Up-regulation of CX3CL1 *via* Nuclear Factor-κB–dependent Histone Acetylation Is Involved in Paclitaxel-induced Peripheral Neuropathy

Dai Li, Ph.D., Zhen-Zhen Huang, Ph.D., Yun-Zhi Ling, Ph.D., Jia-You Wei, Ph.D., Yu Cui, Ph.D., Xiang-Zhong Zhang, Ph.D., He-Quan Zhu, Ph.D., Wen-Jun Xin, Ph.D.

ABSTRACT

Background: Up-regulation of CX3CL1 has been revealed to be involved in the neuropathic pain induced by nerve injury. However, whether CX3CL1 participates in the paclitaxel-induced painful peripheral neuropathy remains unknown. The aim of the current study was to elucidate the involvement of transcriptional factors nuclear factor-κB (NF-κB) and its causal interaction with CX3CL1 signaling in the paclitaxel-induced painful peripheral neuropathy.

Methods: Painful peripheral neuropathy induced by paclitaxel treatment was established in adult male Sprague-Dawley rats. The von Frey test were performed to evaluate neuropathic pain behavior, and real-time quantitative reverse transcription polymerase chain reaction, chromatin immunoprecipitation, Western blot, immunohistochemistry, and small interfering RNA were performed to understand the molecular mechanisms.

Results: The application of paclitaxel induced an up-regulation of CX3CL1 expression in the spinal neurons, which is reduced significantly by NF-κB inhibitor ammonium pyrrolidinedithiocarbamate or p65 small interfering RNA. Blockade of either CX3CL1 (n = 12 each) or NF-κB (n = 12 each) signaling pathway attenuated mechanical allodynia induced by paclitaxel. Chromatin immunoprecipitation further found that paclitaxel induced an increased recruitment of nuclear factor-κB (NF-κB)p65 to the *Cx3cl1* promoter region. Furthermore, an increased acetylation level of H4, but not H3, in *Cx3cl1* promoter region in spinal neurons was detected after paclitaxel treatment, which was reversed by inhibition of NF-κB with ammonium pyrrolidinedithiocarbamate or p65 small interfering RNA.

Conclusions: These findings suggest that up-regulation of CX3CL1 *via* NF-κB–dependent H4 acetylation might be critical for paclitaxel-induced mechanical allodynia. **(ANESTHESIOLOGY 2015; 122:1142–51)**

PACLITAXEL is a first-line chemotherapeutic drug widely used for therapy of various types of cancers, such as lung, breast, and ovarian cancers.[1] The use of paclitaxel is often associated with peripheral neuropathy that predominantly manifests as cold and mechanical hypersensitivity and spontaneous pain in cancer patients.[2,3] Indeed, peripheral neuropathy after the administration of paclitaxel serves as the most common reason for treatment discontinuation or dose reduction rather than tumor progression.[4] Therefore, characterizing the underlying mechanisms is important for clinical use of paclitaxel and relieving associated pain.

Accumulating evidence has demonstrated that CX3CL1, a robust chemoattractant molecule, plays an important role in initiation of neuropathic pain.[5,6] The evidence that inhibition of CX3CL1/CX3CR1 pathway attenuated the hyperalgesia and allodynia in neuropathic pain model[7–9] promoted the identification of CX3CL1 as the potential target for the treatment of paclitaxel-induced pain. Recently, our and peer's studies showed that CX3CL1-induced peripheral axonopathy and ganglionopathy is involved in painful neuropathy after chemotherapeutic drug treatment.[10,11] Although it has been well documented that the CX3CL1/CX3CR1 signaling regulates the interaction between neuronal-microglia in the spinal cord and thereby mediates the development of neuropathic pain,[6,12] involvement of

What We Already Know about This Topic

- Chemotherapy-induced neuropathic pain is very difficult to control, and its mechanisms have not been fully described
- The chemokine CX3CL1 (fractalkine) is felt to support neuropathic pain under some circumstances

What This Article Tells Us That Is New

- Using a rat model of paclitaxel-induced neuropathy, both pharmacological and siRNA-based techniques showed that CX3CL1 supports allodynia in this model
- Chromatin immunoprecipitation experiments demonstrated that an epigenetic mechanism controls CX3CL1 expression in the spinal neurons of neuropathic rats

Drs. Xin and Zhang designed this project and supported this experiment together. Drs. Li and Huang did most of the experiment, so they are co-first authors.

Submitted for publication April 10, 2014. Accepted for publication November 14, 2014. From Department of Physiology and Pain Research Center (D.L., Z.-Z.H., Y.-Z.L., J.-Y.W., Y.C., H.-Q.Z., W.-J.X.), Zhongshan Medical School, and Department of Hematology (X.-Z.Z.), the Third Affiliated Hospital, Sun Yet-Sen University, Guangzhou, Guangdong, People's Republic of China.

 Anesthesiology 2015; 122:1142-51

CX3CL1 in paclitaxel-induced painful peripheral neuropathy still remains unknown.[13–15]

Nuclear factor-κB (NF-κB) is one of the most potent inducible transcriptional factors to modulate the expression of proinflammatory factors such as CXCL10 and CCL2 during neuroinflammation.[16,17] NF-κB exists in homo- or heterodimeric complexes consisting of different members of the Rel protein family.[18] The p50/p65 heterodimer, the most prevalent complex of NF-κB, is bound to inhibitory proteins IκB in the cytoplasm and present in an inactive form in the quiescent cells. Upon activation by varieties of stimuli, IκB is rapidly phosphorylated, ubiquitinated, and then degraded. Subsequently, the active heterodimer of p50/p65 translocates into nucleus and induces DNA transcription by binding to specific promoter elements.[19] NF-κB subunits can recruit several types of histone acetyltransferases to the promoter region of the target gene and facilitate the expression of target gene by changing the acetylation profiles of histones.[20,21] Histone acetylation has been recognized as an important factor to modify the accessibility of the DNA to the transcriptional machinery.[22] Our and peer previous studies have demonstrated that NF-κB in the spinal cord plays a critical role in nerve injury-induced neuropathic pain,[23,24] and the activation of NF-κB induced by paclitaxel is also reported in several cancer cell lines.[25–27]

Therefore, we hypothesize that CX3CL1 mediates the neuronal-glial signaling in the spinal cord and thereby contributes to the development of mechanical allodynia induced by paclitaxel. In the current study, we further explore the involvement of NF-κB and its causal interaction with CX3CL1 signaling and determine their therapeutic potential target in paclitaxel-induced painful peripheral neuropathy.

Materials and Methods

Animals

Male Sprague-Dawley rats (220 to 250 g) were housed in a temperature-controlled room (22 ± 1°C) with a 12-h light-dark cycle. All animal experimental procedures were approved by the Sun Yat-Sen University Animal Care and Use Committee (Guangzhou, Guangdong, People's Republic of China) and carried out in accordance with the guideline of National Institutes of Health on the animal care and the ethical guideline. All animals were randomly assigned to different experimental or control conditions in the current study.

Drug Administration and Behavioral Test

Paclitaxel (Taxol, 6 mg/ml, Bristol-Myers Squibb, New York, NY) was diluted with saline (1:3) and intraperitoneally injected (8 mg/kg, cumulative dose of 24 mg/kg) on 3 alternate days (days 1, 4, and 7). Control animals received an equivalent volume of saline.

* http://www.ambion.com. Accessed February 12, 2013.

