


# LncRNA MEG3-TRPV1 signaling regulates chronic inflammatory pain in rats

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## Abstract

Osteoarthritis (OA) is a common osteoarthropathy with chronic inflammatory pain as the core symptom in middle-aged and elderly people. LncRNA MEG3 (Maternally expressed gene 3) is involved in the development of OA via regulation of angiogenesis, which causes the activation and overexpression of transient receptor potential vanilloid type-1 (TRPV1). In this study, we investigated the mechanism of MEG3-TRPV1 signaling in chronic inflammatory pain (CIP) of rat model. Chronic inflammatory pain was modeled using subcutaneous microinjection of complete Freund's adjuvant (CFA) into the left hind paw of rats. We showed that TRPV1 mRNA and protein were significantly increased, while MEG3 mRNA was significantly decreased, in the DRG and SDH of CFA-induced rats. In addition, intrathecal injection of MEG3-overexpressing lentivirus significantly downregulated TRPV1 expression and alleviated chronic inflammatory pain in CFA-induced rats. Treatment with a TRPV1 antagonist also significantly relieved chronic inflammatory pain in CFA-induced rats. In general, our results reveal that MEG3 alleviates chronic inflammatory pain by downregulating TRPV1 expression. These findings may provide new therapeutic targets in the treatment of patients with OA.

## Keywords

Osteoarthritis, chronic inflammatory pain, MEG3, TRPV1, DRG, SDH

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## Introduction

Osteoarthritis (OA) is the most prevalent degenerative disorder of synovial joints and leads to chronic disability due to chronic inflammatory pain and associated joint dysfunction.<sup>1</sup> The main clinical symptoms of OA include knee joint pain, swelling, deformation, limitation of motion, joint stiffness, and chronic inflammatory pain.<sup>2</sup> Patients with OA often have joint effusion with inflammation of the joint cavity and the synovium, which can damage and destroy the cartilage on the articular surface and lead to secondary synovial inflammation, causing disorders of the synthesis and metabolism of articular cartilage. Currently, no treatments can effectively impede the progression of OA,<sup>3</sup> and surgical joint replacement is ultimately required in many affected patients.<sup>4</sup> There is no consensus on the pathogenesis of primary OA. Various factors, such as age, cartilage metabolism, endocrine conditions, bone and joint strain, high pressure in the joint

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capsule, immune factors, and genetic susceptibility, may be involved in the onset of OA.<sup>5</sup>

Numerous studies suggest that tissue angiogenesis is involved in the formation of lesions observed in OA. The expression of vascular endothelial growth factor (VEGF) is increased in the articular cartilage of animals used to model OA.<sup>5</sup> A previous study assessed mRNA and protein expression of VEGF, and expression of the long non-coding RNA (lncRNA) maternally expressed gene 3 (MEG3), in cartilage samples obtained from OA patients and healthy individuals. The results of that study showed that MEG3 lncRNA is negatively correlated with that of VEGF, suggesting that MEG3 may be involved in the development of OA via regulation of angiogenesis.<sup>6</sup> Moreover, VEGF promotes the activation and upregulates the expression of transient receptor potential vanilloid 1 (TRPV1).<sup>7</sup> However, how MEG3 regulates TRPV1 and the OA-related chronic inflammatory pain is unclear.

In this study, complete Freund's adjuvant (CFA) was used to induce chronic inflammatory pain in a rat model. We showed that chronic inflammatory pain causes downregulation of MEG3 lncRNA and upregulation of TRPV1 expression in CFA-induced rats. These CFA-induced rats were subsequently treated with a MEG3-overexpressing lentivirus and a TRPV1 antagonist to explore the mechanisms of MEG3-TRPV1 signaling in a rat model of chronic inflammatory pain.

## Materials and methods

### Experimental animals

Healthy adult male specific-pathogen free Sprague Dawley (SD) rats weighing 180–200 g were provided by Zhaoyan New Drug Research Center Co., Ltd (Suzhou, China; license number: SCXY [Suzhou] 2018–000, quality control certificate code: 2019120068). The rats were fed a standard rodent pellet feed with free access to the feed and drinking water. The rats were housed in an animal facility maintained at controlled temperature and humidity ( $23 \pm 2^\circ\text{C}$  and 60%, respectively) under a 12-h light-dark cycle. All the rats were adapted to the breeding environment for 7 days before commencement of the experiments.

### Establishment of CFA-induced rat model

Rats were anesthetized using 5% chloral hydrate injected intraperitoneally. Then, using a 1 mL syringe, 100  $\mu\text{l}$  CFA was injected subcutaneously into the left hind paw of the rats under anesthesia. The left hind paws of the rats were compared with right hind paw at 8 h post-injection of CFA to assess the presence of obvious redness and swelling. We also assessed whether the CFA-induced rats demonstrated significantly reduced physical activity, which would indicate successful establishment of this model. Subcutaneous injection of 100  $\mu\text{l}$  saline was used in the left hind paw of control rats.

### Intrathecal injection

Intrathecal injection was performed as described previously.<sup>8–10</sup> Under isoflurane inhalation anesthesia, the rat was placed in the ambulatory position, and the left hand explored the hip nodes, and felt upward to the L4-5 spinous process gap as the puncture point, the skin was tensed outward, and the microsyringe was held to enter the needle from the gap vertically and slowly, and the drug was injected after the syringe was withdrawn with cerebrospinal fluid when the tail-flicking action appeared. Tail flicks during injection indicated successful intrathecal injection.

