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CXCL12/CXCR4 axis: an emerging neuromodulator in pathological pain

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Abstract: The roles of chemokine C-X-C motif ligand 12 (CXCL12) and its receptor chemokine C-X-C motif receptor 4 (CXCR4) reveal this chemokine axis as an emerging neuromodulator in the nervous system. In the peripheral and central nervous systems, both CXCL12 and CXCR4 are expressed in various kinds of nociceptive structures, and CXCL12/CXCR4 axis possesses pronociceptive property. Recent studies have demonstrated its critical roles in the development and maintenance of pathological pain, and both neuronal and glial mechanisms are involved in this CXCL12/CXCR4 axis-mediated pain processing. In this review, we summarize the recent development of the roles and mechanisms of CXCL12/CXCR4 axis in the pathogenesis of chronic pain by sciatic nerve injury, human immunodeficiency virus-associated sensory neuropathy, diabetic neuropathy, spinal cord injury, bone cancer, opioid tolerance, or opioid-induced hyperalgesia. The potential targeting of CXCL12/CXCR4 axis as an effective and broad-spectrum pharmacological approach for chronic pain therapy was also discussed.

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Introduction

Pathological pain is chronic pain lasting for more than 3 months clinically, and it is divided into three categories (neuropathic pain, inflammatory pain, and cancer pain) according to pathophysiology (Kuner, 2010). Damage by an injury or a disease produces inflammatory molecules that alter the property of primary sensory nerve and dorsal root ganglion (DRG). As a consequence of peripheral sensitization, the perception and conduction of pain signals become abnormal in the peripheral nerve system (PNS). These excitatory signals then change neural plasticity in the spinal cord and the higher center in the central nerve system (CNS). Accordingly, central sensitization is established via various mechanisms and contributes to the generation and maintenance of chronic pain (Basbaum et al., 2009; Latremoliere and Woolf, 2009).

Chemokine is a small and secreted protein with the property of leukocyte chemoattractant. On the basis of their molecular structure, chemokines are divided into four subfamilies, including C-C, C-X-C, X-C, and C-X3-C (Zlotnik and Yoshie, 2000). Chemokines exert their function by binding to chemokine receptors that belong to the G-protein-coupled receptor (GPCR) family (Allen et al., 2007). In the nervous system, chemokine signaling modulates neurotransmission and neuronal-glial cross-talk (Rostene et al., 2011). Recently, a growing body of preclinical studies has suggested that chemokine axis functions as an important modulator in chronic pain processing. Chemokine C-X3-C motif ligand 1/chemokine C-X3-C motif receptor 1 (CX3CL1/CX3CR1) and chemokine C-C motif ligand 2/chemokine C-C motif receptor 2 (CCL2/CCR2) are two well-studied chemokine axes in pain research, and they modulate the peripheral and central processing of pathological pain via multiple mechanisms (White et al., 2007; Thacker et al., 2009; Van Steenwinckel et al., 2011, 2015; Li et al., 2015).

CXCL12 (also called stromal cell-derived factor 1, SDF-1) belongs to the C-X-C subfamily of chemokine and exerts

its function by binding to CXCR4. Chemokine C-X-C motif receptor 7 (CXCR7) is newly considered another receptor of CXCL12 (Levoye et al., 2009), but to date, there is no evidence of the involvement of CXCR7 in pain processing. The pair of CXCL12 and CXCR4 is expressed in the PNS and CNS in both constitutive and inducible manners. The distribution of CXCL12/CXCR4 axis has been reported nearly in all cell types in the nervous system, including neuron, astrocyte, and microglia (Li and Ransohoff, 2008). As CXCR4 is the member of GPCR family, the CXCL12/CXCR4 axis was found to be associated with a series of downstream signaling pathways such as phosphatidylinositol 3-kinase, nuclear factor kappa light-chain-enhancer of activated B cells (NF-κB), nuclear factor of activated T cells, mitogen-activated protein kinases (MAPKs) (like extracellular signal-regulated kinases [ERKs], c-Jun N-terminal kinases [JNKs], and p38), and signal transducers and activators of transcription pathways in the nervous system (Li and Ransohoff, 2008). Not only does CXCL12/CXCR4 axis modulate the neuromodulation, neuroprotection, and neuronal-glial interaction in normal conditions, it is also involved in the neurological disorder caused by human immunodeficiency virus (HIV) infection, tumor, stroke, and multiple sclerosis (Li and Ransohoff, 2008). In the last few years, an increasing number of pain research in CXCL12/CXCR4 axis have shown that it is an emerging neuromodulator in pathological pain (Bhangoo et al., 2009; Dubovy et al., 2010; Luo et al., 2014; Shen et al., 2014). Thus, in this article, the evidence for the involvement of CXCL12/CXCR4 axis in pathological pain is reviewed.

