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

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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)
CX3CL1 in paclitaxel-induced painful peripheral neuropathy still remains unknown.\textsuperscript{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.\textsuperscript{16,17} NF-κB exists in homo- or heterodimeric complexes consisting of different members of the Rel protein family.\textsuperscript{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.\textsuperscript{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.\textsuperscript{20,21} Histone acetylation has been recognized as an important factor to modify the accessibility of the DNA to the transcriptional machinery.\textsuperscript{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,\textsuperscript{23,24} and the activation of NF-κB induced by paclitaxel is also reported in several cancer cell lines.\textsuperscript{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. Intrathecal injection was performed according to the our previously described method.\textsuperscript{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.\textsuperscript{29} The experimenter who conducted the behavioral tests was blinded to all treatments. All animals survived throughout the experiments after drugs treatment.

\textbf{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′-GCAUCCGAGCCAACAUAUA dTdT-3′ (sense) 3′-dTdT CGUAGGUCUGGUUGUUAUU-5′ (antisense) siRNA2, target sequence 2: CTCAAGATCTGCCGAGTAA 5′-CUCAAGAUCGCGAGUAA dTdT-3′ (sense) 3′-dTdT GAGUUCUGACGGCUCAUU-5′ (antisense) siRNA3, target sequence 3: GCAGTTCGATGCTGATGAA 5′-CGAGUUCGAGUCUAGAA dTdT-3′ (sense) 3′-dTdT CGUAAAGCUACGACUACUU-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.

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

Immunohistochemistry was performed as we previously described. 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 (1:1000, Torrey Pines Biolabs), phosphorylated NF-κB p65 (Ser311) (1:1000, 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 manufacturer’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. 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

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**Table 1**

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<th>Gene Name</th>
<th>Primer Sequences</th>
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<tr>
<td>CX3CL1</td>
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<td>NF-κB p65</td>
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<td>β-actin</td>
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**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).
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′-GCTGCCCTGACCTAAT-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, 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.

<table>
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<th>Table 1. Specific Primer Sequences</th>
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<td>Gene</td>
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<tr>
<td>Interleukin (IL)-6</td>
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<td>CCL1</td>
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<tr>
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<td>CX3CL1</td>
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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.
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 (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) (**P < 0.01 vs. corresponding vehicle group). 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. 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...
group (fig. 4B). Double immunostaining results revealed that NF-κB 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]; 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, thereby facilitating the expression of target genes.
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.

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 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.
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 causes 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 acetyltransferases 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
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Competing Interests
The authors declare no competing interests.

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