## Original Article
# The cyclic AMP response element-binding protein antisense oligonucleotide induced anti-nociception and decreased the expression of KIF17 in spinal cord after peripheral nerve injury in mice

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Abstract: Backgrounds: The cyclic AMP response element-binding protein (CREB) plays an important role in neuropathic pain. Kinesin superfamily motor protein 17 (KIF17) is involved in long-term memory formation. CREB could increase the level of KIF17 when activated by synaptic input. This study is to investigate the role and mechanism of CREB antisense oligonucleotide (ODN) in neuropathic pain induced by chronic constriction injury (CCI) in mice. Results: CCI surgery decreased thresholds of mechanical allodynia and thermal hyperalgesia whereas CREB antisense oligonucleotide ODN significantly attenuated these pain behaviors (P < 0.05). CCI significantly induced the protein expression of phosphorylated CREB (pCREB) and KIF17, but not KIF5B, in the spinal cord of CCI mice (P < 0.05). Additionally, the mRNA expression of CREB and KIF17 was significantly increased by CCI (P < 0.05). However, CREB antisense ODN significantly decreased the protein expression of pCREB and KIF17 (but not KIF5B), and the mRNA expression of CREB and KIF17 (P < 0.05). Conclusions: CREB antisense oligonucleotide ODN may reduce neuropathic pain through targeting CREB and decreasing the expression of pCREB and KIF17.

Keywords: Neuropathic pain, CREB, KIF17, antisense oligonucleotide

## Introduction

Central sensitization is one of the mechanisms underlying long-standing neuropathic pain. It refers to the increased activity resulting from synaptic plasticity established in somatosensory neurons in the dorsal horn of spinal cord following peripheral noxious stimuli. Neuropathic pains, caused by injuries to the nervous system or the abnormal physiology of the nervous system, have many clinical characteristics, such as persistent spontaneous pain, hyperalgesia and allodynia. Due to limited understanding of the mechanisms for neuropathic pain modulation, the therapeutic effect of neuropathic pain is not ideal. Thus, understanding potential mechanisms of induction and maintenance of neuropathic pain is essential for developing more efficient treatments for neuropathic pain.

As a constitutive transcription factor, cyclic AMP response element-binding protein (CREB) is found to be involved in the maintenance of neuropathic pain [1, 2]. It has been shown that CREB phosphorylation played an important role in many kinds of pain models, such as inflammation pain [3, 4], neuropathic pain [1, 2, 5-7] and chronic muscle pain [8]. In addition, the nociceptive behaviors induced by inflammation and neuropathic pain in these models have been shown to be accompanied with the induction of phosphorylated CREB (pCREB) [7, 9]. Ma et al. and Wang et al. reported that blocking the induction of CREB or pCREB by intrathecal injection of CREB antisense oligonucleotide (ODN) could inhibit the nociceptive behaviors induced by peripheral nerve injury [1, 2]. The mechanism underlying the role of CREB in neuropathic pain is still unclear.

The kinesin superfamily (KIF) consists of microtubule based motor proteins and is involved in the transportation of membrane organelles, protein complexes, and mRNAs [10]. Kinesin superfamily motor protein 17 (KIF17) is an important member of KIF and expresses abundantly in mammalian neurons. It has putative function in the transportation of N-methyl-D-aspartate receptor subunit 2B (NR2B)-containing vesicles in neuronal dendrites [10, 11]. Previous studies have shown that KIF17 plays important roles in long-term potentiation (LTP), memory and learning [12-15]. It might be one of the major functional components transporting NR2B, though the exact mechanism of synaptic plasticity is yet to be elucidated. It has been shown that synaptic plasticity contributes to chronic pain [16]. Interestingly, KIF17 and CREB are functionally related. Instant expression of constitutively active CREB in hippocampal neurons could induce the expression of KIF17 at both mRNA and protein levels [12]. Inhibition of CREB activity by expressing a dominant-negative form of CREB caused significant decrease of KIF17 expression [12]. These data suggested that the activation of CREB could positively regulate KIF17. In addition, KIF5B is another member of the KIF family. According to the report by Wong et al [15], we suppose that CREB does not contain the KIF5B coding gene.

The aim of this present study is to investigate the role and mechanism of CREB antisense ODN in neuropathic pain. Chronic constriction injury (CCI) of the sciatic nerve was established to induce neuropathic pain. CREB oligonucleotides were intrathecally administrated. Expression levels of CREB, pCREB, KIF17 and KIF5B were detected.

## Methods and materials

### Experimental animals

Adult (7-8 weeks old) male C57BL/6 mice weighing 20-25 g were obtained from the Model Animal Research Center of Nanjing University. They were kept on a 12-h light/dark schedule with standard rodent chow and water ad libitum and maintained at 21-24°C. The experimental protocol was approved by the Animal Care and Use Committee of the Medical College of Nanjing University and complied with the guidelines for the use of laboratory animals of Medical College of Nanjing University [17]. All efforts were made to minimize animal suffering and to reduce the number of animals used.