CXCL12/CXCR4 axis and nociception

Distribution on nociceptive structures

CXCL12/CXCR4 axis is widely distributed on nociceptive structures in the PNS and CNS, which implicates its contribution to pain signal transduction. In the PNS, CXCL12/CXCR4 axis was found at the peripheral terminal of the primary sensory fiber in the glabrous skin (Reaux-Le Goazigo et al., 2012). In the sciatic nerve, it was reported that CXCL12/CXCR4 axis was colocalized with calcitonin gene-related peptide (CGRP)-positive and -negative axonal nerve fiber (Reaux-Le Goazigo et al., 2012). CXCR4 was also expressed in Schwann cells in the sciatic nerve (Kury et al., 2002). In DRG, CXCL12 and CXCR4 shared similar expression patterns and were predominantly localized in the small- and medium-diameter neurons. Moreover, double immunolabeling work showed that CXCL12/CXCR4 axis was distributed in DRG neurons with vanilloid receptor 1, CGRP or isolectin-B4 (IB4)-containing subpopulation and some cells without these immunoreactivity (Oh et al., 2001; Reaux-Le Goazigo et al., 2012). In the spinal cord, the expression of this chemokine axis was restricted mainly at the central terminal of primary sensory fiber in the superficial laminae I–III of the dorsal spinal cord and colocalized with CGRP-containing neuron (Reaux-Le Goazigo et al., 2012).

Although CXCL12/CXCR4 axis is distributed in the spinal cord, there are some conflicts on the results of the cellular localization of CXCL12 and CXCR4. Shen et al. (2014) reported that CXCL12 was expressed predominantly in astrocyte, while CXCR4 was found in spinal neuron and microglia with weak immunoreactivity. Reaux-Le Goazigo et al. (2012) found that CXCL12 and CXCR4 were expressed mainly in the spinal neurons and to a lesser extent in the spinal microglia and astrocyte. Using immunoelectron microscopy (Reaux-Le Goazigo et al., 2012), it was found that CXCL12 and CXCR4 were detected in neuronal organelles including the Golgi apparatus, rough endoplasmic reticulum, and the structures at the pre- and post-synapse. Moreover, this study demonstrated that the primary neuron may release CXCL12/CXCR4 axis-containing synaptic vesicles from its central terminal to spinal dorsal horn.

Pronociceptive property

Further direct evidence for the pronociceptive property of CXCL12/CXCR4 axis in nociception comes from pharmacological studies. CXCL12 (recombinant CXCL12, same as below) or gp120 (HIV-derived agonist of CXCR4) lowered the threshold for action potential generation *in vitro* and gp120 stimulated substance P release from cultured neonatal DRG neurons (Oh et al., 2001). Then, an animal behavioral test showed that intradermal injection of CXCL12 or gp120 caused mechanical allodynia in hind paw for hours. These results indicated that CXCL12/CXCR4 axis is potentially involved in pain signaling. Peripheral cellular and molecular mechanisms for CXCL12/CXCR4 axis-mediated nociceptive processing are further delineated in Figure 1. CXCL12/CXCR4 axis can sensitize DRG neuron via many pathways, like Src family-kinases (SFKs) and intracellular calcium density $[Ca^{2+}]_i$. The incubation of CXCL12 increased the phosphorylation level of SFKs in DRG in small- to medium-sized sensory neurons *in vivo*, which was reversed by the coadministration of CXCR4 antagonist (Rivat et al., 2014). Moreover, CXCL12 and gp120 increased $[Ca^{2+}]_i$ in single DRG neuron, but not

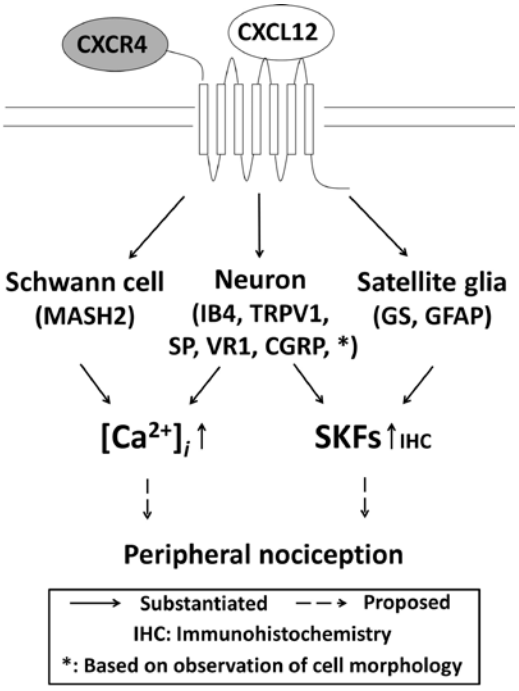

Figure 1: Cellular and molecular mechanisms for CXCL12/CXCR4 axis in peripheral nociception.
Cell-type biomarkers: GS, glutamine synthetase; GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclei; CGRP, calcitonin gene-related peptide; TRPV1, transient receptor potential cation channel subfamily V member 1; SP, substance P; VR1, vanilloid receptor 1; MASH2, mammalian achaete scute homolog 2. Pain pathway: SFKs, Src family-kinases.