Intrathecal injection was preformed according to the our previously described method.[28] In brief, a polyethylene-10 catheter was inserted into the rat's subarachnoid space through L5–L6 intervertebral space, and the tip of the catheter was located at the L5 spinal segmental level. Intrathecal injection of neutralizing antibody against CX3CL1 (10 μg/10 μl, Torrey Pines Biolabs, East Orange, NJ), ammonium pyrrolidinedithiocarbamate (PDTC) (200 ng/10 μl), or NF-κB p65 small interfering RNA (siRNA) (50 μg/15 μl) was initiated 30 min before the first dose of paclitaxel and maintained for 10 days. The withdrawal threshold of foot was determined by applying mechanical stimuli to the plantar surface of the hindpaw using von Frey hairs. The 50% withdrawal threshold was defined as the lowest force that produced five or more responses.[29] The experimenter who conducted the behavioral tests was blinded to all treatments. All animals survived throughout the experiments after drugs treatment.

siRNA Preparation, Transfection, and Screening

Three 19-nt siRNA duplexes targeting rat RelA (NF-κB p65) gene were designed using the siRNA Target Finder and Design Tool* and were commercially obtained from Ribobio (Guangzhou, China). The sequences of these siRNAs were as follows:

siRNA1, target sequence 1: GCATCCAGACCAACAATAA
5′-GCAUCCAGACCAACAAUAA dTdT-3′ (sense)
3′-dTdT CGUAGGUCUGGUUGUUAUU-5′ (antisense)
siRNA2, target sequence 2: CTCAAGATCTGCCGAGTAA
5′-CUCAAGAUCUGCCGAGUAA dTdT-3′ (sense)
3′-dTdT GAGUUCUAGACGGCUCAUU-5′ (antisense)
siRNA3, target sequence 3: GCAGTTCGATGCTGATGAA
5′-GCAGUUCGAUGCUGAUGAA dTdT-3′ (sense)
3′-dTdT CGUCAAGCUACGACUACUU-5′ (antisense)

siRNA, which has no homology to RelA (NF-κB p65) gene, was used as control (Scramble). The efficacy of the RelA siRNAs was tested in a follow-up siRNA screening experiment. HBZY-1 cells were transfected with siRNA using Lipofectamine2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. NF-κB p65 expression levels were determined using quantitative polymerase chain reaction (PCR) and Western blot. Compared with the blank control, RelA messenger RNA (mRNA) expression was suppressed by 84.7 ± 7.1%, 60.7 ± 4.6%, and 34.6 ± 2.8% in cells treated with sequence 1, 2, 3 RelA siRNA, respectively, when measured 24 h after transfection (fig. 1A). In agreement with the PCR results, NF-κB p65 protein expression level was dramatically reduced after treatment with siRNAs of sequence 1 by western blot analysis (fig. 1B). *In vivo* study also confirmed that intrathecal injection of siRNA 1 was effective in suppressing NF-κB p65 protein expression in the spinal cord (fig. 1C). Therefore, the chemically synthesized siRNA 1 was chosen for the subsequent experiments *in vivo*.

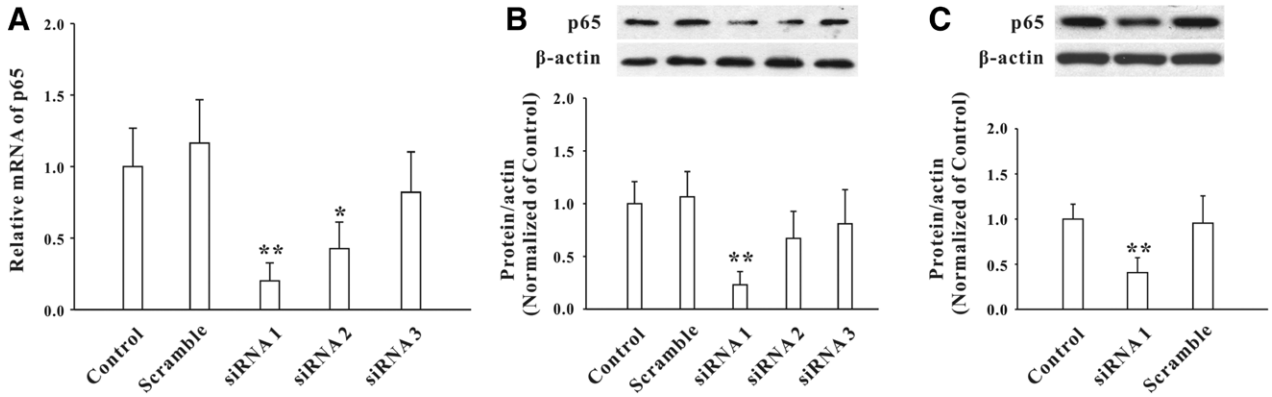

Fig. 1. Determination of efficacy of small interfering RNA (siRNA) in reducing nuclear factor-κB (NF-κB) p65 expression. HBZY-1 cells were transfected with different sequence of siRNA targeting NF-κB p65 or scramble siRNA. After 24 h of transfection, the messenger RNA (mRNA) level of NF-κB p65 was significantly reduced by siRNA1 (**$P < 0.01$) and siRNA2 (*$P < 0.05$), but not by siRNA 3, compared with control group (*A*). Western blot results showed that siRNA1 also decreased the protein expression of NF-κB p65 compared with control group (**$P < 0.01$) (*B*). Intrathecal injection of siRNA1 (50 μg/15 μl for 10 days) suppressed NF-κB p65 expression in the spinal cord compared with control group (**$P < 0.01$) (*C*).

Western Blot

Rats were deeply anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg) at different time points. Spinal cord was removed and sectioned in a cryostat. The spinal dorsal horn punch was taken with a 15-gauge cannula and frozen at −80°C until used. Samples were homogenized on ice in 15 mmol/l Tris buffer containing a cocktail of proteinase inhibitors and phosphatase inhibitors. Protein samples were separated by gel electrophoresis (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and transferred onto a polyvinylidene fluoride membrane. The blots were placed in block buffer for 1 h at room temperature and incubated with primary antibody against CX3CL1 (1:1000, Torrey Pines Biolabs), NF-κB p65 (1:1000, Abcam, Cambridge, United Kingdom), phosphorylated NF-κB p65 (Ser311) (1:1000, Cell Signaling Technology, Danvers, MA), acetylated histone H4 (1:1000, Millipore, Billerica, MA), histone H4 (1:1000, Millipore), acetylated histone H3 (K9) (1:500, Abcam), or histone H3 (1:500, Abcam) overnight at 4°C. The blots were then incubated with horseradish peroxidase–conjugated immunoglobulin G. Electrochemiluminescence (Pierce, Rockford, IL) was used to detect the immune complex. The band was quantified with computer-assisted imaging analysis system (ImageJ, National Institutes of Health, Bethesda, MD).

Immunohistochemistry

Immunochemistry was performed as we previously described.[30] Briefly, rats were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg) and perfused through the ascending aorta with 4% paraformaldehyde. The lumbar spinal cord segments were removed and postfixed in the same fixative overnight. Cryostat sections (16 μm) were cut and processed for immunohistochemistry with primary antibody for CX3CL1 (1:300, Torrey Pines Biolabs), phosphorylated NF-κB p65 (Ser311) (1:300, Cell Signaling Technology), NeuN (1:500; Chemicon, Darmstadt, Germany), glial fibrillary acidic protein (1:500, Chemicon), and OX-42 (1:200, Chemicon). After incubation overnight at 4°C, the sections were incubated with cy3-conjugated and fluorescein isothiocyanate–conjugated secondary antibodies for 1 h at room temperature. The stained sections were then examined with a Leica (Leica, Solms, Germany) fluorescence microscope, and images were captured with a Leica DFC350 FX camera. For quantification of OX-42 immunostaining, the immunoreactive-positive area was analyzed with a Leica Qwin V3 image system.

RNA Extraction and Real-time Quantitative PCR

Total RNA was extracted from the rat's spinal dorsal horn tissues with Trizol reagent (Invitrogen). The reverse transcription was performed using oligo-dT primer and Moloney Murine Leukemia Virus Reverse Transcriptase (Promega, Madison, WI) according to the manufacturer's protocol. Specific primer sequences of the examined mRNA and β-actin for PCR were listed in table 1. Real-time quantitative PCR was performed using SYBR Green qPCR SuperMix (Invitrogen) and the ABI PRISM7500 Sequence Detection System. The reactions were setup based on the manufacture's protocol. PCR conditions were incubation at 95°C for 3 min followed by 40 cycles of thermal cycling (10 s at 95°C, 20 s at 58°C, and 10 s at 72°C). The relative expression ratio of mRNA in rat's spinal tissues was quantified by the $2^{-}\Delta\Delta^{CT}$ method.