### Intrathecal catheter implantation

An intrathecal catheter was implanted in each mouse under the same surgical conditions. Under deep sodium pentobarbital (40 mg/kg, i.p.) anesthesia, the surgical procedures of intrathecal catheterization in this study were conducted according to previously described methods with minor modifications [18]. After intrathecal catheterization, mice were kept in individual cages and allowed to recover for 3 days before establishment of CCI model. Mice with neurologically normal and complete paralysis of the bilateral hind legs after intrathecal administration of 2% lidocaine (2 μl) were used for establishment of CCI.

### CCI surgery

Male mice were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) for surgical procedures. Chronic constriction of the sciatic nerve was performed according to the method described by Bennett and Xie [19]. Briefly, the right sciatic nerve was exposed at the level of the mid-thigh. Three ligatures (5-0 chromic gut, Ethicon, Rome, Italy) were tied loosely around the sciatic nerve with a 1.0-1.5 mm interval between each ligature. The wound was then closed with 4-0 Ethicon silk suture. The injured right hind paw was named as ipsilateral paw and the uninjured left hind paw named as contralateral paw. The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day).

### Intrathecal CREB ODNs administration

The sequences of sense, missense and antisense CREB ODNs were designed as previously reported [20]. Sequences for the ODNs were as follows: antisense ODN, 5'-TGGTCATCTAGTCAC-CGGTG-3'; sense ODN, 5'-CACCGGTGACTAG-ATGACCA-3'; and missense ODN, 5'-GACCT-CAGGTAGTCGTCGTT-3'. The ODNs were phosphorothioate-modified and synthesized by Sangon Biotechnology Company (Shanghai, China). The ODNs were dissolved in saline before administration. The mice were injected intrathecally with 5 μl of saline, 5 μl/5 μg of sense ODN, 5 μl/5 μg of missense ODN and 5 μl/5 μg of antisense ODN, respectively, every 24 h for 6 days. Finally, the injection was fol-

**Table 1.** The paw withdrawal mechanical threshold (PWMT) and the paw withdrawal thermal latency (PWTL) changes of the right hind limb over time in CCI mice (n = 6 per group per day) and sham mice (n = 6 per group per day)

| Days post CCI | PWMT (g) | |
|---|---|---|
| | Sham group | CCI surgery group |
| Day 0 | 1.11 ± 0.11 | 1.03 ± 0.09 |
| Day 7 | 1.01 ± 0.17 | 0.42 ± 0.12* |
| Day 14 | 1.05 ± 0.12 | 0.38 ± 0.12* |
| Days post CCI | PWTL (sec) | |
| | Sham group | CCI surgery group |
| Day 0 | 6.63 ± 0.66 | 6.34 ± 0.49 |
| Day 7 | 7.03 ± 0.77 | 3.26 ± 0.66* |
| Day 14 | 6.88 ± 0.86 | 3.50 ± 0.53* |

Note: *$P < 0.05$, compared with the sham group at the same time point.

lowed by a flush of 5 μl of saline. The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day).

*Mechanical allodynia*

Mice were habituated to the behavioral testing conditions daily for 3 days before initiating baseline testing. Each mouse was placed in individual transparent plastic compartments (8.5 × 11.5 × 14 cm) on a metal mesh floor and allowed to acclimatize for 30 min every time. Von Frey filaments (Stoelting, Wood Dale, IL, USA) with incremental stiffness (0.16-1.4 g) were applied serially to the paw in ascending or descending order of stiffness depending on the foot withdrawal response of the mouse. The maximum and minimum cut-offs were at 1.4 g and 0.16 g, respectively. The filaments were poked vertically against the plantar surface with sufficient force to cause slight bending against the paw and held for 4-5 s with a stimuli interval of approximately 15 seconds. A withdrawal of hind paw upon the stimulus (at least three times out of five applications) was considered as positive responses. Depending on the positive or negative response, the paw withdrawal mechanical threshold (PWMT) was determined by sequentially increasing and decreasing the stimulus strength (the "up-and-down" method). Each mouse was tested five times per stimulus strength. The lowest von Frey filaments which had three or more positive responses were regarded as PWMT.

*Thermal hyperalgesia*

Thermal hyperalgesia was assessed with the paw withdrawal thermal latency (PWTL) to radiant heat. Mice were habituated to the behavioral testing conditions for 3 days before baseline testing. Each mouse was placed in individual transparent plastic compartments (8.5 × 11.5 × 14 cm) on a thin glass platform and allowed to acclimatize for 30 min every time. A radiant thermal stimulator (UGO BASILE 7370 Plantar Test Apparatus, Italy) was focused onto the plantar surface of the hind paw through the glass platform. There were five trials per mouse and 5 min intervals between trials. The infrared intensity was set at 50 (corresponding to 196 mW/cm$^2$), which produced baseline paw withdrawal latencies of 5-10 s. A cut-off time of 20 s was used to avoid tissue damage. The mean latency of withdrawal response of each hind paw was determined by 5 tests.

