FTO up-regulated the m6A levels and down-regulated the mRNA expression and stability of CXCR3. Besides, over-expressed CXCR3 inverted the effects of Lv-sh-FTO on the pain hypersensitivity and inflammation of the SNI mice. These results further indicated that FTO aggravated the NPP progression *via* triggering the demethylation of CXCR3

In conclusion, we demonstrated that CXCR3 can be activated via triggering the demethylation by FTO. FTO-silenced relieved the NPP progression by down-regulating the CXCR3 expressions.

Declarations

Funding. Not applicable.

Disclosure statement. The authors report there are no competing interests to declare.

Data availability statement. Not applicable.

Ethical approval. This study protocol was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University 201900314 on March 14th 2019.

REFERENCES

Agalave NM, Larsson M, Abdelmoaty S, Su J, Baharpoor A, Lundback P, Palmblad K, Andersson U, Harris H, Svensson CI (2014) Spinal HMGB1 induces TLR4-mediated long-lasting hypersensitivity and glial activation and regulates pain-like behavior in experimental arthritis. *Pain* **155**: 1802–1813. https://doi.org/10.1016/j.pain.2014.06.007

Albik S, Tao YX (2021) Emerging role of RNA m6A modification in chronic pain. *Pain* **162**: 1897–1898. https://doi.org/10.1097/j.pain.0000000000002219

Alles SRA, Smith PA, Isom LL (2018) Etiology and pharmacology of neuropathic pain. *Pharmacol Rev* **70**: 315–347. https://doi.org/10.1124/pr.117.014399

Cabej NR (2019) Epigenetics in Health and Disease. *Epigenetic Principles of Evolution*, 2nd edn, pp 647–731. https://doi.org/10.1016/B978-0-12-814067-3.00014-4

Cai W, Zhang Y, Su Z (2020) ciRS-7 targeting miR-135a-5p promotes neuropathic pain in SNI rats *via* inflammation and autophagy. *Gene* **736**: 144386. https://doi.org/10.1016/j.gene.2020.144386

Chen Y, Yin D, Fan B, Zhu X, Chen Q, Li Y, Zhu S, Lu R, Xu Z (2019) Chemokine CXCL10/CXCR3 signaling contributes to neuropathic pain in spinal cord and dorsal root ganglia after chronic constriction injury in rats. *Neurosci Lett* **694**: 20–28. https://doi.org/10.1016/j.neulet.2018.11.021

Ellis A, Bennett DLH (2013) Neuroinflammation and the generation of neuropathic pain. *Brit J Anaesthesia* **111**: 26–37. https://doi.org/10.1093/bja/aet128

Finnerup NB, Kuner R, Jensen TS (2020) Neuropathic pain: from mechanisms to treatment. *Physiol Rev* 101. https://doi.org/10.1152/physrev.00045.2019

Fu Y, Dominissini D, Rechavi G, He C (2014) Gene expression regulation mediated through reversible m-A RNA methylation. *Nature Rev Genet* **15**: 293–306. https://doi.org/10.1038/nrg3724

He DD, Gao Y, Wang S, Xie Z, Song XJ (2020) Systematic administration of B vitamins alleviates diabetic pain and inhibits associated expression of P2X3 and TRPV1 in dorsal root ganglion neurons and proinflammatory cytokines in spinal cord in rats. *Pain Res Manag* **2020**: 3740162. https://doi.org/10.1155/2020/3740162

He L, Li H, Wu A, Peng Y, Shu G, Yin G (2019) Functions of N6-methyladenosine and its role in cancer. *Mol Cancer* **18**: 176–176. https://doi.org/10.1186/s12943-019-1109-9

Hu F, Tong J, Deng B, Zheng J, Lu C (2019) MiR-495 regulates macrophage M1/M2 polarization and insulin resistance in high-fat diet-fed mice *via* targeting FTO. *Pflugers Arch* **471**: 1529–1537. https://doi.org/10.1007/s00424-019-02316-w

Jensen TS, Baron R, Haanp M, Kalso E, Loeser JD, Rice A, Treede RD (2011) A new definition of neuropathic pain. *Pain* **152**: 2204–2205. https://doi.org/10.1016/j.pain.2011.06.017

Ji RR, Chamessian A, Zhang YQ (2016) Pain regulation by non-neuronal cells and inflammation. *Science* **354**: 572–577. https://doi.org/10.1126/science.aaf8924