Chromatin Immunoprecipitation Assays

Chromatin immunoprecipitation (ChIP) assays were performed using the ChIP Assay Kit (Thermo). The rats' spinal dorsal horn were removed quickly and placed in 1% formaldehyde for 2 min. The DNA was fragmented by sonication and digested with micrococcal nuclease. After addition of ChIP dilution buffer, 100 μl of sample was saved as input. Eight microliters of NF-κB p65 antibody (Abcam) or acetylated histone H4 antibody (Millipore) was added to 500 μl precleared

Table 1. Specific Primer Sequences

Gene	Primer	Sequence
Interleukin (IL)-6	Forward	5′-CCACTGCCTTCCCTACTT-3′
	Reverse	5′-TTGCCATTGCACAACTCT-3′
CCL1	Forward	5′-AGAAAGCTGCGCCTTAA-3′
	Reverse	5′-CTCTGGTGCTGGGATGG-3′
IL-1β	Forward	5′-GGATGATGACGACCTGCTA-3′
	Reverse	5′-CACTTGTTGGCTTATGTTCTG-3′
CX3CL1	Forward	5′-CTCCAGCCATCCAGCCATG-3′
	Reverse	5′-CATTTCGTCATGCCGAGGTG-3′
IL-10	Forward	5′-TGGACAACATACTGCTGACAG-3′
	Reverse	5′-GGTAAAACTTGATCATTTCTGACAAG-3′
IL-15	Forward	5′-TGCACGAGTACAGTAACAT-3′
	Reverse	5′-CCTCCAGCTCCTCACAT-3′
β-actin	Forward	5′-AGGGAAATCGTGCGTGACAT-3′
	Reverse	5′-GAACCGCTCATTGCCGATAG-3′

chromatin solution, and the sample was incubated overnight. A "nonantibody" immunoprecipitation was performed as a negative control. Antibody/DNA complexes were captured, washed, eluted, and reverse cross-linked. The DNA was purified from the complexes and input fractions. The precipitated DNA was resuspended in 60 μl of nuclease-free water, and quantitative real-time PCR or semiquantitative PCR was performed on 5 μl of sample as described above. ChIP/input ratio was calculated. Primers 5′-GCTGCCCTGACCATAAAT-3′ and 5′-AGCTGTACGGCACTCACC-3′ were designed to amplify a -1941/-1931 region relative to the transcription start site of rat CX3CL1 promoter, containing NF-κB–binding site.

Statistical Analysis

All data were expressed as means ± SEM and analyzed were using SPSS 13.0 (SPSS, Chicago, IL). Immunohistochemistry and western blot data were analyzed by two-way ANOVA followed by Tukey *post hoc* test. For behavioral analysis, two-way ANOVA with repeated measures followed by Tukey *post hoc* test for all groups and between groups and one-way ANOVA followed by Tukey *post hoc* test for different groups on the same time point were carried out. The criterion for statistical significance was $P < 0.05$. The sample size, which was chosen based on our and peers' experience in painful behavior studies, provides the reason of power analysis.

Results

Paclitaxel-induced Allodynia and Up-regulation of Cytokines in the Spinal Cord

Consistent with our previous study,[29] administration with paclitaxel (3 × 8 mg/kg, cumulative dose 24 mg/kg) induced marked mechanical allodynia compared with the vehicle group (fig. 2A) ($F = 38.379$, $P < 0.01$). In view of the pivotal role of neuroinflammation in dorsal horn in the induction of paclitaxel-induced mechanical allodynia,[31,32] we here determined whether paclitaxel would regulate the expression of cytokines and chemokines. After paclitaxel treatment on alternative days (days 1, 4, and 10), mRNA was extracted from rat spinal cord, and quantitative PCR was performed to examine specific mRNA using primers with different sequences as listed in table 1. As shown in figure 2B, paclitaxel induced increase in the mRNA levels of several examined cytokines at different time points, respectively. Among these cytokine/chemokines, CX3CL1 underwent a significant dynamic change in the pattern consistent with pain behavior induced by paclitaxel. Considering its pivotal role in the communication between neurons and microglia in several settings of neuroinflammatory diseases,[32] CX3CL1 was chosen for the subsequent investigation of its role in the paclitaxel-induced painful peripheral neuropathy.

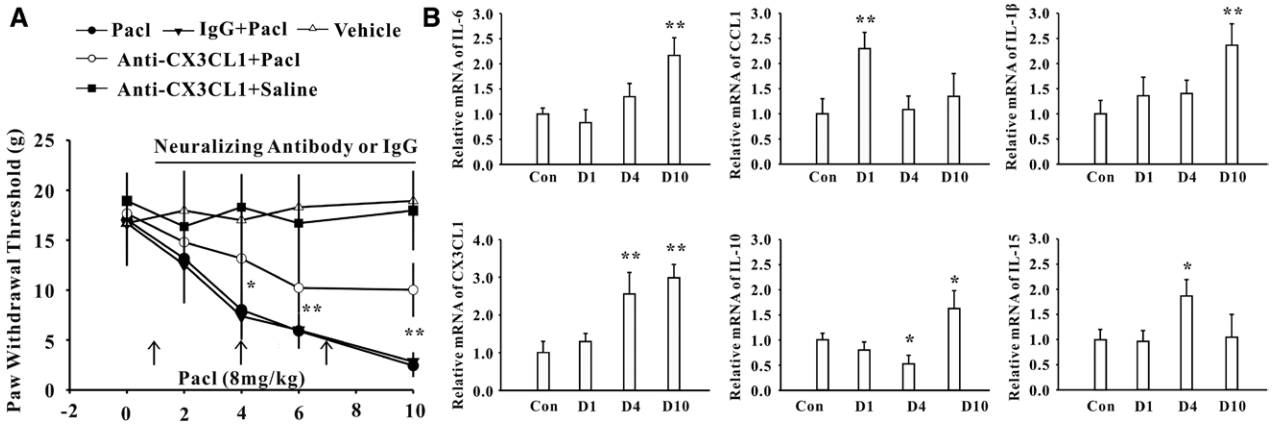

Fig. 2. Paclitaxel (Pacl)-induced allodynia and up-regulation of cytokines in the spinal cord. (*A*) Treatment with paclitaxel (8 mg/kg at day 1, 4, 7) significantly decreased the hindpaw withdraw threshold. Intrathecal injection of neutralizing antibody against CX3CL1 (10 μg/10 μl for 10 days) attenuates mechanical allodynia induced by paclitaxel. n = 12 in each group; *$P < 0.05$, **$P < 0.01$ *vs.* corresponding paclitaxel group. (*B*) The messenger RNA (mRNA) levels of interleukin (IL)-6, CCL1, IL-1β, CX3CL1, IL-10, and IL-15 were surveyed in the spinal dorsal horn of the rats at 1, 4, and 10 days after treatment with paclitaxel. n = 6 in each group; *$P <$ 0.05, **$P < 0.01$. IgG = immunoglobulin G.

