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ORIGINAL ARTICLE

The over-production of TNF-α via Toll-like receptor 4 in spinal dorsal horn contributes to the chronic postsurgical pain in rat

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Abstract
Purpose Many patients suffer from chronic postsurgical pain (CPSP) following surgery, and the underlying mechanisms are poorly understood. In the present work, using the skin/muscle incision retraction (SMIR) model, the role of spinal TLR4/TNF-α pathway in the induction of CPSP was evaluated.
Methods Mechanical allodynia induced by SMIR was established in adult male Sprague–Dawley rats. The von Frey test was performed to evaluate the role of TLR4/TNF-α pathway on the mechanical allodynia. Western-blot and immunohistochemistry methods were adopted to understand the molecular mechanisms.
Results SMIR surgery decreased the ipsilateral 50 % paw withdrawal threshold, lasting for at least 20 days. Western-blot analysis and immunohistochemistry revealed that SMIR surgery significantly upregulated the expression of TLR4 ($p < 0.01$) in glial cells on the ipsilateral side of spinal cord and increased TLR4 occurred on day 5 and was maintained to the end of the experiment (day 20). Similarly, tumor necrosis factor-alpha (TNF-α) was significantly increased on days 5, 10, and 20 on the ipsilateral side of spinal dorsal horn following SMIR surgery. Intraperitoneal injection of an inhibitor of TNF-α synthesis thalidomide at 50 or 100 mg/kg dose (but not 10 mg/kg dose) significantly ameliorated the reduced paw withdrawal threshold induced by SMIR surgery. Importantly, intrathecal delivery of a specific TLR4 antagonist (LPS-RS) at dose of 25 μg significantly attenuated mechanical allodynia and prevented the upregulation of TNF-α induced by SMIR surgery.
Conclusions These findings suggest that the upregulation of TNF-α via TLR4 contributes to the development of CPSP in spinal dorsal horn.

Keywords TLR4 · Chronic postsurgical pain · TNF-α · SMIR

Introduction

Following common operations including thoracotomy, coronary artery bypass surgery, inguinal hernia repair, and Caesarean section, up to 50 % of patients suffer from chronic postsurgical pain (CPSP). It is an important clinical problem that seriously impacts the quality of patients' lives [1, 2]. Due to the deficiency of the mechanism underlying CPSP, effective treatments for CPSP are still limited. In the present study, we explored the molecular mechanism of CPSP by using the skin/muscle incision retraction (SMIR) surgery model [3].

Numerous studies have revealed that inflammation and immune response play an important role in neuropathic pain [4]. Toll-like receptor 4 (TLR4), as an evolutionarily conserved innate immune receptor, is widely expressed in the CNS [5]. Accumulating evidence has shown that TLR4 plays an important role in nerve injury-induced neuropathic pain. For example, nerve injury induced an increase in the production of TLR4 in the spinal cord [6, 7]. Concurrently, LPS-RS pretreatment attenuates pathological pain induced by inflammatory or nerve injury [8, 9]. In addition, inhibition of TLR4 function by intrathecal siRNA against TLR4

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also attenuates neuropathic pain induced by nerve injury and cancer pain [10, 11]. However, whether the TLR4 in spinal cord is involved in the CPSP remains unknown.

Tumor necrosis factor-α (TNF-α) is a soluble 17-kDa protein that predominately mediates immune and inflammatory responses [12] and plays a critical role in facilitating the development of neuropathic pain [13]. Nerve injury unregulated the expression of TNF-α in spinal cord [14] and inhibition of TNF-α signaling markedly attenuates thermal hyperalgesia and mechanical allodynia [15]. It was also reported that local application of TNF-α induced persistent hyperalgesia and allodynia in naive rats [16]. Furthermore, once bound by endogenous or exogenous ligands, activated TLR4 is capable of inducing massive production of inflammatory cytokines such as TNF-α and IL-1β [17, 18]. However, there are no data at present regarding whether TLR4/TNF-α signal pathway is involved in the CPSP induced by SMIR surgery.

In the present study, we evaluate the role of TLR4/TNF-α pathway in spinal cord on SMIR surgery-induced mechanical allodynia. We first examined whether SMIR surgery increased the expression of TLR4 and TNF-α. Furthermore, we explored the role of TLR4 and TNF-α on the development of mechanical allodynia induced by SMIR. Finally, the causal interaction between TLR4 and TNF-α in the SMIR surgery-induced mechanical allodynia were also investigated.