*Western blot*

Mice were killed rapidly by decapitation and the lumbar spinal cord segments were dissected out and frozen on dry ice. Samples were then stored at -80°C for further analysis. Tissue samples were homogenized in lysis buffer. The homogenate was centrifuged at 12,000 × g for 10 min at 4°C and supernatant was removed. The protein concentration was determined by Pierce BCA Protein Assay Kit (GE Healthcare, Chalfont St Giles, UK), following the manufacturer's instructions. Samples (50 μg) were separated on SDS-PAGE (6-12% gradient gel, Nanjing KeyGen Biotech., Co., Ltd., Nanjing, China) and subsequently transferred to polyvinylidene difluoride membranes (Millipore Corporation, Massachusetts, USA). The membranes were blocked with 5% nonfat milk in tris-buffered saline (TBS; pH 7.4; Sigma) for 1 h at room temperature and incubated respectively with primary antibodies of rabbit anti-pCREB (1:1000 dilution; Cell Signaling Technology, Inc., Danvers, Massachusetts, USA), anti-KIF17 (1:1000 dilution; Santa Cruz, California, USA), anti-KIF5B (1:1000 dilution; Santa Cruz, California, USA), and anti-β-actin (1:2000 dilution, Cell Signaling Tech, Massachusetts, USA) at 4°C overnight. Following three consecutive 5 min washes with TBS, the membrane was incubated with secondary antibodies of anti-goat or mouse IgG (1:5000 dilution; Nanjing KeyGen Biotech., Co., Ltd., Nanjing, China). The blots

**Table 2.** The effect of intrathecal administration of CREB oligonucleotides on paw withdrawal mechanical threshold (PWMT) induced by CCI (n = 3-4 per group per day)

| Days post CCI | PWMT (g) | | | |
|---|---|---|---|---|
| | Saline group | Antisense group | Sense group | Missense group |
| Day 0 | 1.14 ± 0.25 | 1.00 ± 0.19 | 1.13 ± 0.12 | 1.13 ± 0.23 |
| Day 7 | 0.42 ± 0.15 | 0.76 ± 0.24* | 0.37 ± 0.15 | 0.34 ± 0.12 |
| Day 14 | 0.36 ± 0.08 | 0.66 ± 0.09*,# | 0.36 ± 0.17 | 0.39 ± 0.11 |

Note: *$P < 0.05$, compared with the saline group at the same time point. #$P < 0.05$, compared with data on Day 0.

**Table 3.** The effect of intrathecal administration of CREB oligonucleotides on paw withdrawal thermal latency (PWTL) induced by CCI (n = 3-4 per group per day)

| Days post CCI | PWTL (sec) | | | |
|---|---|---|---|---|
| | Saline group | Antisense group | Sense group | Missense group |
| Day 0 | 6.58 ± 0.36 | 6.93 ± 1.08 | 6.80 ± 0.84 | 6.70 ± 1.06 |
| Day 7 | 2.74 ± 0.75 | 5.96 ± 0.69* | 2.01 ± 0.74 | 2.40 ± 0.61 |
| Day 14 | 2.60 ± 0.55 | 4.39 ± 0.26*,# | 2.61 ± 0.23 | 2.83 ± 0.78 |

Note: *$P < 0.05$, compared with saline group at the same time point. #$P < 0.05$, compared with data on Day 0.

were developed with enhanced chemiluminescence solution (DuPont NEN, Boston, Massachusetts, USA) for 1 min and exposed to hyper films (Amersham Biosciences, Piscataway, New Jersey, USA) for 1-10 min. Western Blot images were quantified using Quantity One software (Bio-Rad Laboratories, Hercules, California, USA). The density of specific bands was measured with a computer-assisted imaging analysis system (NIH image, version 1.61). β-actin was used as an internal control. The relative expression value was calculated based on the grey value of β-actin.

*Reverse transcription-PCR (RT-PCR)*

Mice were rapidly (< 1 min) killed through decapitation after being anesthetized with pentobarbital. Based on previously described [21], the L3-L5 lumbar spinal cord segments were dissected out and immediately frozen in liquid nitrogen and stored at -80°C. Total RNA was isolated with Trizol (15596-026, Invitrogen, Carlsbad, CA, USA). Then 5 μg RNA was reverse transcribed into cDNA with M-MLV reverse transcriptase (PC0002, Fermentas, Vilnius, Lithuania). The cDNA was used as a template for PCR amplification with Taq DNA polymerase (EP-0702, Fermentas, Vilnius, Lithuania). Primers

for CREB, KIF17 and GAPDH were designed and synthesized by Nanjing KeyGEN Biotech., Co., Ltd. (Nanjing, China. The primer sequences were as follows. CREB, upstream primer sequence, 5'-CAGCCACAGA-TTGCCACAT-3', and downstream primer sequence, 5'-GCTCCTCCCTGGGTAAT-GG'; KIF17, upstream primer sequence, 5'-CCCGCA-GACAACAATTACGA-3', and downstream primer sequence, 5'-CATACGAGTTACG-CATGGCAGT-3' and GAPDH, upstream primer sequence, 5'-GAGACCTTCAACACCC-CAGC-3', and downstream primer sequence, 5'-CAC-AGAGTACTTGCGCTCAG-3'). PCR amplification was performed with 5 min at 95°C and 28 cycles of 30 sec at 95°C, 30 sec at 57°C, and 35 sec at 72°C. Five microliters of amplified cDNA was electrophoresed on 2% agarose gel and stained with ethidium bromide. The intensity of each PCR band was analyzed using gel imaging analytical system (Gel Doc XR; Bio-Rad Laboratories, Hercules, California, USA). Samples without the addition of reverse transcriptase (negative controls) yielded no detectable product.