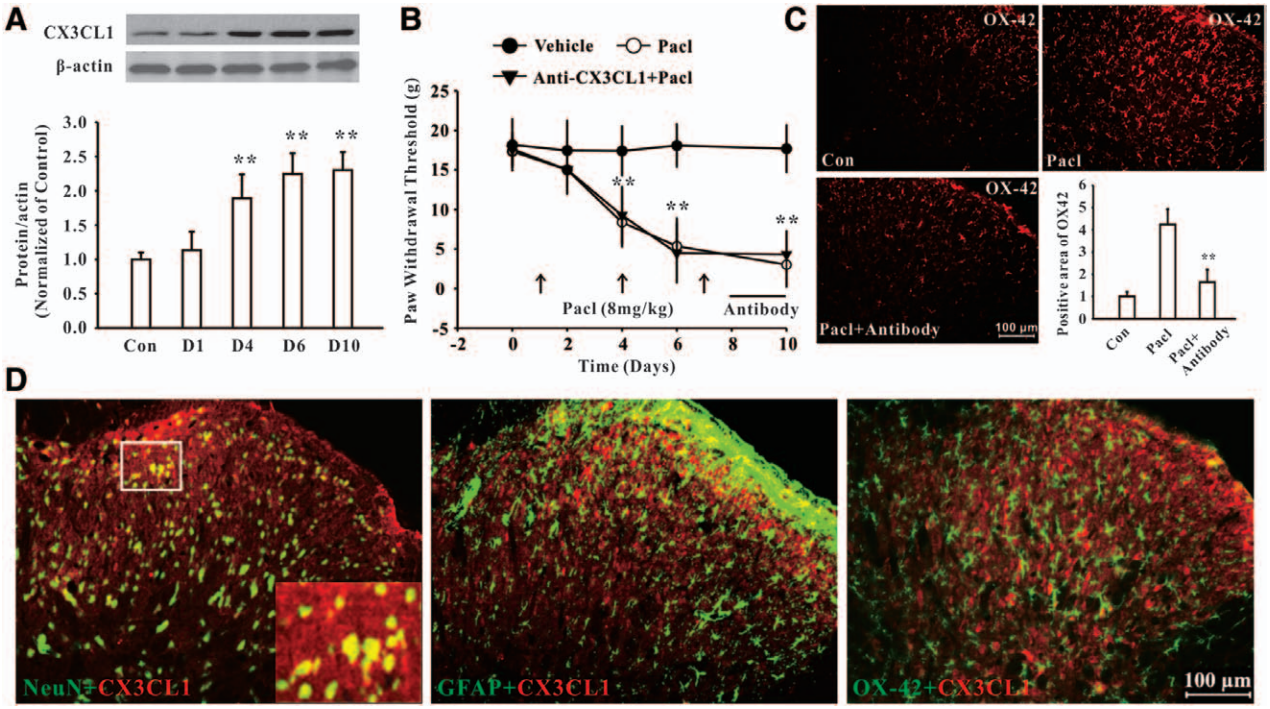

Fig. 3. Treatment with paclitaxel (Pacl) induced the up-regulation of CX3CL1 in the spinal neurons. (*A*) Representative blots and histogram showed up-regulation of CX3CL1 induced by paclitaxel treatment. n = 6 in each group; $^{**}P < 0.01$ *vs.* corresponding control group (Con). (*B*) Intrathecal injection of CX3CL1 neutralizing antibody for two consecutive days on day 8 after paclitaxel treatments did not attenuates mechanical allodynia compared with the paclitaxel group. n = 12 in each group; $^{**}P < 0.01$ *vs.* corresponding vehicle group. (*C*) Intrathecal injection of CX3CL1 neutralizing antibody inhibits the increased OX-42 immunostaining induced by paclitaxel n = 6 in each group; $^{**}P < 0.01$ *vs.* corresponding paclitaxel group. *Scale bar*, 100 μm. (*D*) The immunofluorescence staining of CX3CL1 (*red*) was colocalized with NeuN (neuron marker, *green*) but not glial fibrillary acidic protein (GFAP) (astrocyte marker, *green*) or OX-42 (microglia marker, *green*). *Scale bar*, 100 μm.

Up-regulation of CX3CL1 Contributes to Paclitaxel-induced Allodynia

Consistent with CX3CL1 mRNA change, western blot results revealed that paclitaxel induced an increase in the CX3CL1 protein expression in the spinal cord compared with the control group. Up-regulation of CX3CL1 induced by paclitaxel started on day 4 and maintained to the end of the experiment (day 10) (fig. 3A). To define the role of CX3CL1 in paclitaxel-induced peripheral neuropathy, neutralizing antibody against CX3CL1 (10 μg/10 μl) was intrathecally administrated for 10 days. Behavioral results demonstrated that blocking CX3CL1 significantly attenuated paclitaxel-induced mechanical allodynia compared with the paclitaxel group (fig. 2A) (drug *vs.* paclitaxel in withdrawal threshold, 13.17 [4.41] *vs.* 8.03 [2.63]; 10.22 [2.50] *vs.* 5.91 [1.77]; and 10.03 [2.7] *vs.* 2.44 [1.15], on days 4, 6, and 10, respectively; n = 12 each) ($F = 5.618$, $P < 0.01$). Intrathecal injection of the neutralizing antibody alone had no effect on the mechanical withdrawal thresholds in the naive rats. To further investigate the specific role of CX3CL1 in the maintenance of paclitaxel-induced neuropathic pain, intrathecal injection of neutralizing antibody against CX3CL1 was initiated on day 8 and continued for another 2 days after paclitaxel treatments. The results showed no significant difference in mechanical allodynia between antibody + paclitaxel group and paclitaxel group when behavioral tests was performed on day 10 (fig. 3B), suggesting that CX3CL1 might not contribute to the maintenance of paclitaxel-induced allodynia. In addition, immunohistochemistry results showed that CX3CL1 neutralizing antibody significantly suppressed the increased OX-42 expression induced by paclitaxel application (fig. 3C). Double immunostaining also showed that CX3CL1 was exclusively located in the spinal neurons but not in astrocytes or microglia (fig. 3D). These findings suggested that up-regulation of CX3CL1 in spinal neurons might be involved in chemotherapeutic drug-induced neuroinflammation and mechanical allodynia.

NF-κB p65-mediated Up-regulation of CX3CL1 Induced by Paclitaxel

NF-κB, an important transcriptional factor, regulates the expression of many cytokines and chemokines.[33] To determine whether NF-κB signaling participated in the paclitaxel-induced CX3CL1 up-regulation, phosphorylated NF-κB p65 level after paclitaxel treatment was first examined. The expression of phosphorylated p65 at Ser311 was significantly increased on days 4 and 10 after paclitaxel treatment (fig. 4A). Consistent with western blot result, immunohistochemistry staining showed that paclitaxel treatment significantly increased p-p65 in the spinal cord compared with control