### Thermal paw withdrawal latencies (PWLs)

A Thermal Plantar Analgesia Meter was used to adjust the intensity of radiant heat to that suitable for stimulation. Each rat was placed on a glass plate and covered with a transparent plexiglass cover. The rat was then allowed to adapt to this environment for 30 min until the animal became quiet. The temperature of the glass plate was maintained at  $26 \pm 0.5^\circ\text{C}$ . The light source used to provide illumination under the glass plate was aimed at the left hind paw and adjusted to avoid the left paw pad. The bottom of the paw was then irradiated, and irradiation was stopped when the rat rapidly lifted or licked the paw. To obtain the mean value, duration of thermal radiation was recorded as thermal PWL three times in total, with an interval of 10 min between recordings. The longest thermal radiation interval was preset at 20 s; to prevent scalding, thermal radiation was terminated if the rat still showed no response at this interval.

### Mechanical paw withdrawal thresholds (PWTs)

Each rat was first placed on a metal grid for a 30 min adaptation to the environment until the animal became quiet. A standardized von Frey filament was used to stimulate the middle of the left hind plantar and avoid the right paw pad.<sup>11</sup> A low level of force was applied until the cilia were bent slightly into s-shape, which was designated as the standard for full force. Each stimulation lasted for 3–5 s with a resting interval of 15–20 s, and presence or absence of paw withdrawal behavior was observed. The paw withdrawal threshold was determined by progressively increasing strength from 8.0 g. Rats showing an escape reaction were designated as “X” (i.e. positive), and in the next stimulation, the next lowest filament was used. Rats showing no escape reaction were designated as “O” (i.e. negative), and in the next stimulation, the next highest filament was used. When the combination of “OX” or “XO” occurred in the same rat, stimulation was performed two more times to obtain a series of “O” or “X” combined sequence. The 50% PWT for each rat was calculated using the following equation:  $50\% \text{ PWT (g)} = 10^6 (xf + k\delta - 4)$ , where  $xf$  represents the logarithmic value of the last von Frey filament in the sequence,  $k$  represents the

corresponding value of the sequence obtained in the  $k$  value table, and  $\delta$  represents the average difference of the strength of each fiber filament after taking the log, which was approximately 0.231. The stimulus of “O” occurring three consecutive times was recorded as 26.0 g, and the stimulus of “X” occurring three consecutive times was recorded as 1.0 g. If the calculated 50% PWT was  $> 26.0$  g or  $< 1.0$  g, 26.0 g was considered the maximum or minimum value, and 1.0 g as considered the minimum value. The measurement time was fixed at 09:00–16:00, and ambient temperature was  $23 \pm 2^\circ\text{C}$ . The procedure was performed by the same experimenter blinded to the grouping used in the present study.

### Tissue collection

After completion of behavioral analyses, the rats were euthanized using an excess dose of chloral hydrate. Then, dorsal root ganglia (DRG) and spinal dorsal horn (SDH) tissues of the left L4-6 lumbar vertebrae were harvested and stored at  $-80^\circ\text{C}$  for later use.

### Western blotting

Phenylmethylsulfonyl fluoride protease inhibitor and RIPA lysis buffer (1: 100) were added to the tissues, and the tissues were lysed using ultrasonic pulverization. After the tissues were homogenized and centrifuged, total protein was extracted and quantified using a bicinchoninic acid assay kit (Thermo Scientific, MA, United States). An appropriate amount of total protein extracted from DRG or spinal cord tissue was added to  $5 \times$  loading buffer and denatured at  $95^\circ\text{C}$  for 5 min. Then, 25  $\mu\text{g}$  protein was loaded into each lane of a 5% stacking gel (at 120 V) and separated in a 10% separating gel via electrophoresis (at 80 V). The separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes at 250 mA for 2 h under cooling conditions. The membranes were then blocked in 5% skim milk at room temperature for 2 h, and independently incubated with the following primary antibodies: rabbit anti-rat TRPV1 (1: 200; GTX54762, GeneTex) and rabbit anti-rat GAPDH (1: 1000; G9545-100, Sigma-Aldrich) overnight at  $4^\circ\text{C}$ . The membranes were then washed in the TBST and incubated with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1: 10000) at room temperature for 2 h. Consequently, the membranes were developed using enhanced chemiluminescence reagent and imaged using Image Lab gel imaging system (Bio-Rad, CA, United States). Grayscale values of the protein bands were then analyzed using ImageJ software.

### Real-time PCR

Total RNA was extracted from 100 mg DRG or SDH tissues using Trizol (Invitrogen). The concentration and purity of the extracted RNA were then assessed using a Nanodrop spectrophotometer. RNA with the purity of 1.8–2.0 was then reverse

transcribed into cDNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Real-time PCR was performed using  $2 \times$  RealStar Green Fast Mixture (GenStar) in accordance with the manufacturer's instructions. Three samples were used per group, and three replicates were used per sample. Relative expression levels of target genes were calculated using  $2^{-\Delta\Delta\text{Ct}}$  method. The primer sequences of target genes *TRPV1* and *MEG3*, and of the reference gene *GAPDH*, were as follows: 5'-CGGAAGACA GACCTGAAGC-3' (forward primer for *TRPV1*); 5'-GTTAGCCGCAGCCTGGACATC-3' (reverse primer for *TRPV1*); 5'-AGACAACAGGCCCGTCCAGGAG-3' (forward primer for *MEG3*); 5'-GAAGA GCGAGTCAG-GAAGCAGTG-3' (reverse primer for *MEG3*); 5'-GACAT GCCGCCTGGAGAAAC-3' (forward primer for *GAPDH*); and 5'-AGCCCAGGATGCCCTTTAGT-3' (reverse primer for *GAPDH*).