Jiang BC, He LN, Wu XB, Shi H, Zhang WW, Zhang ZJ, Cao DL, Li CH, Gu J, Gao YJ (2017) Promoted interaction of C/EBPalpha with demethylated Cxcr3 gene promoter contributes to neuropathic pain in mice. *J Neurosci* **37**: 685–700. https://doi.org/10.1523/JNEUROSCI.2262-16.2016

Koper OM, Kaminska J, Sawicki K, Kemona H (2018) CXCL9, CXCL10, CXCL11, and their receptor (CXCR3) in neuroinflammation and neurodegeneration. *Adv Clin Exp Med* **27**: 849–856. https://doi.org/10.17219/acem/68846

Legler DF, Thelen M (2016) Chemokines: Chemistry, Biochemistry and Biological Function. *Chimia (Aarau)* **70**: 856–859. https://doi.org/10.2533/chimia.2016.856

Li H, Ren Y, Mao K, Hua F, Yang Y, Wei N, Yue C, Li D, Zhang H (2018) FTO is involved in Alzheimer's disease by targeting TSC1-mTOR-Tau signaling. *Biochem Biophys Res Commun* **498**: 234–239. https://doi.org/10.1016/j.bbrc.2018.02.201

Li J, Han Y, Zhang H, Qian Z, Jia W, Gao Y, Zheng H, Li B (2019) The m6A demethylase FTO promotes the growth of lung cancer cells by regulating the m6A level of USP7 mRNA. *Biochem Biophys Res Commun* **512**: 479–485. https://doi.org/10.1016/j.bbrc.2019.03.093

Li J, Zhu L, Shi Y, Liu J, Lin L, Chen X (2019) m6A demethylase FTO promotes hepatocellular carcinoma tumorigenesis *via* mediating PKM2 demethylation. *Am J Transl Res* **11**: 6084–6092

Li K, Tan YH, Feng SY, Fu KY (2021) CXCR3 signalling partially contributes to the pathogenesis of neuropathic pain in male rodents. *J Oral Rehab* **49**: 186–194. https://doi.org/10.1111/joor.13262

Li Y, Guo X, Sun L, Xiao J, Su S, Du S, Li Z, Wu S, Liu W, Mo K, Xia S, Chang YJ, Denis D, Tao YX (2020) N(6)-Methyladenosine demethylase FTO contributes to neuropathic pain by stabilizing G9a expression in primary sensory neurons. *Adv Sci (Weinh)* **7**: 1902402. https://doi.org/10.1002/advs.201902402

Liu F, Wang Z, Qiu Y, Wei M, Li C, Xie Y, Shen L, Huang Y, Ma C (2017) Suppression of MyD88-dependent signaling alleviates neuro-

pathic pain induced by peripheral nerve injury in the rat. *J Neuroinflammation* **14**: 70. https://doi.org/10.1186/s12974-017-0822-9

Lu Y, Jiang BC, Cao DL, Zhang ZJ, Zhang X, Ji RR, Gao YJ (2014) TRAF6 upregulation in spinal astrocytes maintains neuropathic pain by integrating TNF-alpha and IL-1beta signaling. *Pain* **155**: 2618–2629. https://doi.org/10.1016/j.pain.2014.09.027

Ma L, Huang Y, Zhang F, Gao DS, Sun N, Ren J, Xia S, Li J, Peng X, Yu L, Jiang BC, Yan M (2021) MMP24 Contributes to neuropathic pain in an FTO-dependent manner in the spinal cord neurons. *Front Pharmacol* **12**: 673831. https://doi.org/10.3389/fphar.2021.673831

Mathiyalagan P, Adamiak M, Mayourian J, Sassi Y, Liang Y, Agarwal N, Jha D, Zhang S, Kohlbrenner E, Chepurko E, Chen J, Trivieri MG, Singh R, Boucharef R, Fish K, Ishikawa K, Lebeche D, Hajjar RJ, Sahoo S (2019) FTO-dependent N(6)-methyladenosine regulates cardiac function during remodeling and repair. *Circulation* **139**: 518–532. https://doi.org/10.1161/CIRCULATIONAHA.118.033794

Miller MC, Mayo KH (2017) Chemokines from a Structural Perspective. *Int J Mol Sci* **18**: 2088. https://doi.org/ 10.3390/ijms18102088