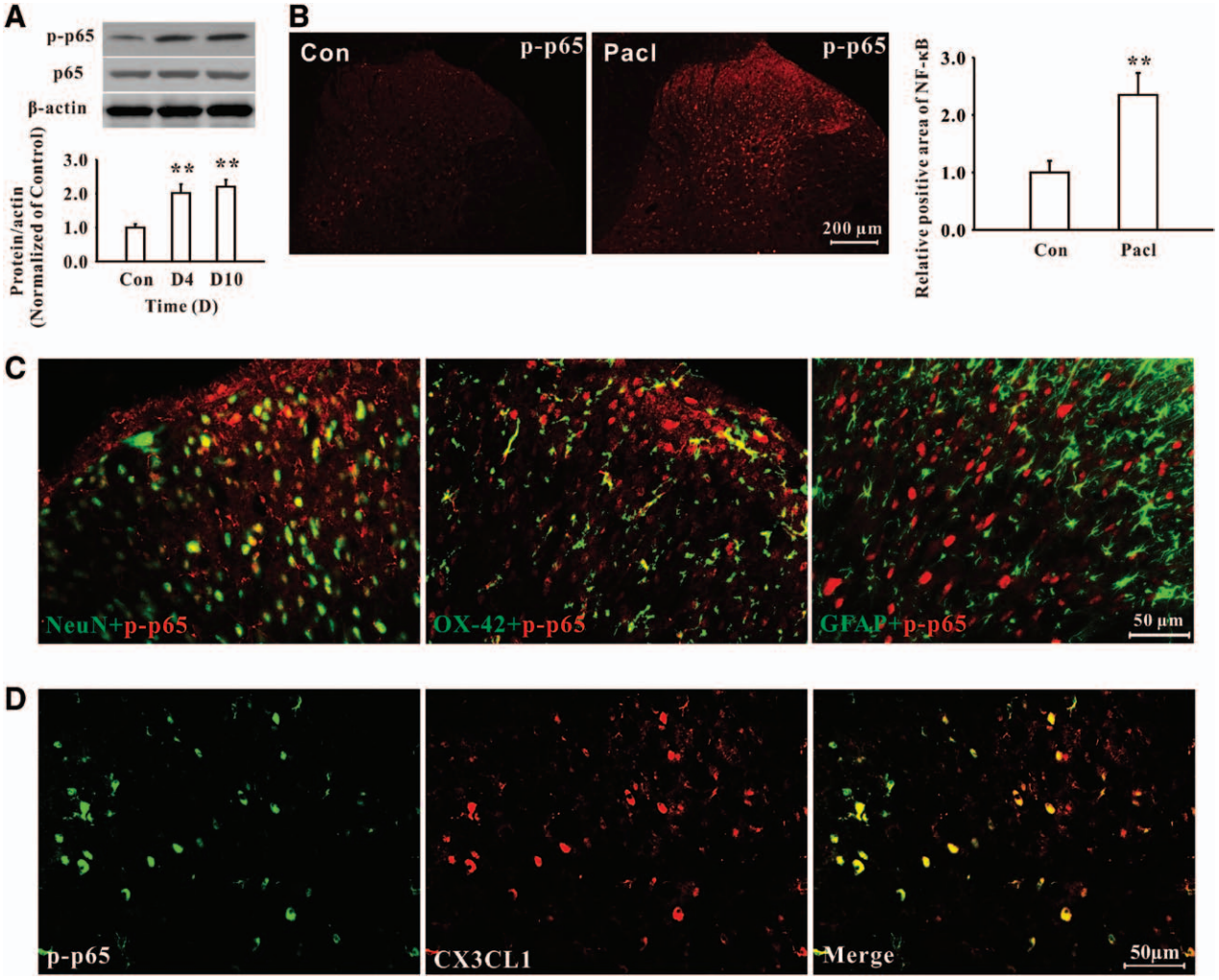

Fig. 4. Increased phosphorylation of NF-κB p65 is induced in the CX3CL1-positive neurons after paclitaxel (Pacl) treatment. (*A*) The expression of phosphorylated p65 at Ser311 was significantly increased after paclitaxel treatment. n = 6 in each group; $^{**}P < 0.01$ *vs.* corresponding control group (Con). (*B*) Increased phosphorylation of p65 was significantly induced after paclitaxel treatment compared with control group. *Scale bar*, 200 μm. (*C*) The immunofluorescence staining of p-p65 (*red*) was colocalized with NeuN (neuron marker, *green*) and OX-42 (microglia marker, *green*) but not with glial fibrillary acidic protein (GFAP) (astrocyte marker, *green*). *Scale bar*, 50 μm. (*D*) Double immunofluorescence staining indicated colocalization of p-p65 with CX3CL1 in the spinal neurons. *Scale bar*, 50 μm.

group (fig. 4B). Double immunostaining results revealed that NF-κB p-p65 was found in the spinal neurons and microglia but not in the astrocyte (fig. 4C). Next, we examined whether transcriptional factor NF-κB p65 modulated CX3CL1 up-regulation after the treatment of paclitaxel. Double immunostaining results demonstrated colocalization of p-p65 and CX3CL1 in the spinal dorsal horn (fig. 4D). Behavior study showed that continuous intrathecal administration of NF-κB p65 inhibitor PDTC (200 ng/10 μl) inhibited mechanical allodynia induced by paclitaxel (fig. 5A) (PDTC *vs.* paclitaxel in withdrawal threshold, 12.53 [3.88] *vs.* 8.34 [2.46]; 10.74 [2.28] *vs.* 6.1 [1.92]; and 10.02 [2.7] *vs.* 2.53 [1.06], on days 4, 6, and 10, respectively) ($F = 6.991$, $P < 0.01$). In addition, intrathecal injection of siRNA targeting NF-κB p65 (50 μg/15 μl) suppressed mechanical allodynia further confirmed the role of NF-κB p65 in the paclitaxel-induced allodynia (fig. 5A) (siRNA *vs.* paclitaxel in withdrawal threshold, 11.9 [3.11] *vs.* 8.34 [2.46]; 11.1 [2.23] *vs.* 6.1 [1.92]; and 9.5 [2.77] *vs.* 2.53 [1.06], on days 4, 6, and 10, respectively; n = 12 each) ($F = 11.937$, $P < 0.01$). Consistent with the inhibitory effect on the phosphorylation of NF-κB p65 (fig. 5B), intrathecal injection of PDTC or specific p65 siRNA reduced CX3CL1 up-regulation at protein (fig. 5B) and mRNA (fig. 5C) levels in the spinal dorsal horn induced by paclitaxel. These results suggest that up-regulation of CX3CL1 after paclitaxel treatment is dependent on NF-κB p65 signaling pathway.

Paclitaxel Treatment Promoted NF-κB p65-mediated Histone H4 Acetylation on the cx3cl1 Gene

Upon activation of NF-κB pathway, NF-κB p65 can bind to target gene promoter and modify acetylation of the histones,[20,21] thereby facilitating the expression of target genes.

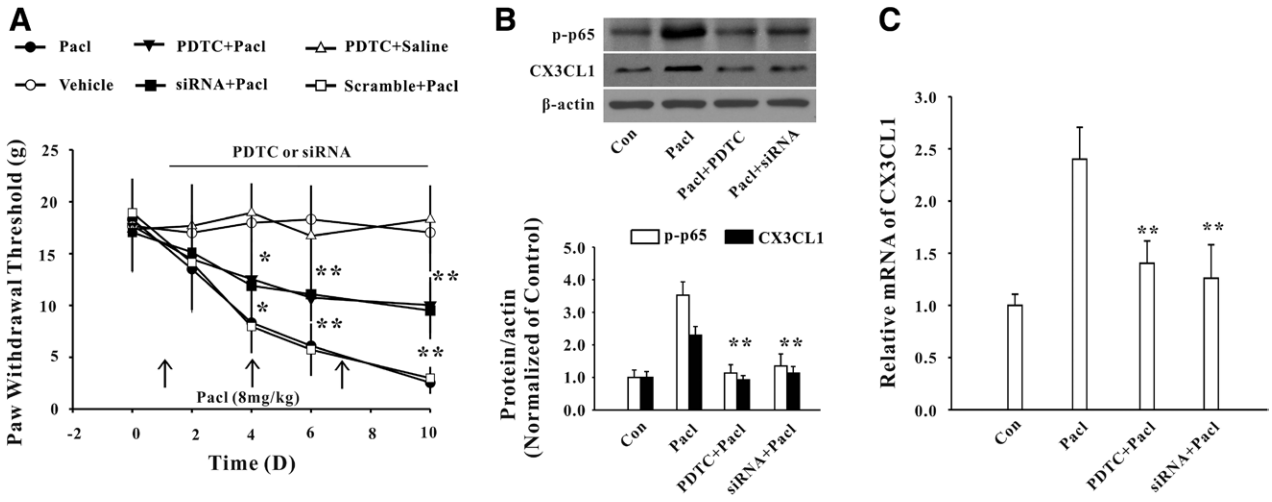

Fig. 5. Inhibition of NF-κB attenuated the up-regulation of CX3CL1 induced by paclitaxel (Pacl). (*A*) Continuous intrathecal injection of p65 inhibitor ammonium pyrrolidinedithiocarbamate (PDTC) (200 ng/10 μl for 10 days) or p65 small interfering RNA (siRNA) (50 μg/15 μl for 10 days) reduced mechanical allodynia in the rats treated with paclitaxel. n = 12 in each group; **P* < 0.05, ***P* < 0.01 *vs.* corresponding paclitaxel group. (*B*) Continuous intrathecal administration of PDTC or p65 siRNA decreased the expression of phosphorylated p65 NF-κB and CX3CL1 in the spinal dorsal horn of the rats receiving paclitaxel on day 10. n = 6 in each group; ***P* < 0.01 *vs*. corresponding paclitaxel. (*C*) PDTC or p65 siRNA reduced up-regulation of CX3CL1 messenger RNA (mRNA) level induced by paclitaxel treatment. n = 6 in each group; ***P* < 0.01 *vs.* corresponding paclitaxel group. Con = control group.

Therefore, we first determined whether application of paclitaxel promoted NF-κB p65 binding to the cx3cl1 gene promoter using ChIP-PCR assay. A potent-binding site of NF-κB p65 in the cx3cl1 gene at position -1941/-1931 was first identified using TFSEARCH and jaspar database. Then, the DNA precipitated by the NF-κB p65 antibody was subjected to PCR using primers designed to amplify a 162-bp fragment (-2029/-1867) of the cx3cl1 promoter flanking the NF-κB–binding site. The results showed that the recruitment of p65 to the cx3cl1 gene promoter was significantly increased after paclitaxel treatment on day 10 compared with control group by quantitative real-time (fig. 6A) and semiquantitative (fig. 6B) PCR analysis. Next, we examined whether treatment with paclitaxel could modify histone acetylation in the cx3cl1 promoter region. The western blot results showed that application of paclitaxel significantly increased global acetylation of H4 (fig. 7A). No alteration of acetylation of H3 (K9) was detected (fig. 7A). For ChIP assay, the DNA precipitated by the acetylated H4 antibody was used for PCR analysis and the cx3cl1 promoter region flanking the NF-κB–binding site was amplified. The results showed that the level of H4 acetylation on the CX3CL1 gene promoter was enhanced after treatment with paclitaxel (fig. 7B). However, the effect of paclitaxel on H4 acetylation was reduced by intrathecal administration of PDTC or p65 siRNA (fig. 7B), indicating that paclitaxel leads to an increase in histone acetylation at the CX3CL1 gene promoter through NF-κB signaling.