Materials and methods

Animals

Male Sprague–Dawley (SD) rats weighing 200–220 g were housed under a 12-h light/dark cycle with free access to food and water at a constant room temperature of 25 °C. Experimental procedures were approved by the local animal care committee and were carried out in accordance with the guidelines of the National Institutes of Health on animal care and ethical guidelines.

Skin/muscle incision and retraction (SMIR) surgery

The SMIR procedure was carried out as previously described [3]. Briefly, under anesthesia with 4 % chloral hydrate, a 1.5–2-cm incision was made in the skin of the medial thigh approximately 4 mm medial to the saphenous vein to expose the muscle of the thigh. An incision (7–10 mm long) was then made in the superficial muscle layer of the thigh to allow the insertion of a micro-dissecting retractor (Biomedical Research Instruments Inc, Silver Spring MD, USA). The skin and superficial muscle of the thigh were retracted by 2 cm for 1 h. Animals were covered with an absorbent bench underpad to minimize heat loss and to prevent dehydration of the surgical site. After the SMIR procedures, tissues in the surgical site were closed with 4.0 silk.

Drug administration and behavioral tests

Intrathecal injection was performed as previously described with minor modification [19]. In brief, laminectomy of the L5 vertebra was performed under anesthesia using sodium pentobarbital. After the dura was probed with an 8-G needle, a polyethylene-10 (PE-10) catheter was inserted into the rat's subarachnoid space through the L5/L6 intervertebral space, and the tip of the catheter was implanted at the L5 spinal segmental level. TLR4 inhibitor LPS-RS (5, 25 μg and 50 μg/10 μl, i.t., InvivoGen), or TNF-α synthesis inhibitor thalidomide (10, 50, and 100 mg/kg, i.p., Sigma) was initiated 30 min prior to the surgical procedures and maintained for 10 days. The same volume of saline was applied at the same schedule as in the control.

Behavioral tests were performed before and on days −1, 1, 5, 10, and 20 after the SMIR surgery. The paw withdrawal threshold 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 [20]. The experimenter who conducted the behavioral tests was blind to all of the treatments. Animals without mechanical allodynia were excluded from the subsequent experiments.

Western blot

Rats were deeply anesthetized using sodium pentobarbital (50 mg/kg, i.p.) at various time points. The L3/4 spinal cord segments were removed and homogenized on ice in 15 mmol/l Tris buffer containing a cocktail of proteinase inhibitors and phosphatase inhibitors. The protein samples were separated via gel electrophoresis (SDS–PAGE) and transferred onto a PVDF membrane. The membranes were placed in blocking buffer for 1 h at room temperature and incubated in a primary antibody against TLR-4 (1:1000, Abcam), or TNF-α (1:500, Santa Cruz) overnight at 4 °C. Then, the membranes were incubated in horseradish peroxidase-conjugated IgG (1:8000, Jackson). An enhanced chemiluminescence (ECL) solution (Pierce) was used to detect the immunocomplexes. Each band was quantified using a computer-assisted imaging analysis system (NIH ImageJ).

Immunohistochemistry

Immunochemistry was performed according to our previously described method [21]. Briefly, the rats were

anesthetized using sodium pentobarbital (50 mg/kg, i.p.) and perfused with 4 % paraformaldehyde through the ascending aorta. The L3/4 spinal cord were removed, post-fixed in the same fixative for 3 h, and transferred to 30 % sucrose overnight. Cryostat sections (25 μm) were cut and processed for immunohistochemistry using primary antibodies against TLR4 (1:500, Abcam), TNF-α (1:200, Santa Cruz), and anti-neuronal nuclei (NeuN, neuronal marker, 1:500, CST), anti-glial fibrillary acidic protein (GFAP, astrocyte marker, 1:1000, Chemicon) or anti-ibal (microglia marker, 1:500, CST) antibody. Following incubation overnight at 4 °C, the sections were incubated for 1 h at room temperature with Cy3-conjugated secondary antibodies (1:500, Jackson) and FITC-conjugated secondary antibody (1:250, Jackson). The TLR4-positive and TNF-α-positive areas were examined using a fluorescence microscope (Leica, Germany), and images were captured using a Leica DFC350 FX camera.

Data analysis

All data were expressed as mean ± SEM and analyzed using SPSS 13.0 (SPSS, USA). 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 for power analysis.