*Statistical analysis*

All data were expressed as mean ± SD (standard deviation). Western blotting, behavioral and RT-PCR data were analyzed by repeated measures ANOVA. The post hoc Newman-Keuls tests were performed to determine sources of differences, when significant main effects were observed. Differences were considered to be statistically significant at the level of α = 0.05.

**Results**

*CCI surgery decreases thresholds of hind paw withdrawal to mechanical and thermal stimuli*

To determine the effect of CCI surgery on pain behaviors, PWMT and PWTL were measured before surgery (day 0) and on day 7 and day 14

after CCI surgery. As shown in **Table 1**, the thresholds for PWMT and PWTL in CCI surgery group were similar to those of sham group on day 0. However, on day 7 and day 14, the thresholds for PWMT and PWTL in CCI surgery group were lower than those of sham group. Compared to sham group, the PWMT and PWTL decreased significantly in CCI surgery group on day 7 and day 14 (**Table 1**, P < 0.05, n = 6). The results showed that CCI surgery induced changes in pain behaviors, suggesting that mice model with CCI was successfully established.

*Intrathecal administration of CREB antisense ODN alleviates pain behaviors induced by CCI*

To determine the effect of CREB oligonucleotides on pain behaviors induced by CCI, PWMT and PWTL were measured on before surgery (day 0) and on day 7 and day 14 after CCI surgery. CCI mice were given a daily intrathecal catheter on postoperative day 1-6 and received 5 μg/5 μL of CREB antisense ODN, sense ODN, missense ODN, and saline (n = 6-8). The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day). According to the treatments, mice in CCI surgery group were divided into the saline group, antisense group, sense group, and missense group. Before CCI operation (Day 0), no differences in PWMT and PWTL were observed among the four groups (**Tables 2** and **3**). On day 7 and day 14 post CCI, the thresholds for PWMT and PWTL in the four groups decreased. Pain thresholds in both sides of hind paw in sham group and contralateral hind paw in CCI group had no significant change (data not shown). However, the thresholds for PWMT and PWTL of the ipsilateral hind limb in antisense group was significantly higher on day 7 and day 14 post surgery (**Tables 2** and **3**, P < 0.05), compared with the saline group. Meanwhile there were no significant differences in PWMT and PWTL among other three groups. These results suggest the pain behaviors induced by CCI was alleviated by CREB antisense oligonucleotide ODN.

*The expression of pCREB increases in spinal cord after CCI*

To observe the changes of pCREB in CCI mice, Western Blot analysis was performed on day 7 and day 14 after CCI surgery. Mice in sham group were used as negative control. Representative and quantitative Western Blot results were shown in **Figure 1A** and **1B**, respectively. Expression level of pCREB in the spinal cord increased on day 7 post CCI. On day 14 post CCI, the pCREB level was further increased. Statistically, compared with mice in sham group, mice underwent CCI had significantly higher levels of pCREB on day 7 and day 14 post CCI (P < 0.05). This data indicate that CCI surgery induced expression of pCREB in spinal cord.

*The mRNA levels of CREB in spinal cord of CCI mice decreases after intrathecal administration of CREB antisense ODN*

To investigate whether the injection of CREB antisense ODN would influence CREB transcription in CCI mice, we detected CREB mRNA level by RT-PCR. CREB oligonucleotide injection was performed as described in Materials and Methods. The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day). Representative and quantitative RT-PCR results were shown in **Figure 1C** and **1D** respectively. The mRNA expression of CREB within the spinal cord was significantly reduced on postoperative day 7 and 14 in CCI mice receiving the CREB antisense ODN, compared to CCI mice receiving sense ODN, missense ODN and saline (P < 0.05; n = 3-4). Furthermore, the mRNA expression levels of CREB on day 14 of CCI mice were significantly increased compared to those on day 7 (P < 0.05; n = 3-4). No significant difference was observed among CCI mice treated with saline, CREB sense ODN or CREB missense ODN. Thus CREB antisense ODN decreased CREB mRNA transcription in CCI mice.