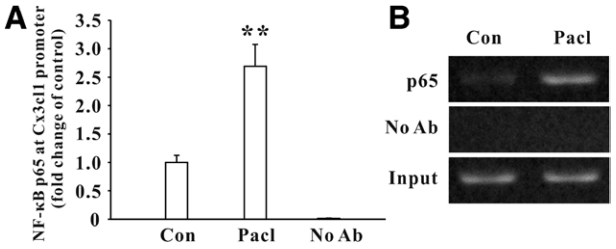

Fig. 6. Treatment with paclitaxel (Pacl) enhanced recruitment of nuclear factor-κB (NF-κB) p65 to the cx3cl1 gene promoter. Chromatin immunoprecipitation (ChIP) assays were performed with or without NF-κB p65 antibody. Increased recruitment of NF-κB p65 to the cx3cl1 gene promoter induced by paclitaxel was demonstrated by quantitative real-time polymerase chain reaction (*A*) and semiquantitative PCR (*B*). n = 7 in each group; ***P* < 0.01 *vs*. corresponding control group (Con). No Ab = normal immunoglobulin G without specific antibody.

Discussion

It has been recently suggested that neuroinflammation might be involved in the chemotherapeutic drug-induced painful neuropathy. In the current study, we showed that application of paclitaxel induced an up-regulation of CX3CL1 protein and mRNA expression in the spinal neurons. Administration of the paclitaxel also increased the phosphorylation of NF-κB p65 in the CX3CL1-expressing spinal neurons. ChIP assay further demonstrated that paclitaxel increased recruitment of NF-κB p65 to, as well as acetylation of histone H4 on, the *cx3cl1* gene promoter in the spinal dorsal horn. Inhibition of NF-κB with PDTC or siRNA significantly decreased H4 acetylation on the *cx3cl1* gene promoter region and suppressed the upsurge of CX3CL1 expression induced by paclitaxel. Blocking NF-κB-CX3CL1 signaling pathway also attenuated mechanical allodynia induced by paclitaxel. These findings suggested that up-regulation of CX3CL1 *via* NF-κB–mediated H4 acetylation contributes to paclitaxel-induced mechanical allodynia.

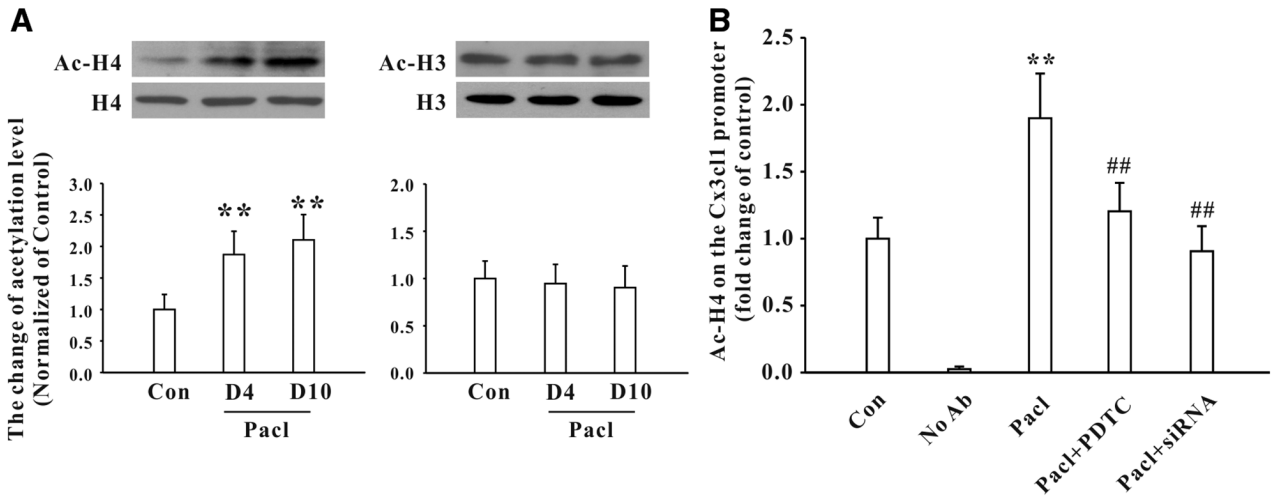

Fig. 7. Nuclear factor-κB (NF-κB) p65 activity is required for the increased acetylation of histone H4 on the cx3cl1 promoter region induced by paclitaxel (Pacl). (*A*) Treatment with paclitaxel significantly increased the global acetylation of histone H4, but not H3 K9, in the spinal dorsal horn. n = 6 in each group; $^{**}P < 0.01$ *vs.* corresponding control group (Con). (*B*) Chromatin immunoprecipitation (ChIP) assay were performed with or without acetylated H4 antibody. Increased acetylation of histone H4 on the cx3cl1 promoter region flanking NF-κB p65-binding site was reduced by ammonium pyrrolidinedithiocarbamate (PDTC) or p65 small interfering RNA (siRNA) in rats receiving paclitaxel treatment. n = 7 in each group; $^{**}P < 0.01$ *vs.* corresponding control group, $^{\#\#}P < 0.01$ *vs.* corresponding paclitaxel group. No Ab = normal immunoglobulin G without specific antibody.

CX3CL1, a well-characterized chemokine released by neurons, has been identified to mediate painful responses in several pathological pain models.[9,34,35] Our recent results that paclitaxel cause apoptosis and macrophage infiltration in the dorsal root ganglion *via* CX3CL1-suggested expression of CX3CL1 in dorsal root ganglion are involved in the paclitaxel-induced painful peripheral neuropathy.[36] In addition, Elizabeth reported that CX3CL1/CX3R1 signal in the sciatic nerve participated in the vincristine-induced painful peripheral neuropathy.[11] These research suggested that CX3CL1 in the peripheral nervous system might be a critical molecular in chemotherapeutic drug-induced peripheral neuropathy. In the current study, our data first revealed that paclitaxel treatment also increase the expression of CX3CL1 in dorsal horn neurons. Our results are supported by the recent report that application of paclitaxel up-regulated the chemokine CCL2 expression in the spinal cord.[37] Although it is generally believed that paclitaxel does not penetrate blood–brain barrier,[38,39] low concentrations of paclitaxel can be detected in spinal cord after systemic treatment.[40] It has been reported that paclitaxel can directly impair neuronal activities.[41] Thus, further study is needed to evaluate whether the up-regulation of spinal CX3CL1 is induced by a direct effect of paclitaxel or through its effects on peripheral targets, these results implied that CX3CL1 *via* action on the central and peripheral nervous system might be a critical molecular in paclitaxel-induced peripheral neuropathy.

Previous studies have established a pivotal role of spinal microglia activation in the induction and maintenance of central sensitization and behavioral mechanical allodynia in several models of neuropathic pain.[9,42] The activation of spinal microglia was reported with high-dose (cumulative dose 36 mg/kg)[43] but not low-dose (cumulative dose 8 mg/kg)[44] paclitaxel treatment. In our current studies, microglia activation was measured as an increase in the immunostaining area of OX-42 in the spinal dorsal horn. It has been reported that CX3CR1 is only expressed in the microglia and required for neuropathic pain facilitation.[6,45] It is possible that CX3CL1 up-regulation *via* binding CX3CR1 activated the microglia in the spinal dorsal horn and mediated the paclitaxel-induced allodynia. Our previous study reported that inhibition of microglia activity by minocycline significantly suppressed mechanical allodynia after paclitaxel.[15,29] Here, blockade of CX3CL1 signaling markedly inhibited microglia activation and mechanical allodynia, which further confirmed the involvement of microglia in chemotherapeutic drug-induced painful neuropathy.