Results

SMIR induced mechanical allodynia and TLR4 upregulation

In the previous study, skin/muscle incision and retraction (SMIR) surgery induced the mechanical allodynia of rat [3]. Consistently, we found that SMIR surgery significantly evoked a persistent mechanical allodynia in ipsilateral paw compared with the pre-surgery baseline ($p < 0.01$; Fig. 1a). A decrease of 50 % paw withdrawal threshold was significant on day 5 and persisted to the end of the experiment (day 20) after SMIR surgery (Fig. 1a). Body weight of all the animals was measured each time before the behavioral test. SMIR surgery did not alter the body weight gain compared with the naive rats (data not shown). Furthermore, Western-blot results showed that SMIR increased the expression of TLR4 in spinal cord in a time-dependent manner compared with the pre-surgery. Upregulation of TLR4 occurred on day 5, peaked on day 10, and maintained to the end of the experiment (day 20) (Fig. 1b). Immunohistochemistry staining showed that the expression of TLR4 in the ipsilateral side of spinal dorsal horn has significantly higher levels on day 10 following SMIR surgery compared to the contralateral tissue (Fig. 2a). The double staining revealed that TLR4 in the spinal dorsal horn was co-localized with GFAP (a marker for astrocyte) and Iba1 (a marker for microglia), but not with NeuN (a marker for neuron) (Fig. 2b). These results suggested that the SMIR surgery-induced TLR4 upregulation mainly occurs on the glial cells in the ipsilateral side of spinal dorsal horn.

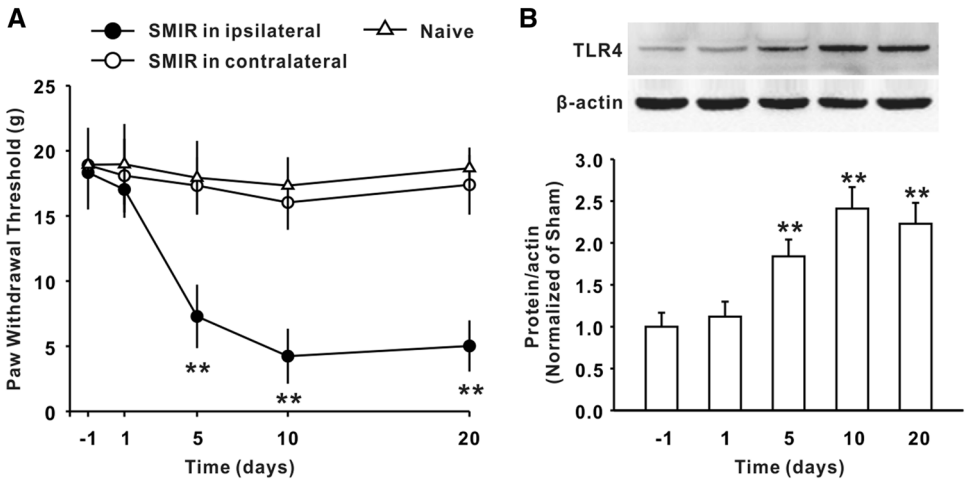

Fig. 1 SMIR surgery induced significant mechanical allodynia and increased the expression of TLR4. **a** SMIR surgery induced a marked and prolonged mechanical allodynia. On days 5, 10, and 20 after SMIR surgery, the SMIR surgery rats showed a significant decrease in 50 % paw withdrawal threshold relative to the pre-surgery baseline (day −1). ($n = 12$/group, $^{**}p < 0.01$). **b** Representative Western blot revealed that the expression of TLR4 was significantly increased on days 5, 10, and 20 in spinal cord following SMIR surgery compared with the pre-surgery ($n = 6$/group, $^{**}p < 0.01$)