### Lentivirus transduction

A lentivirus overexpressing *MEG3* and control empty viruses, constructed at Applied Biological Materials, Inc, were used at the viral titer of  $1.07 \times 10^9$  IU/ml. At day 14 after behavioral analyses, the rats were randomly selected for the collection of left DRG and SDH tissues of the L4-6 lumbar vertebrae under regional anesthesia. The collected tissues were postfixed in 4% paraformaldehyde overnight and then placed into 10, 20 and 30% sucrose solutions for sequential gradient dehydration until the tissues sank to the bottom of the container. The tissues were then embedded in OCT embedding agent and cryosectioned. Consequently, the sections were dried at room temperature, and virus transfection was assessed under a fluorescence microscope.

### Statistical analysis

All the data were shown as mean  $\pm$  SEM. Comparisons between two groups were made using two-tailed t test. Data on the PWT, PWL and two-factor analysis were analyzed by two-way ANOVA. Statistically significant difference was indicated as follows:  $***p < 0.001$ ,  $**p < 0.01$ , and  $*p < 0.05$ . The statistical analysis was performed with the software of GraphPad Prism 8.

## Results

### Chronic inflammatory pain in CFA-induced rats

Subcutaneous injection of CFA into the left hind paw of rats in the CFA group produced obvious redness and swelling in these limbs compared with right hind paw, and the activity of each rat was considerably reduced. The rats in the control group subcutaneously injected with the same amount of saline into the left hind paw showed no significant difference between the left hind paw and the right hind paw, and no significant changes in animal activity were observed.

Thermal and mechanical pain thresholds of the rats were measured before subcutaneous injection of CFA, and then at 8 h and at 1, 3, 7 and 14 days after subcutaneous injection of CFA. The rats in the CFA group showed obvious pain sensitivity after CFA injection. Compared with control rats, the rats in the CFA group had a significant decrease in PWTs at 8 h and at 1, 3, 7 and 14 days after subcutaneous injection of CFA (Figure 1(a)). PWLs in the CFA group were significantly lower than those in the control group at 8 h, and at 1 and 3 days, after subcutaneous injection of CFA (Figure 1(b)).

MEG3 lncRNA is negatively correlated with TRPV1 mRNA in the DRG and SDH of CFA-induced rats

Left L4-6 DRG and SDH tissues were collected for real-time PCR analysis of MEG3 lncRNA before subcutaneous injection of CFA and at 8 h and 1, 3, 7 and 14 days after subcutaneous injection of CFA. Our results indicate that compared with that in control rats, MEG3 lncRNA in the DRG and SDH of the CFA-induced rats was decreased significantly at 1, 3, 7 and 14 days after subcutaneous injection of CFA (Figures 2(a) and (b)). Left DRG and SDH tissues of L4-6 were collected before the subcutaneous injection of CFA and then at 8 h and at 1, 3, 7 and 14 days after subcutaneous injection of CFA; the tissues were analyzed using real-time PCR and western blotting. Our results show that compared with that of the control rats, TRPV1 mRNA in the DRG of CFA-induced group was increased significantly at 8 h and at 1, 3, 7 and 14 days after subcutaneous injection of CFA (Figure 2(c)). Compared with that of the control rats, TRPV1 mRNA in the SDH of the CFA-induced rats was significantly increased at 1, 3, 7 and 14 days after subcutaneous injection of CFA (Figure 2(d)). The results of western blotting indicate that TRPV1 expression in the DRG and SDH of the CFA-induced rats was significantly increased at 8 h and at 1, 3, 7 and 14 days after subcutaneous injection of CFA (Figure 3 and Figure 4). These results indicate that the expression of TRPV1 was increased in CFA-induced rats.

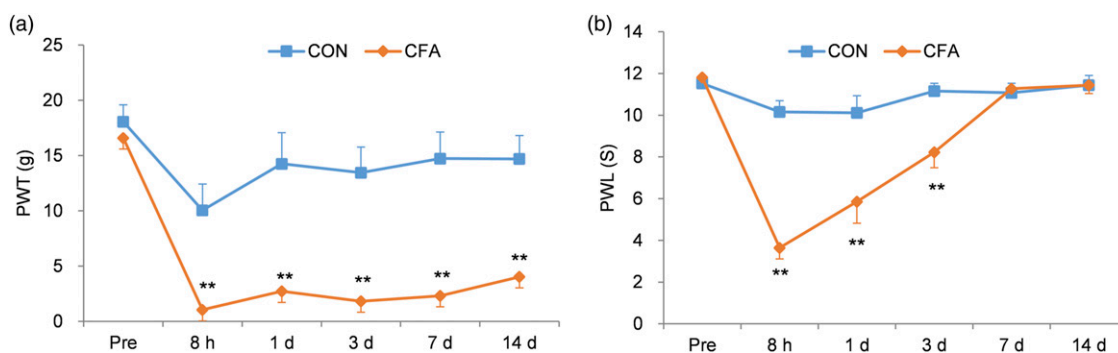
### TRPV1 antagonists alleviate chronic inflammatory pain in CFA-induced rats

Next, we integrated changes in the mechanical and thermal pain thresholds with the time course of TRPV1 expression in the DRG and SDH of CFA-induced rats. Our results indicate that pain sensitivity and molecular detection were relatively stable in these rats at 3 days after subcutaneous injection of CFA. Hence, we selected day 3 post subcutaneous injection of CFA as the time point for performing drug intervention in these rats. After re-establishing the CFA-induced rat model, we intrathecally injected each rat in the CFA group with 10  $\mu$ l TRPV1 receptor blocker capsaizepine (CPZ; dissolved in 10% DMSO at 2 mg/mL). Each rat in the control group was intrathecally injected with 10  $\mu$ l 10% DMSO. Thermal and mechanical pain thresholds in these rats were measured before intrathecal injection, and at 0.5, 1, 2, 4, 6, 8 and 12 h after intrathecal injection. Compared with control rats, intrathecal injection of TRPV1 antagonist in the CFA-induced rats significantly promoted increases in PWTs and PWLs (Figure 5), and significantly reversed CFA-induced inflammatory pain, particularly at 6 h after intrathecal injection.