Saffarpour S, Nasirinezhad F (2017) Functional interaction between N-methyl-D-aspartate receptor and ascorbic acid during neuropathic pain induced by chronic constriction injury of the sciatic nerve. *J Basic Clin Physiol Pharmacol* **28**: 601–608. https://doi.org/10.1515/jbcpp-2017-0015

Szentes V, Gazdag M, Szokodi I, Dezsi CA (2018) The role of CXCR3 and associated chemokines in the development of atherosclerosis and during myocardial infarction. *Front Immunol* **9**: 1932. https://doi.org/10.3389/fimmu.2018.01932

Tokunaga R, Zhang W, Naseem M, PuSNIni A, Berger MD, Soni S, McSkane M, Baba H, Lenz HJ (2018) CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation – A target for novel cancer therapy. *Cancer Treat Rev* **63**: 40–47. https://doi.org/10.1016/j.ctrv.2017.11.007

Vilgelm AE, Richmond A (2019) Chemokines modulate immune surveillance in tumorigenesis, metastasis, and response to immunotherapy. *Front Immunol* **10**: 333. https://doi.org/10.3389/fimmu.2019.00333

Vincenzetti S, PuSNIarelli S, Huang Y, RiSNIutelli M, LambertuSNI C, Volpini R, Scuppa G, Soverchia L, Ubaldi M, Polzonetti V (2019) Biomarkers mapping of neuropathic pain in a nerve chronic constriction injury mice model. *Biochimie* **158**: 172–179. https://doi.org/10.1016/j.biochi.2019.01.005

Wang YW, Zhang X, Chen CL, Liu QZ, Xu JW, Qian QQ, Li WY, Qian YN (2017) Protective effects of Garcinol against neuropathic pain – Evidence from *in vivo* and *in vitro* studies. *Neurosci Lett* **647**: 85–90. https://doi.org/10.1016/j.neulet.2017.03.015

Wang X. Liu Q (2021) Dexmedetomidine relieved neuropathic pain and inflammation response induced by CCI through HMGB1/TLR4/NF-κB signal pathway. *Biol Pharm Bull* **2021**. https://doi.org/10.1248/bpb.b21-00329

Xie W, Strong J, Zhang J (2017) (154) Close relationship between peripheral nerve regeneration and pain behaviors in two rat models of neuropathic pain. *J Pain* **18**: S15–S15. https://doi.org/10.1016/j.jpain.2017.02.060

Yadav R, Weng HR (2017) EZH2 regulates spinal neuroinflammation in rats with neuropathic pain. *Neuroscience* **349**: 106–117. https://doi.org/10.1016/j.neuroscience.2017.02.041

Yu J, Hu X, Chen H, Yang Q, Li H, Dong Y, Zhang Y, Wang J, Jin J, Wu Y, Li J, Ge J, Meng X (2021) DNA methylation of FTO promotes renal inflammation by enhancing m 6 A of PPAR-α in alcohol-induced kidney injury. *Pharmacol Res* **163**: 105286. https://doi.org/10.1016/j.phrs.2020.105286

Zang Y, Chen SX, Liao GJ, Zhu HQ, Wei XH, Cui Y, Na XD, Pang RP, Xin WJ, Zhou LJ, Liu XG (2015) Calpain-2 contributes to neuropathic pain following motor nerve injury *via* up-regulating interleukin-6 in DRG neurons. *Brain Behav Immun* **44**: 37–47. https://doi.org/10.1016/j.bbi.2014.08.003

Zhang X, Chen Q, Shen J, Wang L, Cai Y, Zhu KR (2020) miR-194 relieve neuropathic pain and prevent neuroinflammation via targeting FOXA1. *J Cell Biochem* **121**: 3278–3285. https://doi.org/10.1002/jcb.29598

Zheng Y, Hou X, Yang S (2019) Lidocaine potentiates SOCS3 to attenuate inflammation in microglia and suppress neuropathic pain. *Cell Mol Neurobiol* **39**: 1081–1092. https://doi.org/10.1007/s10571-019-00703-6

Zhou D, Zhang S, Hu L, Gu YF, Cai Y, Wu D, Liu WT, Jiang CY, Kong X, Zhang GQ (2019) Inhibition of apoptosis signal-regulating kinase by paeoniflorin attenuates neuroinflammation and ameliorates neuropathic pain. *J Neuroinflammation* **16**: 83. https://doi.org/10.1186/s12974-019-1476-6