*The protein levels of pCREB induced by CCI in spinal cord decreases after intrathecal administration of CREB antisense ODN*

We next investigated whether intrathecal administration of CREB antisense ODN could also affect pCREB protein expression in the spinal cord in CCI mice. CREB oligonucleotide injection was performed as described in Materials and Methods. The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day). Western Blot showed that the expression of pCREB in the spinal cord was significantly reduced in mice treated with CREB antisense ODN on day 7 and
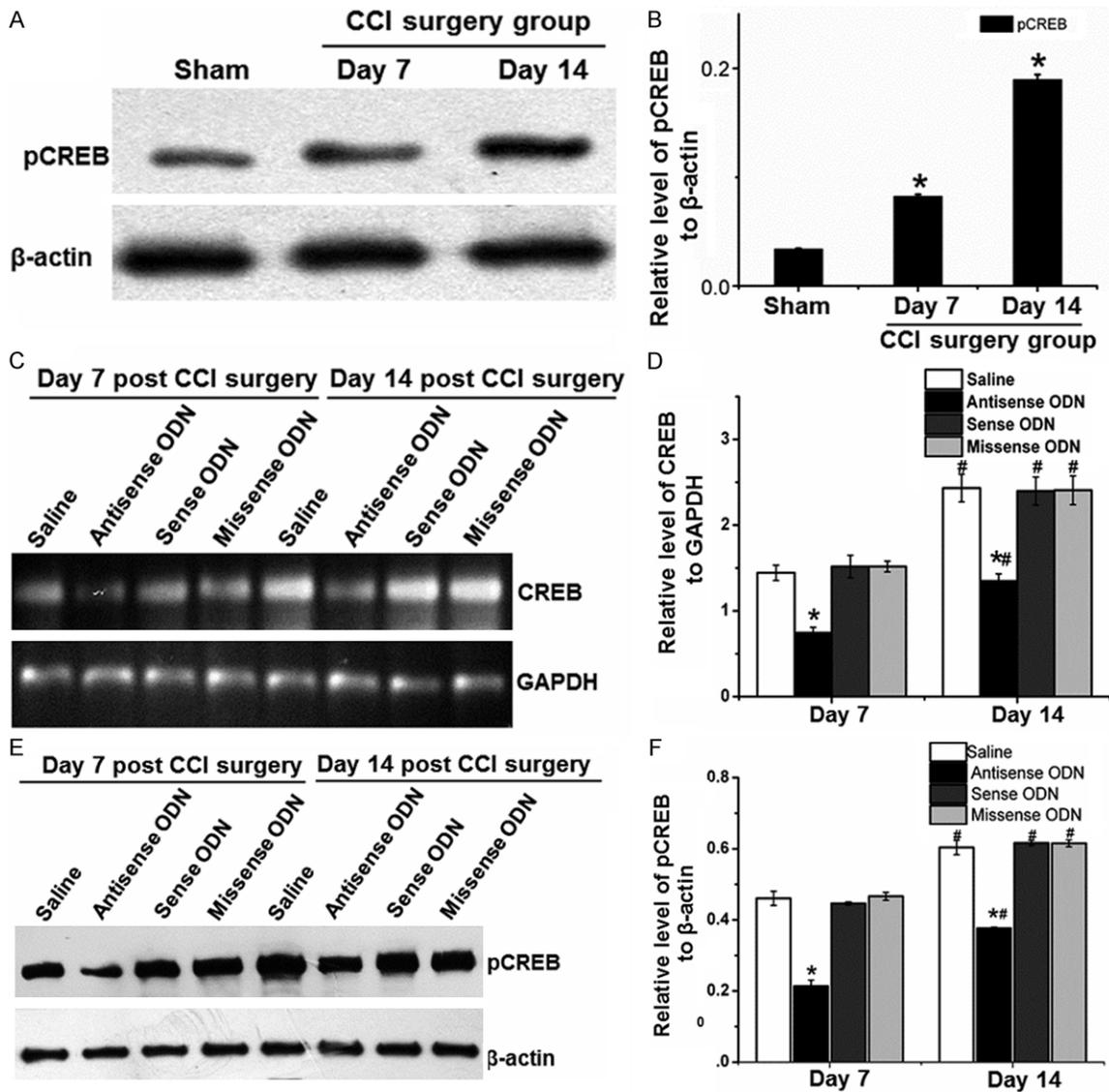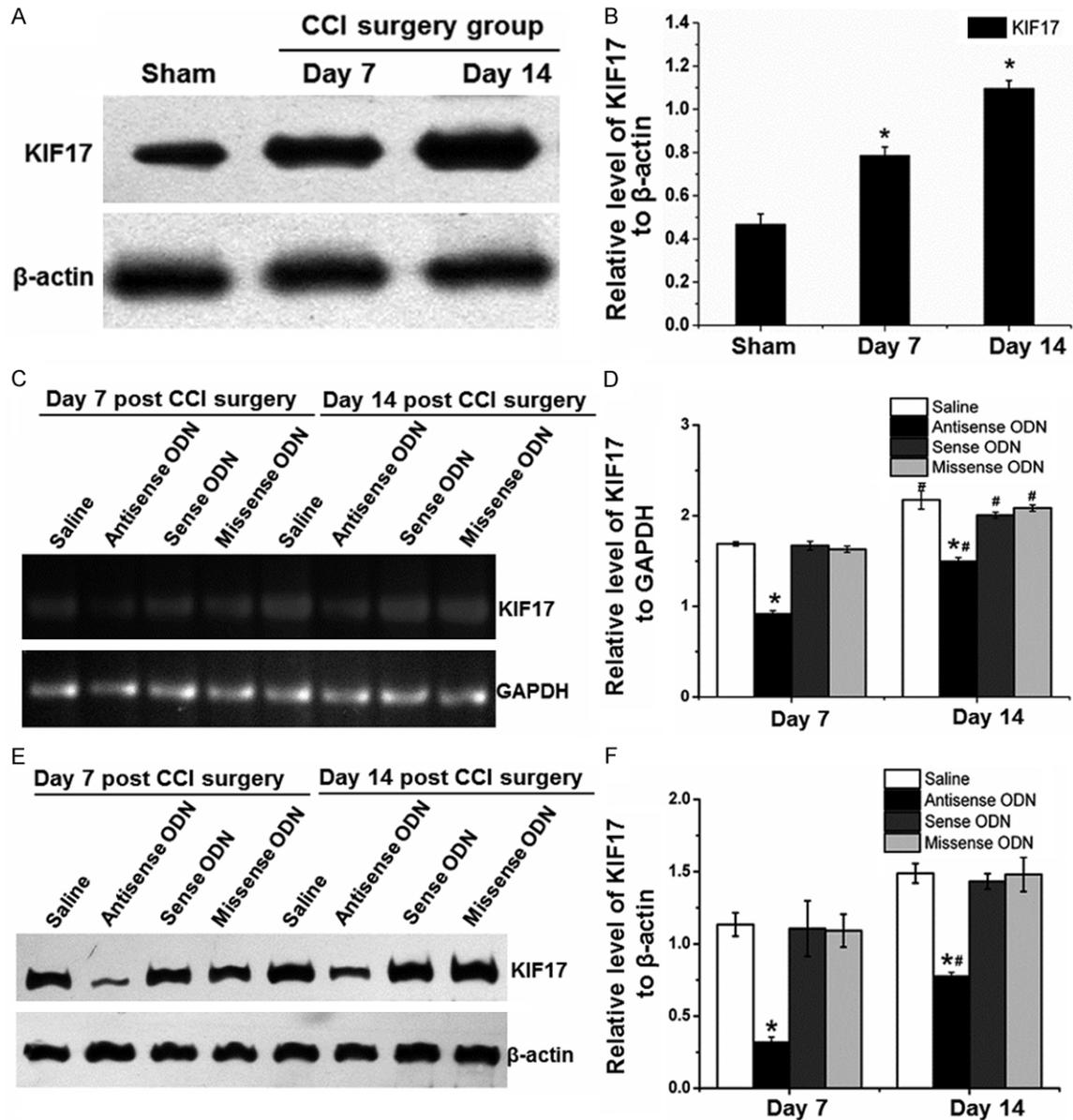
Figure 1. The expression of pCREB and CREB mRNA in spinal cord after CCI. CCI mice were given a daily intrathecal catheter on postoperative day 1-6 and received 5 µg/5 µL of CREB antisense ODN, sense ODN, missense ODN, and saline (n = 6-8). The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day). A. Western Blot analysis was conducted to detect the expression levels of pCREB after CCI. Representative Western Blot results were shown. B. Quantitative Western Blot results were shown. *P < 0.05, compared with sham group. C. CREB mRNA expression after ODN injection was analyzed by RT-PCR. Representative RT-PCR results were shown. D. Quantitative RT-PCR results were shown. E. Expression level of pCREB protein after ODN injection was detected by Western Blot analysis. Representative Western Blot results were shown. F. Quantitative Western Blot results were shown. Data represented mean ± SD of three independent experiments. *P < 0.05, compared with mice received normal saline. #P < 0.05, Day 14 VS Day 7 in each group.