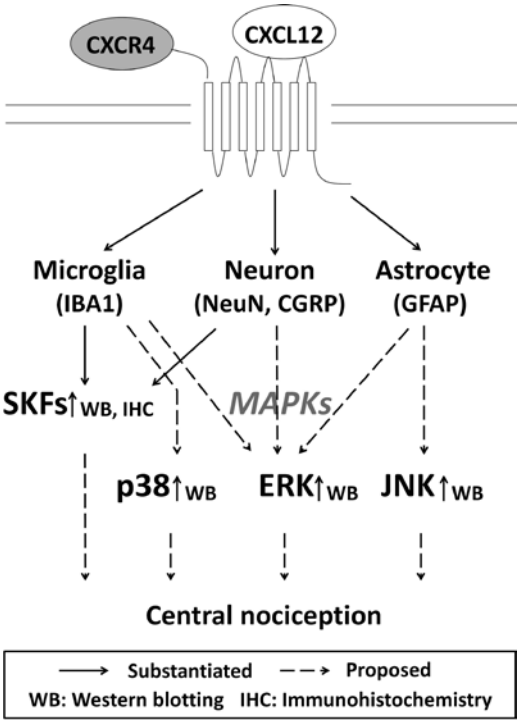

Figure 2: Cellular and molecular mechanisms for CXCL12/CXCR4 axis in central nociception.
Cell-type biomarkers: GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclei; CGRP, calcitonin gene-related peptide; IBA1, ionized calcium-binding adapter molecule 1. Pain pathways: SFKs, Src family-kinases; MAPK, mitogen-activated protein kinases; ERK, extracellular signal-regulated kinases; JNK, c-Jun N-terminal kinases.

fibroblasts, *in vitro*, which was isolated from normal rats (Oh et al., 2001). CXCL12 increased the phosphorylation of SFKs in satellite glia *in vivo* (Rivat et al., 2014) and $[Ca^{2+}]_i$ in Schwann cells *in vitro* (Kury et al., 2003), which might initiate pain-related cellular events. Glial cells, like Schwann cell and satellite glia, can thus be involved in the peripheral nociceptive property of CXCL12/CXCR4 axis.

CXCL12/CXCR4 chemokine axis also exhibits pronociceptive property in the CNS. In intact animals, a single intrathecal injection of CXCL12 produced transient mechanical allodynia, and such allodynia was reversed by pretreatment of CXCR4 neutralizing antibody (12G5) (Reaux-Le Goazigo et al., 2012) or CXCR4 antagonist (AMD3100) intrathecally (Hu et al., 2015). Central mechanisms for CXCL12/CXCR4 in chronic pain are also delineated in Figure 2. In a bone cancer pain model, the intrathecal administration of AMD3100 attenuated mechanical allodynia and thermal hyperalgesia and decreased spinal levels of c-fos (biomarker of neural activity), glial fibrillary acidic protein (GFAP; biomarker of astrocyte activation), and ionized calcium-binding adapter molecule 1 (IBA1; biomarker of microglia activation) (Shen et al., 2014), indicating that neuronal, astrocytic, and microglial mechanisms can contribute to the role of CXCL12/CXCR4 axis in central pain processing. Multiple molecular pain pathways might account for the central pronociceptive property of CXCL12/CXCR4 axis. In normal rats, the intrathecal injection of CXCL12 increased the phosphorylation of ERK, JNK, and p38 pathways in the spinal dorsal horn, and such activation was reversed by the pretreatment of AMD3100 (Hu et al., 2015). Central MAPK pathways are essential for the development and maintenance of various types of pathological pain in a cell type-specific manner. For example, in the spinal cord under pathological pain conditions, ERK was activated in neuron, astrocyte, and microglia; JNK was activated in astrocyte; and p38 was activated in microglia (Ji et al., 2009). Thus CXCL12/CXCR4 might orchestrate pain-related cellular activities in the spinal cord by modulating the activation of MAPK pathways. Moreover, SFKs pathway can be involved in the central role of CXCL12/CXCR4 axis in pain. The intrathecal injection of CXCL12 increased phosphorylation of SFKs in microglia and some

scattered second-order neuron, but not astrocyte, in the lumber spinal cord of normal rats (Rivat et al., 2014).