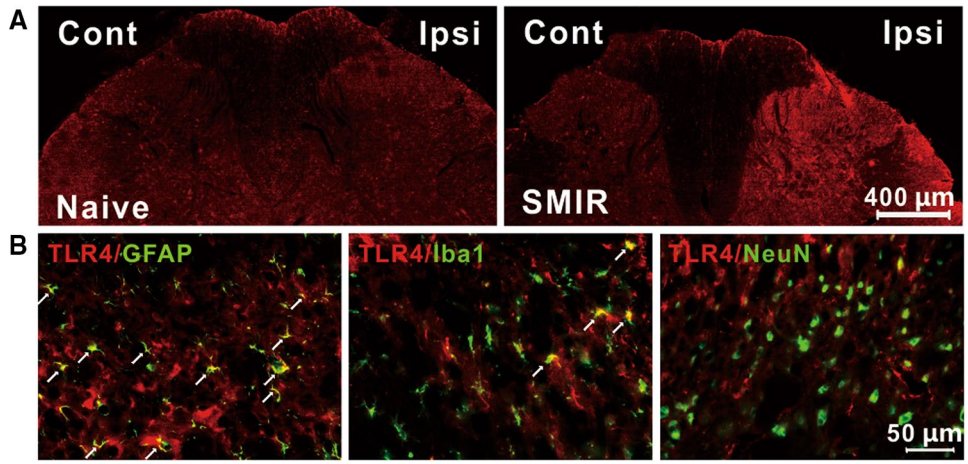

Fig. 2 The upregulated TLR4 occurred in glial cells in the ipsilateral side of spinal dorsal horn following SMIR surgery. **a** Immunohistochemistry experiments showed that the expression of TLR4 in the ipsilateral side of spinal dorsal horn is significant on day 10 after SMIR surgery compared with the contralateral side ($n = 6$/group). **b** The upregulated TLR4 was primarily expressed in GFAP-positive and Iba1-positive cells, but not in NeuN-positive cells ($n = 6$/group)

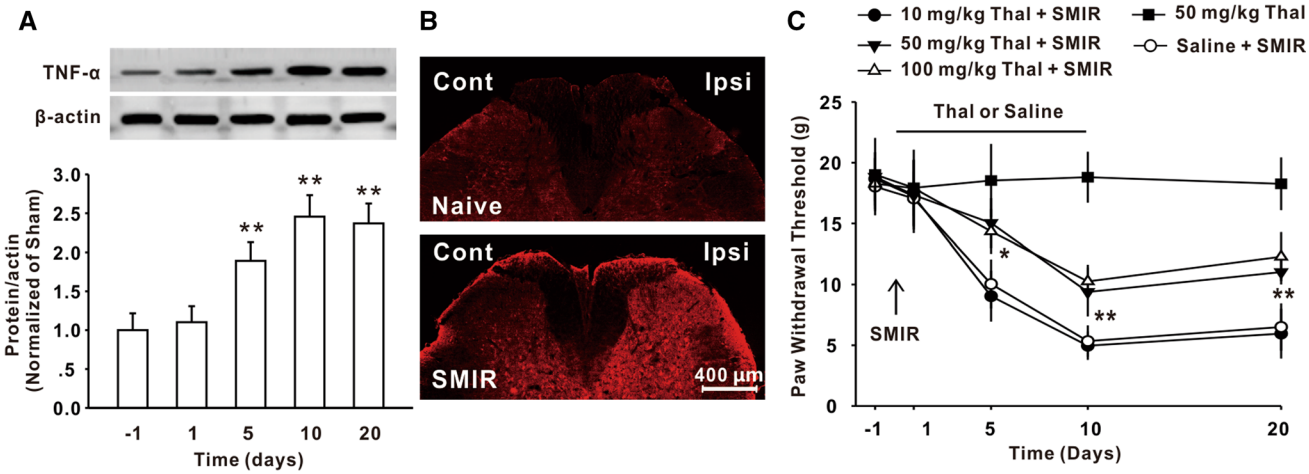

Fig. 3 Upregulation of TNF-α mediated the SMIR-induced mechanical allodynia. **a** Representative Western blot showed that there is a significant increase of TNF-α on days 5, 10, and 20 in spinal cord following SMIR surgery compared with the pre-surgery ($n = 6$/group, $^{**}p < 0.01$). **b** On day 10 after SMIR surgery, TNF-α level in the ipsilateral spinal dorsal horn was markedly higher compared to the contralateral side ($n = 6$/group). **c** Treatment with thalidomide (50 or 100 mg/kg, i.p.) for 10 days significantly attenuated SMIR surgery-induced mechanical allodynia ($n = 12$/group, $^{*}p < 0.01$, $^{**}p < 0.01$ versus the SMIR group)

Upregulation of TNF-α mediated the SMIR-induced allodynia

Accumulating evidence indicates that proinflammatory cytokines TNF-α plays an important role in pathological pain induced by inflammatory or nerve injury [13, 16]. We examined the level of TNF-α in the spinal cord following SMIR surgery via Western-blot analysis. The results revealed that the expression of TNF-α was significantly enhanced in the SMIR group ($p < 0.01$; Fig. 3a). Compared to the pre-surgery, although not on day 1, upregulation of TNF-α occurred on days 5, 10, and 20 after SMIR surgery (Fig. 3a). Moreover, immunohistochemistry results showed that the level of TNF-α expression is higher in the ipsilateral side than the contralateral side of the spinal dorsal horn on day 10 after SMIR surgery (Fig. 3b). In view of the consistency of the time course between TNF-α up-regulation and 50 % paw withdrawal threshold decrease following SMIR surgery, the role of TNF-α in the SMIR-induced allodynia was investigated. As the mechanical allodynia reached a peak on day 10 following SMIR surgery, thalidomide, an inhibitor of TNF-α synthesis, was intraperitoneally injected at various doses (10, 50, and 100 mg/kg) 30 min prior to SMIR surgery and maintained once daily for 10 days. The behavioral results showed that 50 and 100 mg/kg (but not 10 mg/kg) of thalidomide significantly attenuated mechanical allodynia induced by SMIR surgery ($p < 0.01$; Fig. 3c). No dose-dependent manner was observed for the inhibitory effect of thalidomide on the SMIR-induced allodynia. In addition, intrathecal injection of the thalidomide alone had no effect on the mechanical withdrawal thresholds in the naive rats (Fig. 3c).

