Src-family protein tyrosine kinases: A promising target for treating chronic pain

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Abstract

 Despite the growing knowledge of the mechanisms of chronic pain, the treatment of this disorder in the clinic remains a major challenge. Src-family protein tyrosine kinases (SFKs), a group of non-receptor protein tyrosine kinases, have been implicated in neuronal development and synaptic plasticity. SFKs are critical for the regulation of N-methyl-D-aspartic acid receptor (NMDAR) 2B subunit phosphorylation by various transmembrane receptors, e.g., G-protein coupled receptors (GPCRs), Eph receptors (EphBRs), increased intracellular calcium, epidermal growth factor (EGF) and other growth factors, and thus contribute to the development of chronic pain. SFKs have also been regarded as important points of convergence of intracellular signalling components for the regulation of microglial functions and the immune response. Additionally, the intrathecal administration of SFK inhibitors significantly alleviates mechanical allodynia in different chronic pain models. Here, we reviewed the current evidence for the role of SFKs in the development of chronic pain caused by complete Freund’s adjuvant (CFA) injection, peripheral nerve injury (PNI), streptozotocin (STZ) injection and bone metastasis. Moreover, the role of SFKs in the development of morphine tolerance is also discussed. The regulation of SFKs therefore has emerged as a potential therapeutic target for the treatment of chronic pain in terms of safety and efficacy.

1. Introduction

Chronic pain is a major public health issue, and is generally categorized as cancer pain or chronic non-cancer pain, including inflammatory pain, neuropathic pain and idiopathic/dysfunctional pain [1]. The current clinical therapeutic strategies for chronic pain are ineffective because of their limited effects or their various unwanted side effects [2,3]. Unfortunately, despite decades of efforts, few effective analgesic treatments have been developed, and treating chronic pain remains a major clinical challenge [4]. Therefore, it is crucial to provide a better understanding of the cellular and molecular mechanisms of chronic pain.

Src-family protein tyrosine kinases (SFKs), a group of non-receptor protein tyrosine kinases, have been implicated in neuronal development and synaptic plasticity [5,6]. There are at least nine members of this family, namely: Src, Lck, Hck, Blk, Fyn, Lyn, Fgr, Yes and Yrk, which share a modular structure comprising unique Src homology 2 (SH2), Src homology3 (SH3) and kinase catalytic domains [7,8]. It has been reported that at least five SFK members, Src, Fyn, Lck, Yes and Lyn are ubiquitously expressed in the central nervous system (CNS) [6,9–11]. However, Blk, Hck and Fgr are only expressed in specific tissues (Table 1) [7,12]. The activation of SFKs is strictly regulated by...
the phosphorylation and dephosphorylation of tyrosine residues [6,13].

Recently, a number of studies have demonstrated that aberrant SFK activity may be a key element in the development of chronic pain. Here, we reviewed the current evidence for the role of SFKs in the development of chronic pain caused by complete Freund’s adjuvant (CFA) injection, peripheral nerve injury (PNI), streptozotocin (STZ) injection and morphine tolerance is also discussed.

2. Possible mechanisms of SFKs in pain processing

Convincing evidence has shown that the activation of spinal N-methyl-D-aspartic acid receptors (NMDARs) is implicated in neuronal sensitization in chronic pain [14,15]. An array of transmembrane receptors (e.g., G-protein coupled receptors (GPCRs), EphB receptors (EphBRs), increase intracellular calcium and the epidermal growth factor (EGF)) which may cause robust SFKs activation within the NMDAR in the spinal cord, leading to pain hypersensitivity [7,16]. Of the five SFK members expressed in the CNS, Src and Fyn are known to catalyse NMDARs by phosphorylating GluN2B at Tyr1472 [17,18]. The intrathecal application of broad-spectrum SFK inhibitors potently prevents phosphorylation-mediated enhancement of the NMDAR 2B subunit as well as chronic pain [14,19].