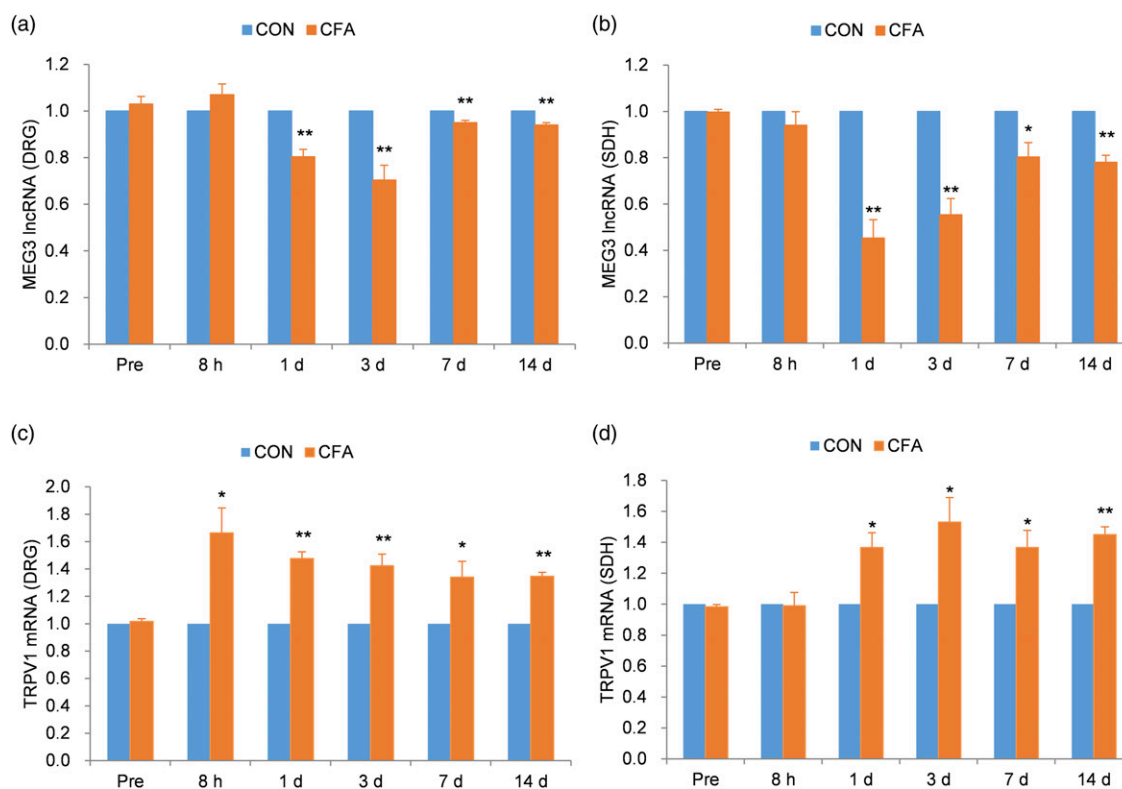
Intrathecal injection of MEG3-overexpressing lentivirus significantly reverses TRPV1 expression in the DRG and SDH of CFA-induced rats

After the intrathecal injection of a MEG3-overexpressing lentivirus into the DRG and SDH tissues of CFA-induced rats at day 14 after CFA induction, transfection efficiency was assessed using a fluorescence microscope. Strong fluorescence signals, indicating GFP expression, were distributed in most of the DRG neurons and SDH tissues (Figures 6(a) and (b)), indicating that the DRG and SDH of these rats were successfully infected with MEG3-overexpressing lentiviruses.

Behavioral analyses in these rats were performed at 14 days after the intrathecal injection of MEG3-overexpressing lentivirus. Then, left DRG and SDH tissues were collected for analysis using real-time PCR and western blotting. The results of western blotting indicate that after the intrathecal



**Figure 1.** The mechanical and thermal pain threshold of CFA rats was decreased. (a) PWT was significantly reduced in CFA group rats from 8 h to 14 days compared with control group rats (saline injection) (two-way ANOVA analysis,  $n = 6$ ,  $**p < 0.01$ ). (b) PWL was significantly reduced in CFA group rats from 8 h to 3 days compared with control group rats (saline injection) (two-way ANOVA analysis,  $n = 6$ ,  $**p < 0.01$ ).



**Figure 2.** MEG3 lncRNA was decreased and TRPV1 mRNA was increased in CFA rats. (a) Quantification of QPCR assays showing significant decrease of MEG3 lncRNA in L4-6 DRG of CFA rats from 1 day to 14 days compared with control rats (two-way ANOVA analysis,  $n = 3$ ,  $**p < 0.01$ ). (b) Quantification of QPCR assays showing significant decrease of MEG3 lncRNA in L4-6 SDH of CFA rats from 1 day to 14 days compared with control rats (two-way ANOVA analysis,  $n = 3$ ,  $*p < 0.05$ ). (c) Quantification of QPCR assays showing significant upregulation of TRPV1 mRNA in L4-6 DRG of CFA rats from 8 h to 14 days compared with control rats (two-way ANOVA analysis,  $n = 3$ ,  $*p < 0.05$ ). (d) Quantification of QPCR assays showing significant upregulation of TRPV1 mRNA in L4-6 SDH of CFA rats from 1 day to 14 days compared with control rats (two-way ANOVA analysis,  $n = 3$ ,  $*p < 0.05$ ).

injection of MEG3-overexpressing lentivirus, the expression of TRPV1 in the DRG and SDH tissues of CFA-induced rats was significantly downregulated compared with that of control rats (Figure 6(c)). Real-time PCR also showed that TRPV1 mRNA in the DRG and SDH tissues of CFA-induced rats was downregulated after the intrathecal injection of MEG3-overexpressing lentivirus compared with that of control rats (Figure 6(d)). These results indicate that MEG3 overexpression significantly reversed TRPV1 expression in the DRG and SDH tissues of rats with CFA-induced chronic inflammatory pain.