CXCL12/CXCR4 axis and pathological pain

Different events, which occur in the PNS and CNS, may account for a unique set of neurochemical and cellular changes in different types of chronic pain. For example, the pattern of spinal glial activation differs under various pathological pain conditions (Watkins and Maier, 2002; Cao and Zhang, 2008). As CXCL12/CXCR4 axis contributes to the neuroinflammation and the neuronal-glial interaction (Li and Ransohoff, 2008; Grace et al., 2014), it is speculated that this chemokine axis may participate in the processing of different types of pathological pain. The involvement of CXCL12/CXCR4 axis in the processing of pathological pain is overviewed and elaborated in the following sections.

CXCL12/CXCR4 axis and neuropathic pain

Neuropathic pain is caused by a lesion or disease on the peripheral or central somatosensory nervous system and characterized by spontaneous ongoing or short pain and amplified pain response after nonnoxious or noxious stimuli (Baron et al., 2010). As CXCL12/CXCR4 axis plays essential roles in the biological response to nerve injury (Ji et al., 2004) and in neuromodulation (Guyon and Nahon, 2007; Rostene et al., 2007) in the nervous system, it is highly possible that this chemokine pair would be involved in the pain processing following neuropathy.

CXCL12/CXCR4 axis and sciatic nerve injury

Animal models of sciatic nerve injury are widely used in studies of peripheral neuropathic pain (Jaggi et al., 2011), especially in CXCL12/CXCR4-related research. The involvement of CXCL12/CXCR4 axis in sciatic neuropathy was first found in a rat model of peripheral neuropathic pain, in which lysophosphatidylcholine (LPC) was applied into the gastrocnemius muscle to cause mechanical allodynia (Bhangoo et al., 2007a). In this model, immunostaining work showed that pain hypersensitivity was associated with the increased number of DRG neurons expressing CXCR4, while CXCL12 expression pattern was not affected. By assessing $[Ca^{2+}]_i$ in DRG cells acutely isolated from LPC-treated rats *in vitro*, the number of DRG cells responding to CXCL12 increased following PLC administration. In another study, sciatic nerve injury induced by LPC increased the frequency of micturition and caused visceral pain hypersensitivity (Foster et al., 2011). It was found that CXCL12 increased $[Ca^{2+}]_i$ in somatic- and bladder-associated DRG neurons that were isolated from these PLC-treated animals. These findings implicate that CXCL12/CXCR4 axis might contribute to the pathogenesis of these complications of peripheral neuropathy. Previously, it was found that unilateral nerve injury excited neurons, activated satellite glia, and initiated macrophage invasion at both ipsilateral and contralateral DRG (Dubový et al., 2006, 2007), but the mechanisms underlying these phenomena remain unclear. Recently, it was shown that chronic constriction injury (CCI) operation induced chronic pain, which was associated with the increased expression of CXCR4 in neuron and satellite glia in bilateral lumbar and cervical DRG (Dubovy et al., 2010). It was also shown that macrophages exhibiting CXCL12 were recruited to the lumbar DRG following CCI. Additionally, intraperitoneal AMD3100 reversed thermal hyperalgesia induced by CCI. Therefore, these results implicate that CXCL12/CXCR4 axis may modulate the responses of bilateral DRG to unilateral nerve injury.

In a partial sciatic nerve ligation (pSNL) model, we first reported the central roles of CXCL12/CXCR4 axis in neuropathic pain processing (Luo et al., 2014). Our study showed that a single intrathecal injection of AMD3100 attenuated established hyperalgesia by pSNL in a dose-dependent manner. Using real-time polymerase chain reaction (PCR) and Western blotting, it was found that such intrathecal AMD3100 injection downregulated the activation of glia-related pain pathways, like MAPKs (JNK, p38) and NF-κB (p65), and modulated the spinal expression of pain molecules, like substance P and intracellular adhesive molecule-1 (proinflammatory molecule and indicator of the permeability of the blood-brain barrier; Lee and Benveniste, 1999). These findings further indicate that besides the neurons, multiple downstream mechanisms associated with glia-related pain pathways were involved in CXCL12/CXCR4 axis-mediated central processing of neuropathic pain.

CXCL12/CXCR4 axis and HIV-associated sensory neuropathy

HIV-associated neuropathic pain occurs in one third of AIDS patients. Although the pathophysiology of this pain disorder remains largely unclear, the mechanisms involving gp120, immune pathogenic factors, and nucleoside