SFKs are also considered key points of convergence of various intracellular signalling components for the regulation of the immune response and microglial functions (Fig. 1) [6,20,21]. Microglia are involved in both the innate and adaptive immune responses in the CNS [22]. In addition, our previous studies have demonstrated that microglia are activated in the spinal cord in cancer pain [23,24]. Many lines of studies have indicated that peri-scientifically administered interleukin (IL)-1β or tumor necrosis factor (TNF-α) may be up stream of SFKs activation under both physiological and pathological conditions [21,25]. SFKs are considered to be crucial activators of toll-like receptors (TLRs), and thus play an important role in regulating the activation of nuclear factor (NF)-κB and overexpression of pro-inflammatory cytokines (e.g., IL-1β, TNF-α, IL-6) [5,26]. Positive feedback-mediated by an autocrine mechanism contributes to the development of chronic pain [25]. In addition, the intrathecal administration of SFK inhibitors may reduce the expression of these cytokines. Mitogen-activated protein kinases (MAPKs), which are mainly activated in the microglia, have been shown to be associated with the pathogenesis of chronic pain [20]. SFKs are vital intermediates for various signalling pathways and lead to the activation of MAPKs and the downstream extracellular signal-related kinase (ERK) to mediate pathological pain status [27–29]. As demonstrated by Li et al., the peri-sciatic administration of recombinant TNF-α into the sciatic nerve of adult rats may trigger positive feedback in the spinal cord, and ultimately induce the overexpression of cytokines after SFK/MAPK activation [27]. These cytokines may control the direction of plastic changes at C-fibre synapses, contributing to peripheral sensitization in the spinal cord [30]. Moreover, pretreatment with the SFK inhibitor PP2 reversed MAPK activation in spinal microglia as well as mechanical allodynia induced by recombinant TNF-α injection.

3. The role of SFKs in chronic pain and morphine tolerance

3.1. SFKs and inflammatory pain

Inflammation serves as a defensive barrier during innate immune responses, triggered by physical injury or infection with bacteria, viruses, or fungi [26]. Inflammatory pain is associated with maladaptive plastic changes and the activation of immune cells in the peripheral or central nociceptive networks [31]. Transcription factors, such as NFκB, and a variety of inflammation genes, inducing inducible nitric oxide synthase (iNOS), TNF-α and cyclooxygenase (COX)-2, are involved in the inflammatory process [32]. As shown by Igwe et al., treatment with wt-NF-κB double stranded oligodeoxynucleotides suppresses c-Src and CFA-induced COX-2 expression in dorsal root ganglia (DRG) neurons.
suggesting that Src activation is involved in NF-κB activation [33]. A very recent study found that phosphorylation levels of Src is an upstream regulatory molecule of NF-κB. Moreover, Momordica cochinchinensis Spreng, also known as gac or red melon, can reduce the production of NF-κB, iNOS and COX-2 in LPS-activated RAW264.7 cells by directly inhibiting Src/Syk activation [26]. Hence, the c-Src/NF-κB interaction may represent an alternative therapeutic target for the treatment of inflammatory pain.

Previous studies have shown that the phosphorylation of the NMDAR 2B subunit by Src or Fyn served as a key step in enhancing NMDAR 2B subunit function in the spinal cord after the intradermal injection of CFA [16,34]. Various transmembrane receptors such as GPCR/protein kinase A (PKA), GPCR/protein kinase C (PKC), and EphBRs are involved in this process [16,35,36]. Furthermore, the intrathecal administration of the Src inhibitor PP2 delays the onset of CFA-induced mechanical allodynia [35]. In addition to spinal mechanisms, supraspinal mechanisms appear to be involved in the role of Src in pain transmission. As reported by Xu et al., CFA-treatment enhances the spontaneous firing of arcuate nucleus (ARC) neurons, which is suppressed by the NMDAR antagonist Ro25-6981 and the Src inhibitor PP2 [14]. This finding suggests that ARC Src/GluN2BR activation may contribute to inflammatory pain.

3.2. SFKs and neuropathic pain

Neuropathic pain refers to the aberrant functioning of a pathologically altered in the CNS [6]. The hallmarks of neuropathic pain are enhanced sensitivity to noxious stimuli (hyperalgesia) and abnormal pain responses to innocuous stimuli (tactile allodynia) [37,38]. Despite increasing knowledge of the mechanisms of chronic pain, the treatment of neuropathic pain remains a major challenge in clinical practice [1]. Various animal models, such as models of PNI-, diabetes-, spinal cord injury (SCI)- and chemotherapy-induced pain, have been established to study the mechanisms of neuropathic pain of different aetiologies [1,38]. Recently, a growing body of evidence has indicated that SFKs play a critical role in neuropathic pain caused by PNI and diabetes (Fig. 2) [38,39].