Inhibition of TLR4 prevented the TNF-α upregulation and attenuated mechanical allodynia induced by SMIR surgery

Consider the above results that the time course of TLR4 upregulation is similar with the TNF-α increase. Next, we evaluated the role of TLR4 in SMIR-induced mechanical allodynia and TNF-α up-regulation by intrathecal injection of a TLR4 inhibitor LPS-RS. The behavioral results indicated that consecutive intrathecal injection of LPS-RS for 10 days at doses of 25 and 50 μg (but not 10 μg) significantly attenuated the mechanical allodynia induced by SMIR surgery ($p < 0.01$; Fig. 4a). Likely, LPS-RS has neither an inhibitory effect of dose-dependent manner nor an effect of the 50 % withdrawal threshold of the naive rats (Fig. 4a). To investigate the role of TLR4 in the maintenance of SMIR-induced allodynia, intrathecal injection of LPS-RS was initiated on day 10 and continued for another 10 days following SMIR surgery. The results showed no significant difference in mechanical allodynia between LPS-RS + SMIR group and SMIR group when behavioral tests were performed on day 20 (Fig. 4b), which indicated that TLR4 might not contribute to the maintenance of SMIR-induced allodynia. Western-blot results showed that intrathecal injection of thalidomide prevented the expression of TNF-α (Fig. 4c). Importantly, consecutively intrathecal injection of LPS-RS significantly prevented the overproduction of TNF-α in the spinal cord induced by SMIR surgery on day 12 (Fig. 4c), which suggested that TLR4, as an important upstream molecular, mediated TNF-α overproduction following SMIR surgery.

Discussion

The present study is the first to determine the involvement of TLR4/TNF-α signaling pathway in the spinal cord in mechanical allodynia following SMIR surgery, although accumulating evidence has indicated that TLR4 or TNF-α play a critical or important role in pathological pain, respectively [22, 23]. Our results showed that SMIR surgery significantly induced a prolonged mechanical allodynia in the ipsilateral paw and increased TLR4 expression in the glial cells in the ipsilateral side of the spinal dorsal horn. In addition, TNF-α expression in the ipsilateral side of the spinal dorsal horn is also markedly upregulated following SMIR surgery and application of thalidomide significantly attenuated the SMIR surgery-induced mechanical allodynia. We further found that intrathecal injection of TLR4 inhibitor LPS-RS prevented the upregulation of TNF-α and attenuated the mechanic allodynia induced by SMIR surgery. These findings suggest that upregulated TNF-α expression via TLR4 in spinal dorsal horn contributes to the CPSP.

CPSP induced by various common surgical procedures is a widespread clinical problem that has a significant impact on the quality of life of patients [2]. However, the underlying mechanism of this remained largely unclear. In the present study, SMIR surgery was employed to model persistent postsurgical pain. We found that SMIR surgery only induced mechanical allodynia in the ipsilateral paw. This result is consistent with a peer's report [3]. Recently, it has been recognized that TLR4 signaling plays an important role in the behavioral hypersensitivity in chronic pain