reverse transcriptase inhibitors (NRTIs) have been proposed (Verma et al., 2005). CXCR4 is a coreceptor of HIV-1 gp120 and plays vital roles in the invasion of virus to host cells (Alkhatib, 2009). The intradermal injection of gp120 or CXCL12 in rat hind paw caused transient allodynia (Oh et al., 2001). Therefore, as a pronociceptive component, CXCL12/CXCR4 axis would be potentially a therapeutic target for HIV-associated sensory neuropathy. Intraperitoneal injection of 2′,3′-dideoxycytidine (ddC; an NRTI) caused sciatic nerve injury and mechanical allodynia and increased mRNA level of CXCL12 and CXCR4 in DRG non-neuronal cells *in vivo* (Bhangoo et al., 2007b). In cultured DRG isolated from rats treated with ddC or vehicle, ddC treatment increased the number of cells responding to CXCL12 by monitoring $[Ca^{2+}]_i$, indicating functional CXCR4 was increased in DRG following NRTI treatment. Moreover, the intraperitoneal administration of AMD3100 transiently reversed established mechanical allodynia by ddC treatment. These results are the first to report the involvement of CXCL12/CXCR4 axis in DRG processing of NRTI-induced neuropathy. Perineural administration of gp120 caused sciatic nerve injury and persistent pain in rat hind paw (Zheng et al., 2014). Herpes simplex virus vectors expressing anti-inflammatory cytokine, interleukin 10 (IL-10), was constructed and injected into ipsilateral hind paw of gp120-treated rats, which reversed the established mechanical allodynia. In this gp120-induced neuropathic pain model, overexpressed exogenous IL-10 downregulated the phosphorylation of p38 and decreased the protein levels of tumor necrosis factor-α (TNF-α), CXCL12, and CXCR4 at both DRG and spinal dorsal horn by Western blotting. This study demonstrated that both central and peripheral CXCL12/CXCR4 axis might contribute to pain processing of HIV-associated sensory neuropathy. In a model combining the application of perineural gp120 and intraperitoneal ddC, it was shown that such dual-drug treatment increased CXCL12 signaling in sensory neuron in DRG and intraperitoneal AMD3100 reversed mechanical allodynia induced by gp120 and ddC, but not hyperalgesia only induced by gp120 (Bhangoo et al., 2009). This research uncovers that gp120, but not ddC, may induce hyperalgesia via a CXCR4-independent manner.

CXCL12/CXCR4 axis and diabetic neuropathy

Diabetic neuropathy occurs in one quarter of diabetic patients, and its mechanisms remain largely unknown (Albers and Pop-Busui, 2014). In an *in vivo* work, two animal models of type II diabetes (mice treated with high-fat diet [HFD] and transgenic mice without leptin receptor) were used to study the peripheral roles of CXCL12/CXCR4 axis in painful diabetic neuropathy (Menichella et al., 2014). In two models of type II diabetes, mice showed mechanical allodynia, and the intraperitoneal administration of AMD3100 reversed such neuropathic pain for hours. CXCL12 increased $[Ca^{2+}]_i$ in DRG sensory neurons *in vivo*, which were acutely isolated from mice with HFD-induced diabetes. The infiltration of CXCR4-expressing CD3 positive T cells to DRG was also shown in this study. All those results suggest the potential roles of CXCL12/CXCR4 axis-targeted therapy in the management of painful diabetic neuropathy.

CXCL12/CXCR4 axis and spinal cord injury

Neuropathic pain is not uncommon after spinal cord injury, and its pathology and therapeutic strategy are unclear (Finnerup, 2013). As CXCL12/CXCR4 axis orchestrates cellular events involved in the recovery process following the spinal cord injury (Tysseling et al., 2011; Jaerve et al., 2012), this chemokine axis might contribute to the pathophysiology of spinal cord neuropathy. The spinal expression and distribution of CXCL12/CXCR4 were reported in a rat model of spinal cord injury (Knerlich-Lukoschus et al., 2011). Spinal cord contusion increased the expression of CXCL12/CXCR4 axis in the dorsal horn and dorsal column. Double immunolabeling work showed that spinal CXCL12/CXCR4 axis was expressed on neurons, astrocytes, and microglia/macrophages in rat with spinal cord injury. This is the first study that showed the association of CXCL12/CXCR4 axis with central neuropathic pain, which needs to be warranted by further pharmacological and behavioral evidence.

CXCL12/CXCR4 axis and cancer pain

Various types of factors such as prostaglandins, TNF-α, and endothelin secreted by tumor and/or the extension of tumor tissue sensitize or excite the primary afferent sensory neuron in DRG. Persistent noxious stimuli from tumor tissue also sensitize sensory structures in the spinal cord and the higher centers in the brain (Mantyh et al., 2002). CXCL12/CXCR4 axis plays important roles in many aspects of the cancer biology, including angiogenesis and metastasis (Teicher and Fricker, 2010). Current research focuses on the central role of CXCL12/CXCR4 axis in pain processing following oncogenesis. In a rat model of bone cancer pain, astrocytic mechanisms for the involvement of CXCL12/CXCR4 axis in cancer pain processing were found