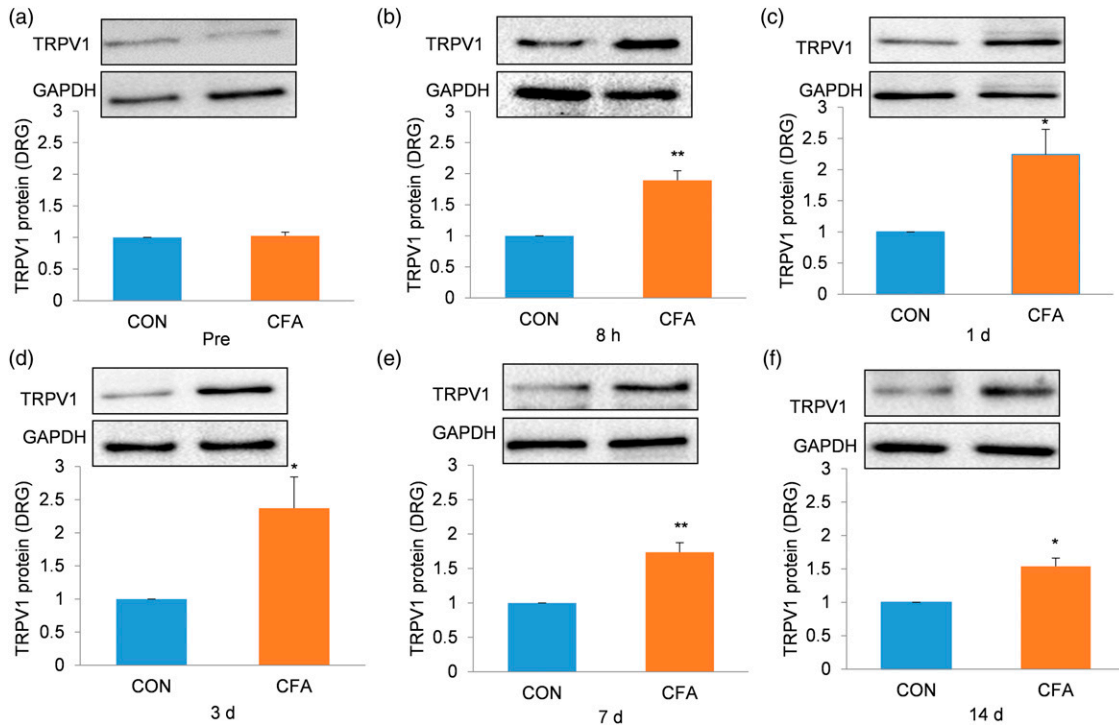
Intrathecal injection of MEG3-overexpressing lentivirus alleviates chronic inflammatory pain in CFA-induced rats

Next, 10  $\mu$ l lentivirus overexpressing MEG3 (used at the viral titer of  $1 \times 10^9$  IU/ml) was injected intrathecally, while an equivalent amount of empty virus, also injected intrathecally, was used as control. CFA induction to model chronic inflammatory pain was performed at day 3 after injection of MEG3-overexpressing lentivirus or empty control virus. Thermal and mechanical pain thresholds in these rats were measured before and at 8 h, 1 day, 3 days, 7 days, 14 days after subcutaneous injection of CFA. Our results show that