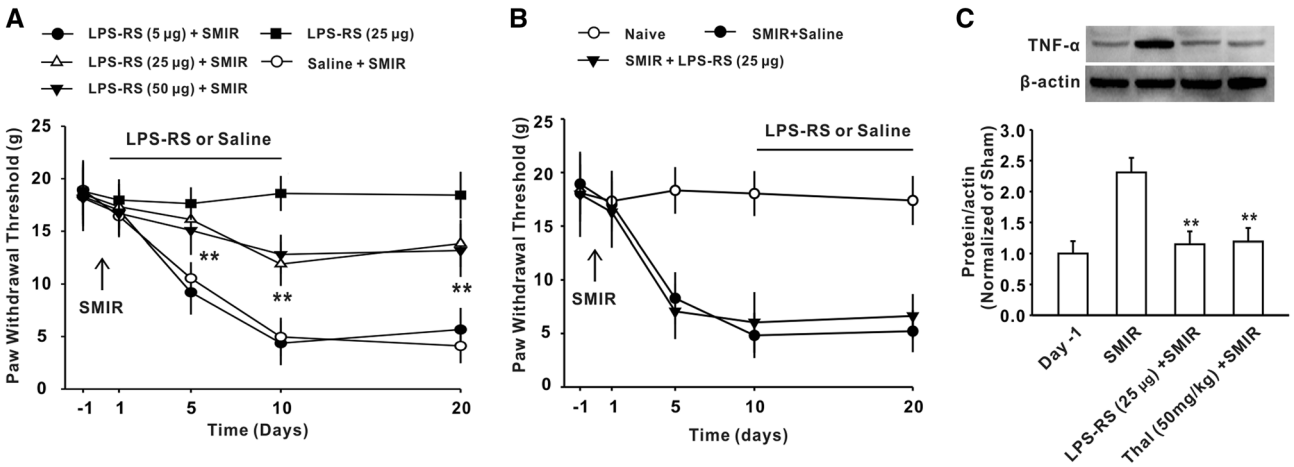

Fig. 4 Inhibition of TLR4 by LPS-RS attenuated mechanical allodynia and prevented TNF-α upregulation induced by SMIR surgery. **a** Intrathecal injection of TLR4 inhibitor LPS-RS (25 or 50 μg) for 10 days significantly attenuated SMIR-induced mechanical allodynia ($n = 12$/group, $^{**}p < 0.01$ versus the SMIR group). **b** Intrathecal injection of LPS-RS at 25 μg for 10 consecutive days on day 10 following SMIR did not attenuate mechanical allodynia compared with the SMIR group ($n = 12$/group). **c** LPS-RS or thalidomide prevented TNF-α upregulation on day 10 following SMIR surgery ($n = 6$/group, $^{**}p < 0.01$ versus the SMIR group)

induced by nerve injury or infection. For example, nerve injury induced an increase in the production of TLR4 in the spinal cord [6, 7]. Concurrently, inhibition of TLR4 significantly attenuated or prevented neuropathic pain induced by nerve injury and cancer pain [10, 11]. According to the phenomenon of mechanical allodynia, we also found that SMIR surgery significantly increased TLR4 expression in the ipsilateral side of the spinal dorsal horn. Although the cause of TLR4 upregulation remained unclear in the present study, a recent study showed that SMIR induced activation of microglia and astrocyte in the spinal cord [24]. We also found that TLR4 is co-expressed on the microglia and astrocyte, so it is possible that activated glial cell via signal pathway such as p38 et al increased the TLR4 expression following SMIR surgery. Furthermore, intrathecal injections of TLR4 inhibitor LPS-RS significantly ameliorated the reduced paw-withdrawal threshold in the SMIR group, but did not affect the paw-withdrawal threshold of the naive group. Together with the reports of fellow investigators, our results suggest that TLR4 signaling in the spinal cord plays an important role in the SMIR surgery-induced mechanical allodynia.

Accumulating evidence showed that proinflammatory cytokines such as TNF-α or IL-1β played an important role in neuropathic pain induced by nerve injury or inflammation [25]. For example, TNF-α was significantly upregulated in the DRG and spinal cord following nerve injury or inflammation and inhibition of TNF-α prevented neuropathic pain [26–28]. The present research found that SMIR surgery also significantly increases the expression of TNF-α in the ipsilateral side of spinal dorsal horn. This result was supported by the recently report that TNF-α upregulation mediated by P2X7 receptor participated in the mechanical allodynia induced by SMIR surgery [24]. Moreover, several studies have shown that TLR4 induced the activation of glia cells in the CNS [11, 29], synthesized and released proinflammatory cytokines by the activated glia cell participated in the development and maintenance of inflammatory pain and neuropathic pain [30, 31]. In addition, recent studies have shown that SMIR surgery increased expression of proinflammatory cytokines including IL-1β and IL-6 as well as TNF-α in spinal cord and DRG [32]. In the present study, inhibition of TLR4 prevented SMIR surgery-induced TNF-α upregulation in the spinal cord. Thalidomide treatment only attenuated (but did not totally prevent) mechanical allodynia. These finding implied that other mechanisms are involved in the postsurgical pain. Although the present study did not examined whether SMIR surgery activate the glia cell or enhance other cytokines expression in the spinal cord, the peer's and our results implied that it is possible that TLR4-mediated the activation of glia cell or cytokines releases in the spinal cord contributed to the chronic postsurgical pain.