(Shen et al., 2014). In this study, the tumor cell implantation (TCI) into rat tibial cavity produced persistent cancer pain and upregulated the expression of CXCL12 at DRG and spinal cord *in vivo*. As CXCL12 was expressed mainly in spinal astrocytes following TCI, the intrathecal administration of astrocytic activation inhibitor (fluorocitrate) or selective JNK inhibitor (SP600125) blocked such TCI-induced increase in spinal CXCL12 expression. TCI triggered the increase in CXCR4 expression in spinal cord following the surgery, which was colocalized mainly with NeuN (biomarker of neuron) and GFAP and a small amount with IBA1. This study also reported that the intrathecal injection of CXCL12 neutralizing antibody or CXCR4 antagonist (AMD3100) attenuated the initiation and maintenance of cancer pain. Intrathecal AMD3100 also suppressed the activation of neuron (c-Fos), astrocyte (GFAP), and microglia (IBA1) induced by TCI treatment, indicating that CXCL12/CXCR4 axis may contribute to cancer pain processing via neuronal and glial mechanisms in the CNS. In a subsequent research, intrathecal injection of CXCL12 neutralizing antibody attenuated cancer pain and decreased the level of IBA1 following TCI treatment (Liu et al., 2014). The results suggested that microglia might also be involved in CXCL12/CXCR4-mediated cancer pain processing in the CNS. Furthermore, in the same model, it was found that TCI treatment increased the phosphorylation of MAPK pathways and the production of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) in the lumbar spinal cord (Hu et al., 2015). The blockade of CXCL12/CXCR4 axis by AMD3100 or CXCL12 neutralizing antibody or MAPK pathways (by ERK inhibitor [U0126], JNK inhibitor [SP600125], or p38 inhibitor [SB503580]) reversed persistent pain and downregulated the production of these cytokines in a rat bone cancer model. This study indicates that spinal MAPK pathways might be associated with CXCL12/CXCR4-mediated neuroinflammation under cancer condition.

CXCL12/CXCR4 axis and opioid tolerance and hyperalgesia

Although opioid therapy is accepted as an effective treatment for chronic pain, the efficacy decreases with time due to opioid tolerance or opioid-induced hyperalgesia (OIH) (Chan et al., 2011). Recently, the crosstalk between opioid and chemokine receptor provides novel concepts in reducing the complications of clinical opioid use (Parsadaniantz et al., 2015). As a member of the GPCR family, CXCR4 heterodimerizes with opioid receptor (like mu-opioid receptor [MOR] or delta-opioid receptor [DOR]) on cell membrane and modulates opioid-mediated activities under various pathological conditions, such as ischemia-induced myocardial infarction and HIV invasion (Kramp et al., 2011). Therefore, CXCL12/CXCR4 axis is proposed to function in the pathology of opioid tolerance and OIH.

CXCL12/CXCR4 axis and opioid tolerance

CXCR4 was coexpressed with MOR in various areas in rat brain, including the hippocampus, cingulate cortex, and periaqueductal grey (PAG) (Heinisch et al., 2011). In uninjured animals, double immunolabeling work showed that CXCR4 was distributed on cells expressing DOR and MOR at lumbar DRG and spinal dorsal horn, implicating that CXCL12/CXCR4 axis might influence opioid receptor activity (Rivat et al., 2014). Opioid-induced analgesia by [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO), an agonist of MOR, in rats was abolished by the administration of CXCL12 in PAG (Szabo et al., 2002). These findings demonstrated that CXCL12/CXCR4 axis might play a central role in opioid signaling in the CNS. Using electrophysiological tools *in vitro*, it was found that exogenous CXCL12 blocked the response of PAG neuron to morphine-induced hyperpolarization and reduction of input resistance (Heinisch et al., 2011). This study further suggests the involvement of CXCL12/CXCR4 axis in opioid-mediated intracellular events in neurons. PAG injection of DAMGO or [D-Pen(2), D-Pen(5)]-enkehphalin (DPDPE) (agonist of DOR) increased the latency to flick the tail in cold water in rats, and such antinociceptive effect was blocked by pretreated or cotreated of CXCL12 in PAG (Chen et al., 2007). This work indicates that CXCL12/CXCR4 axis might modulate opioid-induced analgesia by interacting with DOR and MOR rapidly in PAG. PAG injection of buprenorphine, an artificial derivative of thebaine and opioid-like peptide, increased the latency to flick the tail in cold water in rats, and such analgesic effect was not affected by coadministrated CXCL12 (Benamar et al., 2011). Then, PAG injection of buprenorphine and methadone caused an analgesic effect in rats in a hot plate test, and such analgesic effect of methadone, but not buprenorphine, was blocked by cotreated gp120 (Palma et al., 2011). Findings from these two studies implicate that buprenorphine could be applied to patients who suffer opioid tolerance by endogenous CXCL12/CXCR4 axis in the CNS.