In addition, the current study revealed an NF-κB–mediated epigenetic mechanism underlying the up-regulation of CX3CL1 after paclitaxel treatment. NF-κB is an important transcription factor, which regulates the expression of many proinflammatory cytokines in various neuropathy diseases.[19,46,47] Furthermore, the activation of NF-κB induced by paclitaxel is also reported in several cancer cell lines.[26,27] Here, we demonstrated that intrathecal injection of NF-κB activation inhibitor PDTC or p65 siRNA significantly reduced up-regulation of CX3CL1 protein and mRNA, as well as the mechanical allodynia. It is noteworthy that activation of NF-κB p65 was significant induced in the spinal neurons and microglia after paclitaxel treatment. Although the role of microglia in the development of paclitaxel-induced allodynia cannot be excluded, the evidence that up-regulation of CX3CL1 was induced in the p-p65-positive spinal neurons suggested that NF-κB p65-dependent CX3CL1 up-regulation contribute to painful neuropathy after treatment of paclitaxel.

Once disassociated with IκB in response to multistimuli, NF-κB may undergo significant translocation to the nucleus and subsequent recruitment to the binding site on target genes, thereby modifying the chromatin structure and gene expression *via* epigenetic mechanism. For example, it was reported that cytokine-induced CX3CL1 expression was accompanied by increased recruitment of NF-κB to the CX3CL1 gene promoter in human lung epithelial cells.[48] NF-κB binding to the promoter is also required for IL-8 gene expression activated by paclitaxel in ovarian cells.[27] In our current study, ChIP assay data showed that the increased binding of p65 to the CX3CL1 gene promoter was detected after paclitaxel treatment. Therefore, decreased CX3CL1 expression by inhibition of NF-κB phosphorylation by PDTC or p65 siRNA might result from reduction in NF-κB translocation to the nucleus and recruitment to CX3CL1 gene.

It is well-known that acetylation of histone increases the accessibility of transcriptional machinery by facilitation of DNA uncoiling.[49] Modification of histone acetylation serves as an important mechanism underlying the epigenetic modulation of the expression of cytokines and chemokines, such as IL-8 and E-selectine, in several pathological conditions. In our current study, we found that enhanced H4 acetylation is closely related to CX3CL1 up-regulation after paclitaxel. It has been demonstrated that NF-κB subunits can recruit histone acetytransferases to the target gene promoter to change the histones acetylation, thereby facilitating the genes expression.[20,21] For example, enhanced transcription of CCL11 after acetylation of histone H4 at gene promoter region is induced in a NF-κB p65-dependent way.[50,51] Here, our ChIP study revealed that paclitaxel induced significantly increased histone H4 acetylation in cx3cl1 promoter region, which was largely reduced by inhibition of NF-κB pathway with PDTC or p65 siRNA. Therefore, combined with the result that inhibition of NF-κB pathway remarkably suppresses the CX3CL1 up-regulation, these data suggest that NF-κB p65-dependent histone acetylation might contribute to paclitaxel-induced up-regulation of CX3CL1.

Acknowledgments

This study was funded by Natural Science Fund (31171034, 81271474, and U1201223) from the National Natural Science Foundation of China, Beijing, People's Republic of China; Fundamental Research Funds for the Central Universities (NO. 12ykpy03) from Sun Yet-Sen University, Guangzhou, People's Republic of China; Program for New Century Excellent Talents in University (NCET-12-0568) from Ministry of Education of China, Beijing, People's Republic of China; and Science and Technology Project in Guangzhou (2014J4100180) from Science and Information Technology of Guangzhou, Guangzhou, People's Republic of China.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Xin: Department of Physiology and Pain Research Center, Zhongshan Medical School of Sun Yat-Sen University, 74 Zhongshan Rd. 2, Guangzhou, China. xinwj@mail.sysu.edu.cn.

References

1. Guastalla JP 3rd, Diéras V: The taxanes: Toxicity and quality of life considerations in advanced ovarian cancer. Br J Cancer 2003; 89(Suppl 3):S16–22
2. Dougherty PM, Cata JP, Cordella JV, Burton A, Weng HR: Taxol-induced sensory disturbance is characterized by preferential impairment of myelinated fiber function in cancer patients. Pain 2004; 109:132–42
3. Tofthagen C, McAllister RD, McMillan SC: Peripheral neuropathy in patients with colorectal cancer receiving oxaliplatin. Clin J Oncol Nurs 2011; 15:182–8
4. Pasetto LM, D'Andrea MR, Rossi E, Monfardini S: Oxaliplatin-related neurotoxicity: How and why? Crit Rev Oncol Hematol 2006; 59:159–68
5. Matsushita K, Tozaki-Saitoh H, Kojima C, Masuda T, Tsuda M, Inoue K, Hoka S: Chemokine (C-C motif) receptor 5 is an important pathological regulator in the development and maintenance of neuropathic pain. Anesthesiology 2014; 120:1491–503
6. Lindia JA, McGowan E, Jochnowitz N, Abbadie C: Induction of CX3CL1 expression in astrocytes and CX3CR1 in microglia in the spinal cord of a rat model of neuropathic pain. J Pain 2005; 6:434–8
7. Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, Streit WJ, Salafranca MN, Adhikari S, Thompson DA, Botti P, Bacon KB, Feng L: Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci U S A 1998; 95:10896–901
8. Watkins LR, Maier SF: Glia: A novel drug discovery target for clinical pain. Nat Rev Drug Discov 2003; 2:973–85
9. Zhuang ZY, Kawasaki Y, Tan PH, Wen YR, Huang J, Ji RR: Role of the CX3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. Brain Behav Immun 2007; 21:642–51
10. Huang ZZ, Li D, Liu CC, Cui Y, Zhu HQ, Zhang WW, Li YY, Xin WJ: CX3CL1-mediated macrophage activation contributed to paclitaxel-induced DRG neuronal apoptosis and painful peripheral neuropathy. Brain Behav Immun 2014; 40:155–65
11. Old EA, Nadkarni S, Grist J, Gentry C, Bevan S, Kim KW, Mogg AJ, Perretti M, Malcangio M: Monocytes expressing CX3CR1 orchestrate the development of vincristine-induced pain. J Clin Invest 2014; 124:2023–36
12. Verge GM, Milligan ED, Maier SF, Watkins LR, Naeve GS, Foster AC: Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. Eur J Neurosci 2004; 20:1150–60
13. Balayssac D, Ferrier J, Descoeur J, Ling B, Pezet D, Eschalier A, Authier N: Chemotherapy-induced peripheral neuropathies: From clinical relevance to preclinical evidence. Expert Opin Drug Saf 2011; 10:407–17
14. Peters CM, Jimenez-Andrade JM, Jonas BM, Sevcik MA, Koewler NJ, Ghilardi JR, Wong GY, Mantyh PW: Intravenous paclitaxel administration in the rat induces a peripheral sensory neuropathy characterized by macrophage infiltration and injury to sensory neurons and their supporting cells. Exp Neurol 2007; 203:42–54
15. Boyette-Davis J, Xin W, Zhang H, Dougherty PM: Intraepidermal nerve fiber loss corresponds to the development of taxol-induced hyperalgesia and can be prevented by treatment with minocycline. Pain 2011; 152:308–13