Finally, it is worthy to note that the saphenous nerve is known to have inputs into the spinal cord at L3 and L4 levels, but not at L5 where the predominant input is from the sciatic nerve. In addition, in our present study, the regions of von Frey stimulation in the plantar surface of the hindpaw were innervated predominantly by the sciatic nerve, not the saphenous nerve [33]. A possible reason for this is that upregulation of TLR4/TNF-α following SMIR surgery induced central sensitization. The central sensitization enhanced mechanical hypersensitivity of the hindpaw dermatomes, which are innervated predominantly by the sciatic nerve. How SMIR evokes expansion of peripheral receptive fields remains to be further determined.

In summary, SMIR surgery significantly enhanced the expression of TLR4 and the production of TNF-α in the spinal dorsal cord. Blocking of TNF-α production significantly attenuated the chronic postsurgical pain. Furthermore, inhibition of TLR4 dramatically attenuated mechanical allodynia and the TNF-α upregulation induced by SMIR surgery in spinal dorsal horn. These findings suggested that the TLR4/TNF-α pathway plays a pivotal role in chronic postsurgical pain.

Conflict of interest Hao Tang received a research grant from Science and Technology Project in Guangdong (2010B031600046). The authors declare that they have no conflict of interest.

References

1. Steyaert A, De Kock M. Chronic postsurgical pain. Curr Opin Anaesthesiol. 2012;25:584–8.
2. Kehlet H, Jensen TS, Woolf CJ. Persistent postsurgical pain: risk factors and prevention. Lancet. 2006;367:1618–25.
3. Flatters SJ. Characterization of a model of persistent postoperative pain evoked by skin/muscle incision and retraction (SMIR). Pain. 2008;135:119–30.
4. Schomberg D, Ahmed M, Miranpuri G, Olson J, Resnick DKMM. Neuropathic pain: role of inflammation, immune response, and ion channel activity in central injury mechanisms. Ann Neurosci. 2012;19:125–32.
5. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140:805–20.
6. Yuan B, Liu D, Liu X. Spinal cord stimulation exerts analgesia effects in chronic constriction injury rats via suppression of the TLR4/NF-kappaB pathway. Neurosci Lett. 2014;581:63–8.
7. Ma YQ, Chen YR, Leng YF, Wu ZW. Tanshinone IIA downregulates HMGB1 and TLR4 expression in a spinal nerve ligation model of neuropathic pain. Evid Based Complement Alternat Med. 2014;. doi:10.1155/2014/639563.
8. Qi J, Buzas K, Fan H, Cohen JI, Wang K, Mont E, Klinman D, Oppenheim JJ, Howard OM. Painful pathways induced by TLR stimulation of dorsal root ganglion neurons. J Immunol. 2011;186:6417–26.
9. Christianson CA, Dumlao DS, Stokes JA, Dennis EA, Svensson CI, Corr M, Yaksh TL. Spinal TLR4 mediates the transition to a persistent mechanical hypersensitivity after the resolution of inflammation in serum-transferred arthritis. Pain. 2011;152:2881–91.