In cultured DRG neuron, it was found that an SFK inhibitor (PP2) increased the constitutive activity of MOR and downregulated the recycling of MOR (Walwyn et al., 2007). In engineered Chinese hamster ovary cells stably expressing DOR, DPDPE promoted intracellular activities (like the recruitment and dephosphorylation of β-arrestin)

associated with DOR *in vitro*, and such effect by DPDPE was suppressed by the coadministration of PP2 (Hong et al., 2009). The results from those studies suggest that SFKs might be involved in the pathology of opioid tolerance by regulating MOR and DOR activities. Recently, how CXCL12/CXCR4 axis caused a loss in morphine analgesia via SFK mechanism was studied (Rivat et al., 2014). This study showed that CXCL12 activated SFK pathway (phosphorylation) on primary DRG neuron *in vitro*, and such effects were blocked by the coincubation of an CXCR4 antagonist (IT1t). it was also shown that the intrathecal injection of CXCL12 increased the phosphorylation of SFKs in a subpopulation of cells containing MOR and DOR at the spinal dorsal horn. Morphine was applied subcutaneously and caused an analgesic effect in rats by increasing painful threshold in paw pressure vocalization test. The intrathecal administration of CXCL12 blocked such morphine-induced analgesic effect, while intrathecal IT1t prolonged the period of such analgesic effect. Moreover, the intrathecal treatment of PP2 blocked the effect by intrathecal CXCL12 on morphine-induced analgesia, and such findings were consistent with their immunohistological evidence at the spinal dorsal horn. Their work implicates that endogenous CXCL12/CXCR4 axis may be a therapeutic target to treat opioid tolerance (Parsadaniantz et al., 2015).

CXCL12/CXCR4 axis and OIH

It has been a long-term concern in anesthesiology that OIH limits the clinical efficacy of opioid therapy in both clinical and laboratory research (Angst and Clark, 2006; Chu et al., 2008). Recently, evidence from an *in vivo* study directly proves that CXCL12/CXCR4 axis was essential to the pathology of OIH (Wilson et al., 2011). In this study, it was found that repeated intraperitoneal injection of morphine induced tactile hyperalgesia, which was attenuated by intraperitoneal administration of AMD3100 in rats. Moreover, repeated intraperitoneal injection of morphine caused an increase in CXCL12 expression and a decrease in CXCR4 expression in DRG *in vivo*. Interestingly, intraperitoneal administration of AMD3100 reversed the decrease in CXCR4 expression in DRG in these morphine-treated rats.

Prospect

Neural plasticity-targeted pain management does not attain the expected purpose for some side effect (like addiction and tolerance) (Dworkin et al., 2003). Given the contribution of chemokine signaling to neuromodulation, chemokine axis-targeted therapy is taken as a novel method for pathological pain management (Gao and Ji, 2010; Ji et al., 2014). Currently, CXCL12/CXCR4 axis has been proven to play an essential role in the pathogenesis of neuropathic pain, cancer pain, and opioid tolerance and hyperalgesia, as shown in Figure 3 for its cellular and molecular mechanisms in pain processing in the PNS and/or CNS under these pathological conditions. Future work will explore the peripheral and central roles of CXCL12/CXCR4 axis in pain processing under pathological conditions, in which the function of this chemokine axis remains still unknown. Output from these studies will help to evaluate whether CXCL12/CXCR4 axis could be an effective and board-spectrum target for chronic pain therapy. The interaction between chemokine and opioid system has been considered an emerging target to improve the efficacy of opioid therapy (Parsadaniantz et al., 2015). As CXCL12/CXCR4 axis is central for the pathology of opioid tolerance (Rivat et al., 2014) and OIH (Wilson et al., 2011), the blockade of CXCL12/CXCR4 axis could be an adjunctive strategy for opioid therapy. Therefore, preclinical and/or clinical studies on this topic are necessary to evaluate such possibility. Numerous drugs have been designed for blocking chemokine signaling; however, the efficacy of these chemokine receptor antagonists is poor in preclinical and clinical studies (Pease and Horuk, 2009a,b). Therefore, the improvement of efficacy of CXCL12/CXCR4 axis-targeted therapy should be the major concern for its future application. Even though some reasons for the poor response have been proposed, like pharmacokinetic properties, species selectivity, and drug metabolism (Gao and Ji, 2010), an effective solution to this problem has not been reported. In our previous study, it was found that the antiallodynia effects of AMD3100 by injecting centrally lasted for 3–4 days (Luo et al., 2014), but such effects by injecting peripherally only lasted for hours (Bhangoo et al., 2007b, 2009; Dubovy et al., 2010; Wilson et al., 2011). Therefore, we hypothesize that the efficacy of CXCL12/CXCR4 axis-related therapy may depend on how it is delivered, and more evidence is needed to prove this.