16. Kaltschmidt B, Kaltschmidt C: NF-kappaB in the nervous system. Cold Spring Harb Perspect Biol 2009; 1:a001271
17. Richmond A: Nf-kappa B, chemokine gene transcription and tumour growth. Nat Rev Immunol 2002; 2:664–74
18. Li Q, Verma IM: NF-kappaB regulation in the immune system. Nat Rev Immunol 2002; 2:725–34
19. Mincheva-Tasheva S, Soler RM: NF-κB signaling pathways: Role in nervous system physiology and pathology. Neuroscientist 2013; 19:175–94
20. Ghizzoni M, Haisma HJ, Maarsingh H, Dekker FJ: Histone acetyltransferases are crucial regulators in NF-κB mediated inflammation. Drug Discov Today 2011; 16:504–11
21. Sheppard KA, Rose DW, Haque ZK, Kurokawa R, McInerney E, Westin S, Thanos D, Rosenfeld MG, Glass CK, Collins T: Transcriptional activation by NF-kappaB requires multiple coactivators. Mol Cell Biol 1999; 19:6367–78
22. Vettese-Dadey M, Grant PA, Hebbes TR, Crane- Robinson C, Allis CD, Workman JL: Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA *in vitro*. EMBO J 1996; 15:2508–18
23. Niederberger E, Geisslinger G: The IKK-NF-kappaB pathway: A source for novel molecular drug targets in pain therapy? FASEB J 2008; 22:3432–42
24. Zang Y, He XH, Xin WJ, Pang RP, Wei XH, Zhou LJ, Li YY, Liu XG: Inhibition of NF-kappaB prevents mechanical allodynia induced by spinal ventral root transection and suppresses the re-expression of Nav1.3 in DRG neurons *in vivo* and *in vitro*. Brain Res 2010; 1363:151–8
25. Gocho T, Uwagawa T, Furukawa K, Haruki K, Fujiwara Y, Iwase R, Misawa T, Ohashi T, Yanaga K: Combination chemotherapy of serine protease inhibitor nafamostat mesilate with oxaliplatin targeting NF-κB activation for pancreatic cancer. Cancer Lett 2013; 333:89–95
26. Mabuchi S, Ohmichi M, Nishio Y, Hayasaka T, Kimura A, Ohta T, Kawagoe J, Takahashi K, Yada-Hashimoto N, Seino-Noda H, Sakata M, Motoyama T, Kurachi H, Testa JR, Tasaka K, Murata Y: Inhibition of inhibitor of nuclear factor-kappaB phosphorylation increases the efficacy of paclitaxel in *in vitro* and *in vivo* ovarian cancer models. Clin Cancer Res 2004; 10:7645–54
27. Lee LF, Haskill JS, Mukaida N, Matsushima K, Ting JP: Identification of tumor-specific paclitaxel (Taxol)-responsive regulatory elements in the interleukin-8 promoter. Mol Cell Biol 1997; 17:5097–105
28. Xu JT, Xin WJ, Wei XH, Wu CY, Ge YX, Liu YL, Zang Y, Zhang T, Li YY, Liu XG: p38 activation in uninjured primary afferent neurons and in spinal microglia contributes to the development of neuropathic pain induced by selective motor fiber injury. Exp Neurol 2007; 204:355–65
29. Liu CC, Lu N, Cui Y, Yang T, Zhao ZQ, Xin WJ, Liu XG: Prevention of paclitaxel-induced allodynia by minocycline: Effect on loss of peripheral nerve fibers and infiltration of macrophages in rats. Mol Pain 2010; 6:76
30. Gong QJ, Li YY, Xin WJ, Zang Y, Ren WJ, Wei XH, Li YY, Zhang T, Liu XG: ATP induces long-term potentiation of C-fiber-evoked field potentials in spinal dorsal horn: The roles of P2X4 receptors and p38 MAPK in microglia. Glia 2009; 57:583–91
31. Naguib M, Xu JJ, Diaz P, Brown DL, Cogdell D, Bie B, Hu J, Craig S, Hittelman WN: Prevention of paclitaxel-induced neuropathy through activation of the central cannabinoid type 2 receptor system. Anesth Analg 2012; 114:1104–20
32. Ransohoff RM, Cardona AE: The myeloid cells of the central nervous system parenchyma. Nature 2010; 468:253–62
33. Baeuerle PA, Henkel T: Function and activation of NF-kappa B in the immune system. Annu Rev Immunol 1994; 12:141–79
34. Lindia JA, McGowan E, Jochnowitz N, Abbadie C: Induction of CX3CL1 expression in astrocytes and CX3CR1 in microglia in the spinal cord of a rat model of neuropathic pain. J Pain 2005; 6:434–8
35. Clark AK, Yip PK, Grist J, Gentry C, Staniland AA, Marchand F, Dehvari M, Wotherspoon G, Winter J, Ullah J, Bevan S, Malcangio M: Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. Proc Natl Acad Sci U S A 2007; 104:10655–60
36. Huang ZZ, Li D, Liu CC, Cui Y, Zhu HQ, Zhang WW, Li YY, Xin WJ: CX3CL1-mediated macrophage activation contributed to paclitaxel-induced DRG neuronal apoptosis and painful peripheral neuropathy. Brain Behav Immun 2014; 40:155–65
37. Pevida M, Lastra A, Hidalgo A, Baamonde A, Menéndez L: Spinal CCL2 and microglial activation are involved in paclitaxel-evoked cold hyperalgesia. Brain Res Bull 2013; 95:21–7
38. Glantz MJ, Choy H, Kearns CM, Mills PC, Wahlberg LU, Zuhowski EG, Calabresi P, Egorin MJ: Paclitaxel disposition in plasma and central nervous systems of humans and rats with brain tumors. J Natl Cancer Inst 1995; 87:1077–81
39. Heimans JJ, Vermorken JB, Wolbers JG, Eeltink CM, Meijer OW, Taphoorn MJ, Beijnen JH: Paclitaxel (Taxol) concentrations in brain tumor tissue. Ann Oncol 1994; 5:951–3
40. Cavaletti G, Cavalletti E, Oggioni N, Sottani C, Minoia C, D'Incalci M, Zucchetti M, Marmiroli P, Tredici G: Distribution of paclitaxel within the nervous system of the rat after repeated intravenous administration. Neurotoxicology 2000; 21:389–93
41. Figueroa-Masot XA, Hetman M, Higgins MJ, Kokot N, Xia Z: Taxol induces apoptosis in cortical neurons by a mechanism independent of Bcl-2 phosphorylation. J Neurosci 2001; 21:4657–67
42. Sun S, Cao H, Han M, Li TT, Pan HL, Zhao ZQ, Zhang YQ: New evidence for the involvement of spinal fractalkine receptor in pain facilitation and spinal glial activation in rat model of monoarthritis. Pain 2007; 129:64–75
43. Peters CM, Jimenez-Andrade JM, Kuskowski MA, Ghilardi JR, Mantyh PW: An evolving cellular pathology occurs in dorsal root ganglia, peripheral nerve and spinal cord following intravenous administration of paclitaxel in the rat. Brain Res 2007; 1168:46–59
44. Zhang H, Yoon SY, Zhang H, Dougherty PM: Evidence that spinal astrocytes but not microglia contribute to the pathogenesis of Paclitaxel-induced painful neuropathy. J Pain 2012; 13:293–303
45. Milligan ED, Zapata V, Chacur M, Schoeniger D, Biedenkapp J, O'Connor KA, Verge GM, Chapman G, Green P, Foster AC, Naeve GS, Maier SF, Watkins LR: Evidence that exogenous and endogenous fractalkine can induce spinal nociceptive facilitation in rats. Eur J Neurosci 2004; 20:2294–302
46. Gutierrez H, Davies AM: Regulation of neural process growth, elaboration and structural plasticity by NF-κB. Trends Neurosci 2011; 34:316–25
47. Teng FY, Tang BL: NF-kappaB signaling in neurite growth and neuronal survival. Rev Neurosci 2010; 21:299–313
48. Bhavsar PK, Sukkar MB, Khorasani N, Lee KY, Chung KF: Glucocorticoid suppression of CX3CL1 (fractalkine) by reduced gene promoter recruitment of NF-kappaB. FASEB J 2008; 22:1807–16
49. Kazantsev AG, Thompson LM: Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. Nat Rev Drug Discov 2008; 7:854–68
50. Clarke DL, Sutcliffe A, Deacon K, Bradbury D, Corbett L, Knox AJ: PKCbetaII augments NF-kappaB-dependent transcription at the CCL11 promoter *via* p300/CBP-associated factor recruitment and histone H4 acetylation. J Immunol 2008; 181:3503–14
51. Clarke DL, Clifford RL, Jindarat S, Proud D, Pang L, Belvisi M, Knox AJ: TNFα and IFNγ synergistically enhance transcriptional activation of CXCL10 in human airway smooth muscle cells *via* STAT-1, NF-κB, and the transcriptional coactivator CREB-binding protein. J Biol Chem 2010; 285:29101–10