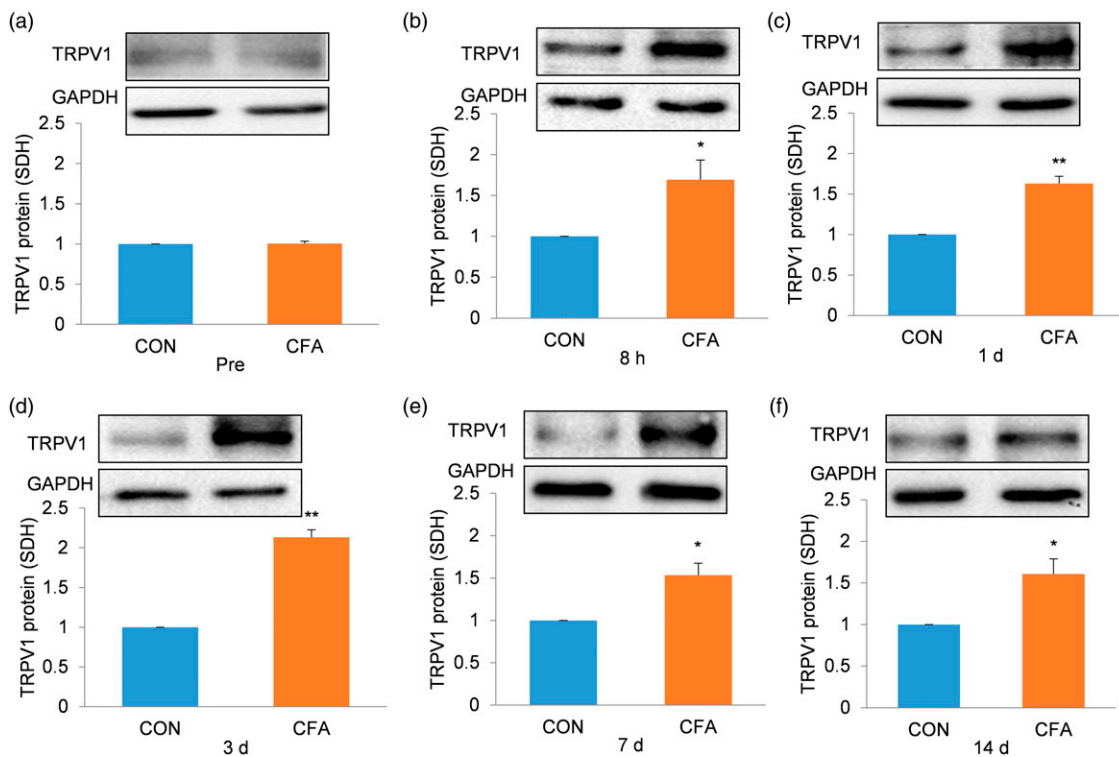
after intrathecal injection of the MEG3-overexpressing lentivirus, PWTs of CFA-induced rats were increased significantly at 7 and 14 days after CFA induction compared with those of control rats (Figure 7(a)). PWLs were also significantly increased in CFA-induced rats at 3 and 7 days after CFA induction compared with those of control rats (Figure 7(b)). These results indicate that intrathecal injection of a MEG3-overexpressing lentivirus significantly alleviated inflammatory pain in CFA-induced rats.

## Discussion

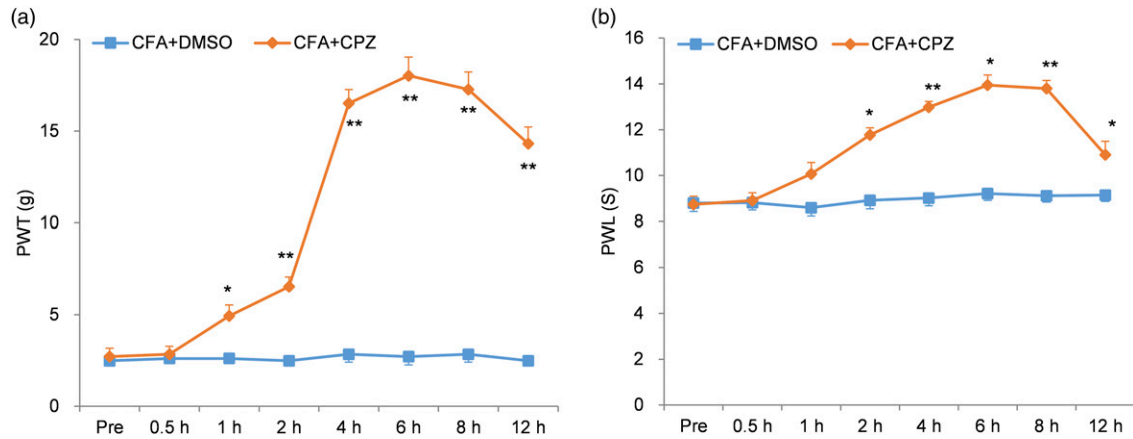
Although the pathogenesis of OA is still unclear, numerous studies have shown that angiogenesis and inflammation in OA are related to the regulation of chondrocyte function, abnormal tissue proliferation and perfusion, ossification, and joint imaging changes. Indeed, angiogenesis, inflammation, tissue hypoxia, and mechanical loading, are involved in the pathogenesis and pain mechanisms of OA.<sup>12</sup> Inflammatory pain is a type of chronic pathologic pain that decreases a patient's quality of life and causes considerable economic losses to society.<sup>13–18</sup>



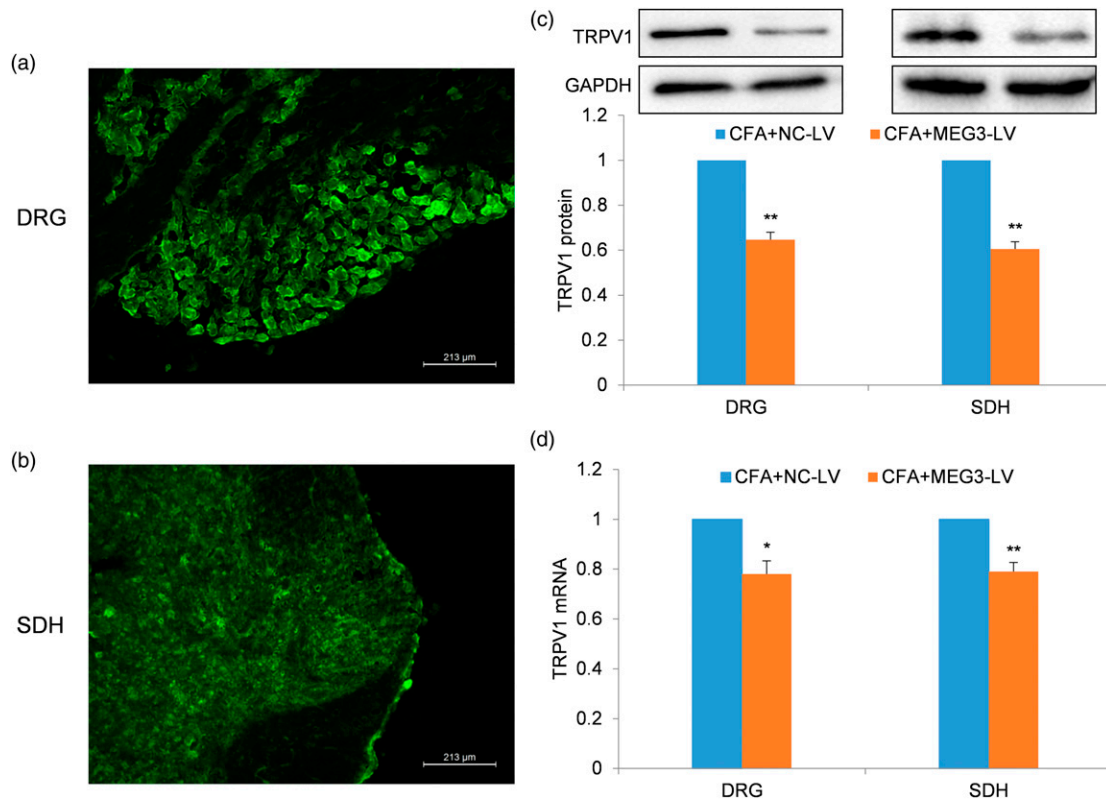
**Figure 3.** The protein expression of TRPV1 in L4-6 DRG of CFA rats was significantly increased from 8 h to 14 days compared with control rats (two-tailed t test,  $n = 3$ ,  $*p < 0.05$ ).