Conclusion

A growing body of preclinical evidence suggests a wide involvement of CXCL12/CXCR4 axis in pathological pain. This chemokine axis modulates peripheral sensitization through neuronal and glial mechanisms. The CXCL12/CXCR4 axis also contributes to central sensitization by

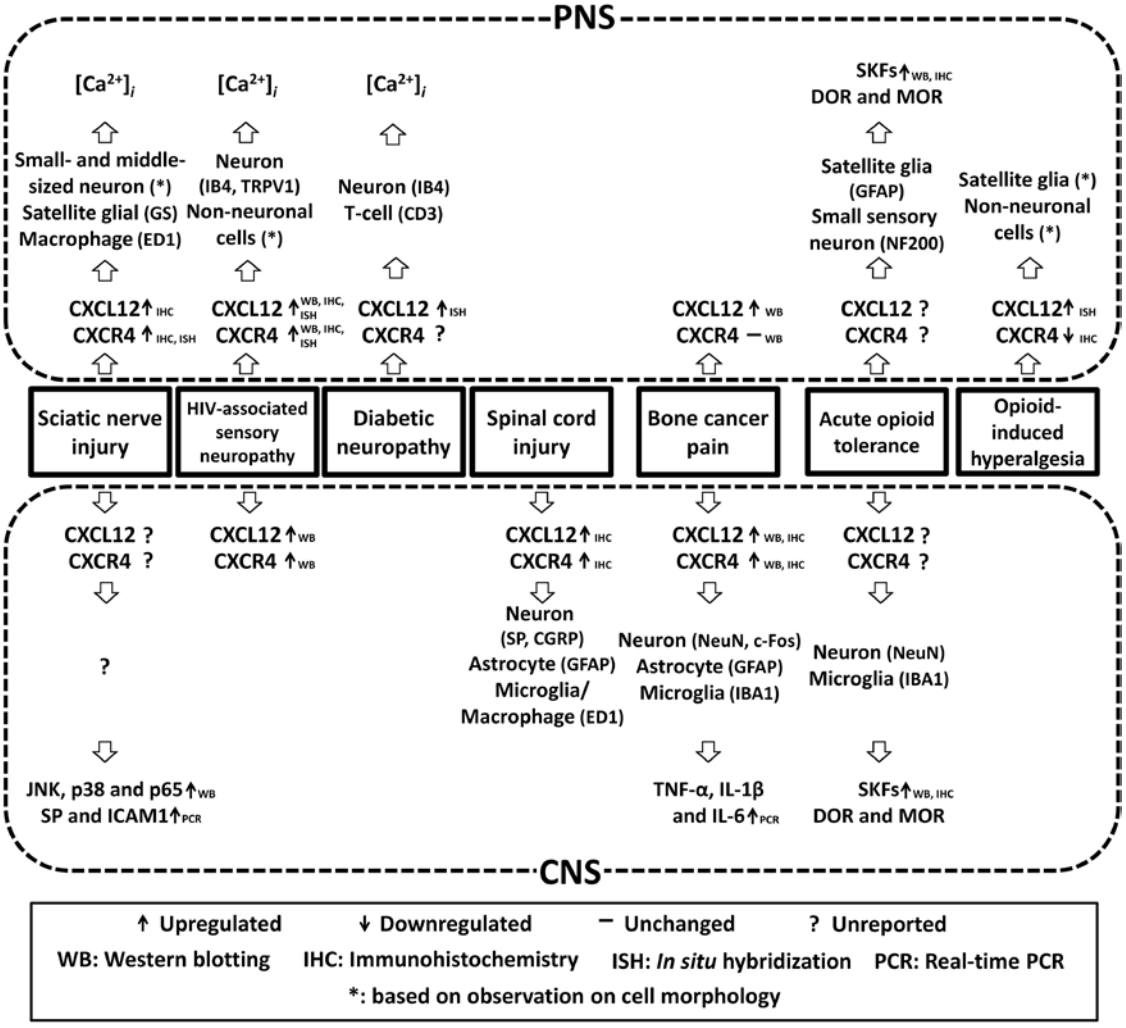

Figure 3: Cellular and molecular mechanisms for the role of CXCL12/CXCR4 axis in the processing of neuropathic pain, cancer pain, and opioid tolerance and hyperalgesia.
Cell-type biomarkers: GS, glutamine synthetase; ED1, also called cluster of differentiation (CD) 68; IB4, isolectin B4; TRPV1, transient receptor potential cation channel subfamily V member 1; CD3, cluster of differentiation 3; GFAP, glial fibrillary acidic protein; NF200, neurofilament-200; SP, substance P; CGRP, calcitonin gene-related peptide; NeuN, neuronal nuclei; IBA1, ionized calcium-binding adapter molecule 1. Pain molecules and pathways: JNK, c-Jun N-terminal kinases; ICAM-1, intracellular adhesive molecule-1; TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β; SFKs, Src family-kinases; MOR, μ-opioid receptors; DOR, δ-opioid receptor.

mechanisms such as neuroinflammation and neuronal-glial interaction. Thus, all these findings inspire coming studies to integrate CXCL12/CXCR4 axis into our current pain knowledge. The potential of CXCL12/CXCR4-targeted therapy in future management of pathological pain requests further extensive exploration.

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