10. Wu FX, Bian JJ, Miao XR, Huang SD, Xu XW, Gong DJ, Sun YM, Lu ZJ, Yu WF. Intrathecal siRNA against Toll-like receptor 4 reduces nociception in a rat model of neuropathic pain. Int J Med Sci. 2010;7:251–9.
11. Lan LS, Ping YJ, Na WL, Miao J, Cheng QQ, Ni MZ, Lei L, Fang LC, Guang RC, Jin Z, Wei L. Down-regulation of Toll-like receptor 4 gene expression by short interfering RNA attenuates bone cancer pain in a rat model. Mol Pain. 2010;6:2–14.
12. Gosselin D, Rivest S. Role of IL-1 and TNF in the brain: twenty years of progress on a Dr. Jekyll/Mr. Hyde duality of the innate immune system. Brain Behav Immun. 2007;21:281–9.
13. Xu QH, Yaksh TL. A brief comparison of the pathophysiology of inflammatory versus neuropathic pain. Curr Opin Anesthesiol. 2011;24:400–7.
14. Raghavendra V, Rutkowski MD, DeLeo JA. The role of spinal neuroimmune activation in morphine tolerance/hyperalgesia in neuropathic and sham-operated rats. J Neurosci. 2002;22:9980–9.
15. Sommer C, Lindenlaub T, Teuteberg P, Schafers M, Hartung T, Toyka KV. Anti-TNF-neutralizing antibodies reduce pain-related behavior in two different mouse models of painful mononeuropathy. Brain Res. 2001;913:86–9.
16. Wei XH, Zang Y, Wu CY, Xu JT, Xin WJ, Liu XG. Peri-sciatic administration of recombinant rat TNF-alpha induces mechanical allodynia via upregulation of TNF-alpha in dorsal root ganglia and in spinal dorsal horn: the role of NF-kappa B pathway. Exp Neurol. 2007;205:471–84.
17. Liu T, Gao YJ, Ji RR. Emerging role of Toll-like receptors in the control of pain and itch. Neurosci Bull. 2012;28:131–44.
18. Tse KH, Chow KB, Leung WK, Wong YH, Wise H. Lipopolysaccharide differentially modulates expression of cytokines and cyclooxygenases in dorsal root ganglion cells via Toll-like receptor-4 dependent pathways. Neuroscience. 2014;267:241–51.
19. LoPachin RM, Rudy TA, Yaksh TL. An improved method for chronic catheterization of the rat spinal subarachnoid space. Physiol Behav. 1981;27:559–61.
20. Zhang J, Su YM, Li D, Huang ZZ, Wei JY, Xue Z, Pang RP, Liu XG, Xin WJ. TNF-alpha-mediated JNK activation in the dorsal root ganglion neurons contributes to Bortezomib-induced peripheral neuropathy. Brain Behav Immun. 2014;38:185–91.
21. Gong QJ, Li YY, Xin WJ, Zang Y, Ren WJ, Wei XH, 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.
22. Leung L, Cahill CM. TNF-alpha and neuropathic pain—a review. J Neuroinflammation. 2010;27:1–11.
23. Sorge RE, LaCroix-Fralish ML, Tuttle AH, Sotocinal SG, Austin JS, Ritchie J, Chanda ML, Graham AC, Topham L, Beggs S, Salter MW, Mogil JS. Spinal cord Toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. J Neurosci. 2011;31:15450–4.
24. Ying YL, Wei XH, Xu XB, She SZ, Zhou LJ, Lv J, Li D, Zheng B, Liu XG. Over-expression of P2X7 receptors in spinal glial cells contributes to the development of chronic postsurgical pain induced by skin/muscle incision and retraction (SMIR) in rats. Exp Neurol. 2014;261C:836–43.
25. Clark AK, Old EA, Malcangio M. Neuropathic pain and cytokines: current perspectives. J Pain Res. 2013;6:803–14.
26. Lu Y, Jiang BC, Cao DL, Zhang ZJ, Zhang X, Ji RR, Gao YJ. TRAF6 upregulation in spinal astrocytes maintains neuropathic pain by integrating TNF-alpha and IL-1beta signaling. Pain. 2014;155:2618–29.
27. Kallenborn-Gerhardt W, Hohmann SW, Syhr KM, Schroder K, Sisignano M, Weigert A, Lorenz JE, Lu R, Brune B, Brandes RP, Geisslinger G, Schmidtko A. Nox2-dependent signaling between macrophages and sensory neurons contributes to neuropathic pain hypersensitivity. Pain. 2014;155:2161–70.
28. Thomas RC, Bath MF, Stover CM, Lambert DG, Thompson JP. Exploring LPS-induced sepsis in rats and mice as a model to study potential protective effects of the nociceptin/orphanin FQ system. Peptides. 2014;61C:56–60.
29. Tanga FY, Nutile-McMenemy N, DeLeo JA. The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. Proc Natl Acad Sci USA. 2005;102:5856–61.
30. Wen YR, Tan PH, Cheng JK, Liu YC, Ji RR. Microglia: a promising target for treating neuropathic and postoperative pain, and morphine tolerance. J Formos Med Assoc. 2011;110:487–94.
31. Landry RP, Jacobs VL, Romero-Sandoval EA, DeLeo JA. Propentofylline, a CNS glial modulator does not decrease pain in post-herpetic neuralgia patients: in vitro evidence for differential responses in human and rodent microglia and macrophages. Exp Neurol. 2012;234:340–50.
32. Chen YW, Tzeng JI, Lin MF, Hung CH, Wang JJ. Transcutaneous electrical nerve stimulation attenuates postsurgical allodynia and suppresses spinal substance p and proinflammatory cytokine release in rats. Phys Ther. 2015;95:76–85.
33. Bajrovic F, Sketelj J. Extent of nociceptive dermatomes in adult rats is not primarily maintained by axonal competition. Exp Neurol. 1998;150:115–21.