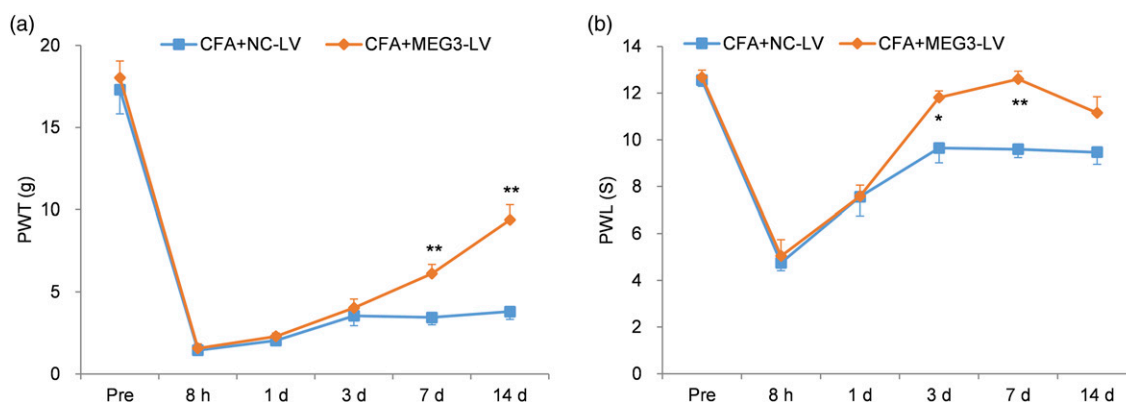
**Figure 4.** The protein expression of TRPV1 in L4-6 SDH of CFA rats was significantly increased from 8 h to 14 days compared with control rats (two-tailed t test,  $n = 3$ ,  $*p < 0.05$ ).



**Figure 5.** The PWT and PWL of CFA rats was increased after Capsazepine (CPZ) injection. (a) The PWT of CFA rats subcutaneous injected with TRPV1 inhibitor (CPZ, dissolved in 10% DMSO at 2 mg/mL) were significantly increased than in control CFA rats injected with same volume of 10% DMSO from 0.5 h to 12h (two-way ANOVA analysis,  $n = 6$ ,  $*p < 0.05$ ). (b) The PWL of CFA rats subcutaneous injected with TRPV1 inhibitor (CPZ, dissolved in 10% DMSO at 2 mg/mL) were significantly increased than in control CFA rats injected with same volume of 10% DMSO from 0.5 h to 12h (two-way ANOVA analysis,  $n = 6$ ,  $*p < 0.05$ ).



**Figure 6.** The expression of TRPV1 was downregulated after the intrathecal injection of MEG3-overexpressing lentivirus. (a) The GFP was expressed in the DRG neurons after the intrathecal injection of a MEG3-overexpressing lentivirus at day 14. (b) The GFP was expressed in the SDH tissues after the intrathecal injection of a MEG3-overexpressing lentivirus at day 14. (c) The protein expression of TRPV1 in L4-6 DRG and SDH tissues of MEG3-overexpressing lentivirus CFA rats was significantly downregulated compared with that of control rats (two-way ANOVA analysis,  $n = 3$ ,  $*p < 0.01$ ). (d) Quantification of QPCR assays showed that TRPV1 mRNA in L4-6 DRG and SDH tissues of MEG3-overexpressing lentivirus CFA rats was downregulated compared with that of control rats (two-way ANOVA analysis,  $n = 3$ ,  $*p < 0.05$ ).



**Figure 7.** MEG3-overexpressing lentivirus treatment attenuated the mechanical and thermal pain of CFA rats. (a) The PWT of CFA rats treated with MEG3-overexpressing lentivirus (intrathecal injection,  $1 \times 10^9$  IU/mL) was significantly increased than in control CFA rats injected with same volume of empty virus from 7 days to 14 days (two-way ANOVA analysis,  $n = 6$ ,  $**p < 0.01$ ). (b) The PWL of CFA rats treated with MEG3-overexpressing lentivirus (intrathecal injection,  $1 \times 10^9$  IU/mL) was significantly increased than in control CFA rats injected with same volume of empty virus from 3 days to 7 days (two-way ANOVA analysis,  $n = 6$ ,  $*p < 0.05$ ).