14 after CCI, when compared with mice treated with saline (**Figure 1E** and **1F**, P < 0.05). In addition, the expression of pCREB on day 14 after CCI was higher than that on day 7 (**Figure 1E** and **1F**, P < 0.05). No significant difference was found among the saline treatment, CREB sense ODN treatment and CREB missense ODN treatment. Therefore, CREB antisense ODN decreased pCREB expression induced by CCI.

*The expression of KIF17 increases in spinal cord after CCI*

To detect the expression level of KIF17 in CCI mice, Western Blot analysis was conducted on day 7 and day 14 after CCI surgery. Mice in sham group were used as negative control. As shown in **Figure 2A** and **2B**, KIF17 expression was increased in CCI mice. And KIF17 expres-
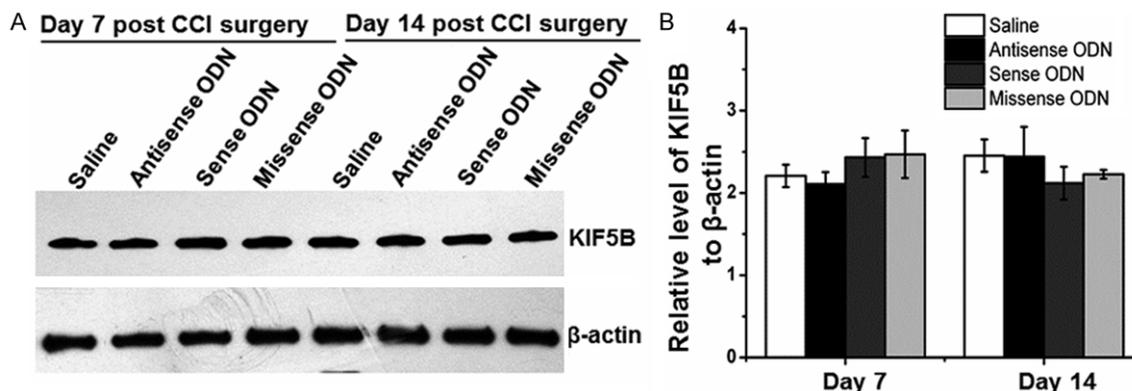
**Figure 2.** The expression of KIF17 in spinal cord after CCI. CCI mice were given a daily intrathecal catheter on postoperative day 1-6 and received 5 µg/5 µL of CREB antisense ODN, sense ODN, missense ODN, and saline (n = 6-8). The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day). A. KIF17 protein expression after CCI was analyzed by Western Blot analysis. Representative Western Blot results were shown. B. Quantitative Western Blot results were shown. *P < 0.05, compared with sham group. C. RT-PCR was performed to analyze KIF17 mRNA expression after ODN injection. Representative RT-PCR results were shown. D. Quantitative RT-PCR results were shown. E. Expression level of KIF17 protein after ODN injection was detected by Western Blot analysis. Representative Western Blot results were shown. F. Quantitative Western Blot results were shown. Data represented mean ± SD of three independent experiments. *P < 0.05, compared with mice received normal saline. #P < 0.05, Day 14 VS Day 7 in each group.

sion on day 14 was higher than that on day 7. Compared with mice in sham group, mice in CCI surgery had significantly higher levels of KIF17 on day 7 and day 14 after CCI surgery (P < 0.05). This result suggests that CCI surgery could induce expression of KIF17 in the spinal cord.

*Intrathecal administration of CREB antisense ODN reduces KIF17 mRNA expression in spinal cord of CCI mice*

To examine how CREB affects the mRNA expression of KIF17 in the spinal cord during the development of neuropathic pain, we

**Figure 3.** Analysis of KIF5B protein expression in the spinal cord of CCI mice. CCI mice were given a daily intrathecal catheter on postoperative day 1-6 and received 5 µg/5 µL of CREB antisense ODN, sense ODN, missense ODN, and saline (n = 6-8). The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day). Expression level of KIF5B protein was detected by Western Blot analysis. β-actin was used as an internal control. A. Representative Western Blot results. B. Quantitative Western Blot results. The relative expression level of KIF5B was calculated based on the intensity of β-actin. Data represented mean ± SD of three independent experiments.

detected the mRNA level of KIF17 in mice treated with saline, CREB antisense ODN, sense ODN and missense ODN by RT-PCR. The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day). Representative and quantitative RT-PCR results were shown in **Figure 2C** and **2D**, respectively. The mRNA expression of KIF17 in the spinal cord were significantly reduced in mice treated with CREB antisense ODN on day 7 and day 14 after CCI surgery, compared to mice treated with saline (P < 0.05). In addition, the expression level of KIF17 mRNA on day 14 was higher than that on day 7 (P < 0.05). There was no significant difference among mice treated with saline, sense ODN and missense ODN. Thus, KIF17 mRNA expression was inhibited by CREB antisense ODN in CCI mice.

*The expression of KIF17 protein is reduced after intrathecal administration of CREB antisense ODN in CCI mice*

We further investigated whether intrathecal administration of CREB antisense ODN could influence KIF17 protein expression in spinal cord of CCI mice. Western Blot analysis was conducted to analyze KIF17 protein expression. CREB oligonucleotide injection was performed as described in Materials and Methods. The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day). As shown in **Figure 2E** and **2F**, the expression of KIF17 protein in the spinal cord was significantly reduced on postoperative day 7 and

day 14 in CCI mice receiving the CREB antisense ODN, compared to mice receiving saline (P < 0.05; n = 3-4). Moreover, the expression of KIF17 protein on day 14 in the spinal cord was significantly increased when compared to that on day 7 (P < 0.05; n = 3-4). The expression of KIF17 protein on day 14 in mice treated with saline didn't change much compared to that on day 7. No significant difference was found among the saline group, CREB sense ODN group and CREB missense ODN group on both day 7 and day 14. This data showed that intrathecal administration of CREB antisense ODN reduced KIF17 protein expression in spinal cord of CCI mice.