In our present study, we used the classic CFA-induced model, involving a subcutaneous injection of CFA into the left hind paw of rats, to study the mechanisms of chronic inflammatory pain. Successfully induced rats in the CFA group showed significantly reduced activity and obvious redness and swelling in their left hind paw compared with the contralateral right hind paw. Our behavioral analyses show that the thermal and mechanical pain thresholds of these rats were significantly reduced after the injection of CFA, indicating that the CFA-induced rat model was successfully established in our study.

TRPV1 is a capsaicin receptor and non-selective cation channel distributed mainly in nociceptive neurons. As a type of ion channel membrane receptor involved in pain transmission, TRPV1 is involved in the perception and transmission of stimuli. TRPV1 also plays a role in mediating various types of pains such as inflammatory, visceral, and cancer-related pain, and even hyperalgesia. TRPV1 is considered a key receptor in pain regulation,<sup>19–21</sup> and plays important roles in pain, inflammation, thermoreception, and regulation of vascular function.<sup>22</sup> TRPV1 can also be activated by numerous factors including inflammation, pain, capsaicin, and mechanical pressure. Long-term activation of TRPV1 promotes vasorelaxation and improves endothelial function.<sup>23</sup> A previous study showed that CFA-induced knee-joint pain in *TRPV1*-knockout animals is significantly reduced compared with that in *TRPV1*<sup>+/+</sup> animals.<sup>24</sup> Other studies showed that treatment using TRPV1 antagonists reduces pain in monoiodoacetic acid-induced arthritis and inhibits the signaling from the nociceptor nerve fibers surrounding the joint to neurons in the spinal dorsal horn.<sup>25–28</sup> Amaya et al. showed that increased TRPV1 expression in DRG neurons under inflammatory conditions may be related to persistent hyperalgesia.<sup>29</sup> TRPV1 is expressed in multiple cell types in the joints. Expression of synovial TRPV1 is increased in OA patients undergoing total joint

replacement.<sup>30</sup> In our present study, the mRNA and protein expression of TRPV1 in the DRG and SDH of CFA-induced rats was significantly increased. The mechanical and thermal pain thresholds of these rats were significantly increased after intrathecal injection of a TRPV1 antagonist. Treatment with TRPV1 antagonist significantly alleviated CFA-induced inflammatory pain and decreased pain-related behavior in these rats. These results were consistent with those obtained in previous studies showing that TRPV1 is involved in the occurrence and progression of chronic inflammatory pain, and that antagonizing TRPV1 activity reduces pain responses.

lncRNAs are long-chain transcribed RNAs containing more than 200 nucleotides.<sup>31</sup> Studies showed that OA pathogenesis is closely related to abnormally expressed lncRNAs such as MEG3, growth arrest-specific transcript 5, tumor necrosis factor  $\alpha$  and heterogeneous nuclear ribonucleoprotein L-related immunoregulatory lincRNA (THRIL), and postmeiotic segregation increased 2.<sup>32</sup>

MEG3 is a new type of lncRNA that play an increasingly important role in the regulation of epigenetics.<sup>33,34</sup> MEG3 is involved in articular cartilage inflammation and may be a novel potential target for OA.<sup>35,36</sup> A previous study showed that MEG3 lncRNA in the cartilage tissues of OA patients is significantly reduced, which is closely related to the degree of cartilage degeneration in these patients<sup>[24]</sup>. Wang et al. showed that MEG3 lncRNA is downregulated in the cartilage tissues of rats with OA.<sup>37</sup> Xu et al. showed that the expression level of MEG3 is downregulated in OA tissues and is negatively correlated with VEGF expression, suggesting that MEG3 may participate in the pathologic processes of OA by regulating angiogenesis.<sup>38</sup> In our present study, the expression of DRG and MEG3 in the SDH of CFA-induced rats was significantly reduced. After the intrathecal injection of a



MEG3-overexpressing lentivirus, the mechanical and thermal pain thresholds in these rats were significantly increased, indicating that intrathecal injection of a MEG3-overexpressing lentivirus significantly alleviated inflammatory pain in these animals. The TRPV1 mRNA and protein were also significantly upregulated in the DRG and SDH of CFA-induced rats treated with intrathecal injection of MEG3-overexpressing lentivirus. This finding suggests that MEG3 may participate in the mechanisms of chronic inflammatory pain by upregulating the activity of TRPV1, and may provide a new target in the treatment of patients with OA.

Based on the above findings, we further explored the upstream and downstream relationship between MEG3 and TRPV1. Treatment with a MEG3-overexpressing lentivirus significantly upregulated TRPV1 expression. These results indicated that MEG3 downregulates the expression of TRPV1 in CFA-induced rats. As far as we know, this study was the first to show that MEG3 downregulated TRPV1 expression and alleviated symptoms of OA in CFA-induced rats. Our findings may provide a new therapeutic target for the treatment of OA. Our study also had several limitations, including limited samples and focus on only CFA model. Further studies would use more samples, animal models, advanced methods and examine additional OA-related chronic inflammatory pain.

### Author contributions

J-W.P. performed experiments, analyzed data, and prepared figures and the manuscript. Y-Y.G., J.W., Y.S. and C-L.Z. performed experiments. X.C, Y.S. L.C. and L.Z. edited manuscript. H-L.Z. and Q.W. designed experiments, supervised the experiments and finalized the manuscript. All the authors have read and approved the paper.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Ethics approval

Care and handling of the animals were approved by the Institutional Animal Care and Use Committee of Soochow University and were in accordance with the guidelines of the International Association for the Study of Pain.

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