*KIF5B protein expression is not significantly changed after intrathecal administration of CREB antisense ODN*

KIF5B, like KIF17, is also a member of the KIF family. To investigate the effect of CREB antisense ODN on KIF5B protein expression in spinal cord of CCI mice, Western Blot analysis was performed. CREB oligonucleotide injection was performed as described in Materials and Methods. The spinal cord samples were collected on postoperative day 7 and 14 (n = 3-4 per group per day). Representative Western Blot results were shown in **Figure 3A** and quantitative results were shown in **Figure 3B**. KIF5B protein expression was not obviously changed in mice with different treatments or in samples from different time points (day 7 and day 14). Statistically, there were no significant changes

in the expression of KIF5B in the spinal cord on day 7 and day 14 (**Figure 3**) (n = 3-4). Thus, KIF5B protein expression in CCI mice was not affected by CCI injury or CREB oligonucleotide administration.

## Discussion

This study showed the involvement of CREB in the central sensitization of nociceptive spinal neurons induced by CCI in mice. Administration of the CREB antisense ODN, attenuated the pain behaviors induced by CCI, and inhibited the upregulation of pCREB. These results indicate that CREB antisense ODN may contribute to the behavioral changes associated with central sensitization through regulating pCREB expression. Our results also showed that KIF17, but not KIF5B, was associated with CREB-modulated neuropathic pain development.

CREB is a constitutively expressed transcription factor and has multiple functions, especially in the nervous system, such as the modulation of gene expression, and synaptic plasticity, including LTP and central sensitization. Recently, it has been shown that CREB is preferentially localized in pain-processing regions in the nervous system, such as neurons in the spinal dorsal horn [1, 2], which suggests that it may contribute to nociceptive processing. Many previous studies have suggested that the activation of CREB in the spinal dorsal horn plays an important role in the processing of pain induced by inflammation [3, 4, 9] and nerve injury [5]. Our results showed that, compared with mice in sham group, the pCREB protein in the spinal cord of CCI mice was significantly increased on day 7 and day 14 after CCI. The upregulation was abolished by CREB antisense ODN. This result was consistent with previous studies [1, 2], indicating that pCREB is an important modulator in the pathogenesis of neuropathic pain induced by CCI.

Hirokawa et al reported that instant expression of constitutively active CREB in hippocampal neurons could induce the expression of KIF17 at both mRNA and protein level [12]. Blocking CREB activity by introducing a dominant-negative form of CREB could lead to significant decreases of KIF17 mRNA [12]. These data suggest that the activation of CREB could positively regulate KIF17 transcription. Wong et al [15] found that CREB phosphorylation could lead to the increase of KIF17 protein expression. However, the underlying mechanism is unknown. The results of this study showed that the expression of KIF17 increased significantly on day 7 and 14 after CCI. Furthermore, our study demonstrated that chronic daily intrathecal administration of CREB antisense ODN alleviated the CCI induced mechanical allodynia and thermal hyperalgesia, and reduced the expression of pCREB and KIF17 in spinal cord of CCI mice, but not KIF5B. These results suggest that the activation of CREB participates in the transcription of KIF17 during the development of neuropathic pain induced by CCI.

KIF17, a recently characterized member of the KIF family, has been proposed to bind to a protein complex containing mLin10 (Mint1/X11) and the NR2B subunit of the NMDA receptors (NMDARs) in vitro. In mammalian brain, NR2B plays important roles in synaptic plasticity, learning and memory [22-24]. Like NMDARs, KIFs also play important roles in synaptic plasticity, learning and memory [12-15]. Newly synthesized NR2B in the nerve cell bodies could be transported to axon and somatodendritic by members of KIF family. KIF17-mediated NR2B trafficking is one of the key regulators of NMDAR formation in neuron [12, 14, 15]. Cellular knockdown or functional blockade of KIF17 could significantly inhibit NR2B expression and its synaptic localization, impairing the function of NR2B. This study suggested that CCI could induce the upregulation of KIF17 expression in the spinal cord on postoperative day 7 and 14. And, pain threshold decreased on postoperative day 7 and 14. The KIF17 expression upregulation was abolished by CREB antisense ODN. These results indicate that KIF17 may play an important role in neuropathic pain development and may act as a downstream effector of CREB.

## Conclusions

In summary, our results demonstrated that mechanical allodynia, thermal hyperalgesia and the elevated expression of CREB and KIF17 induced by CCI could be inhibited by intrathecal injection of CREB antisense ODN. Our study also suggests that CREB may regulate the expression of KIF17 in spinal cord in the development of neuropathic pain induced by CCI.

## Disclosure of conflict of interest

None.

## Abbreviations

CCI, Chronic constriction injury; CREB, cyclic AMP response element-binding protein; KIF, kinesin superfamily; LTP, long-term potentiation; NMDARs, NMDA receptors; NR2B, N-methyl-D-aspartate receptor subunit 2B; ODN, oligonucleotide; pCREB, phosphorylated CREB; PWMT, paw withdrawal mechanical threshold; RT-PCR, Reverse transcription-PCR; TBS, tris-buffered saline.

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