3.2.1. SFKs and peripheral nerve injury

PNI causes aberrant excitability in the CNS, notably in the primary sensory ganglia and the spinal cord [38]. This pathological alteration of nociceptive transmission requires interactions between microglia and other cell types [40]. After PNI, resting microglia are activated through a series of molecular changes. Tsuda et al. indicated that stimulation of the IFN-γ receptor (IFN-γR) in naïve rats activates spinal microglia and produces long-lasting pain hypersensitivity. Conversely, ablating IFN-γR significantly impairs nerve injury-evoked microglial activation and mechanical allodynia. They also found that IFN-γ-stimulated spinal microglia can upregulate Lyn and the P2X4 receptors (P2X4Rs) [38]. Furthermore, Lyn-deficient mice also show suppressed microglial activation in the spinal cord, indicating that Lyn is involved in the molecular changes that underlie microglial activation [6]. Activated spinal microglia also released various pro-inflammatory cytokines, chemokines and neurotrophic factors that regulate pain transmission [41].
3.2.2. SFKs and diabetic neuropathy

Neuropathy, one of the most common complications of diabetes, remains an unsolved clinical problem [47]. It is often resistant to current analgesics because of the cellular and molecular mechanisms of diabetic neuropathy are largely unknown. Previous studies have demonstrated that increased NMDAR activity significantly contributes to central sensitization in diabetic neuropathy [47,48]. Protein tyrosine phosphatase 1B (PTP1B), a ubiquitous enzyme, has been shown to stimulate Src and enhance the tyrosine phosphorylation of NMDAR in the spinal cord, which contributes to the development of diabetic neuropathy [39]. Moreover, the siRNA-mediated knockdown of PTP1B or a PTP inhibitor represses Src activity and reverses mechanical allodynia in STZ-injected rats. These findings demonstrate that Src/GluN2B signalling represents a vital pathway through which PTP1B exaggerates painful responses. The present studies also confirm the activation of spinal microglia in STZ-injected rats, not only through alterations in morphology but also through the activation of intracellular signalling involved in microglia functions. As shown by Tsuda et al., the SFK/ERK signalling pathway is implicated in the process of microglial activation caused by STZ injection [28]. Moreover, the intrathecal administration of U0126, an inhibitor of ERK activation, remarkably alleviates tactile allodynia in diabetic rats.

3.3. SFKs and cancer pain

Treating cancer pain remains a clinical challenge, and current analgesics may be inadequate, therefore, there is a great need for new treatment strategies [49]. The mechanism of cancer pain may consist of components of both neuropathic and inflammatory pain but also involve distinctive characteristics [50]. In this review, we focus on the role of SFKs in pain caused by bone metastasis. There are several factors that contribute to the development of bone cancer pain, including damage to the surrounding nerves and tissue, release of inflammatory mediators, injury to sensory nerve fiber terminals and increased bone degradation [19]. Src, a non-receptor protein tyrosine kinase, is involved in several processes that lead to bone cancer pain, such as cancer growth, angiogenesis and metastasis [19]. Src is widely expressed in osteoclasts, platelets, and neurons [51]. Regarding pain pathologies, it has been widely demonstrated that the activation of Src contributes to bone cancer pain through the phosphorylation of the NMDARs. As shown by Liu et al., the spinal administration of recombinant IL-18 to naive rats can induce pain hypersensitivity, and the activation of GluN2B [4]. Furthermore, the Src inhibitor PP1 remarkably inhibits IL-18-induced GluN2B. Moreover, Src is also a key regulator of bone resorption. Mice lacking the Src gene develop osteopetrosis, mainly due to impaired osteoclastic function. It has been reported that a Src inhibitor can reduce pain hypersensitivity in bone cancer pain rats, and that this effect is associated with both a reduction of NMDAR activity and the inhibition of bone resorption [51].

3.4. SFKs and morphine tolerance

Tolerance of the effects of opioid analgesics is a major clinical issue in chronic pain treatment due to the poor understanding of its core mechanisms [52]. A recent study demonstrated that morphological changes in opioid receptors, and the activation of NMDARs are implicated in the development of opioid tolerance [53]. The µ-opioid receptor (MOR) mediates both the beneficial and adverse effects of opioids [54,55]. It is worth noting that β-Arrestin2, a protein that recruits c-Src to MORs, is critical for the development of morphine tolerance [56]. Additionally, β-arrestin 2+/− mice exhibit upregulate MOR-mediated basal nociception and reverse morphine tolerance. Meanwhile, the inhibition of c-Src in DRG β-arrestin 2+/− neurons increases the expression of the MOR and abolishes opioid-induced desensitization in vitro [57]. Therefore, c-Src, which is recruited by β-arrestin2, is required for the development of morphine tolerance. It is widely known
4. Conclusions

In this review, we summarized the cellular and molecular mechanisms of the role of SFKs in the initiation and development of chronic pain and morphine tolerance (Figs. 3). SFKs are critical for various cellular signalling pathways that promote pain hypersensitivity, suggesting that aberrant SFK activity may be a potential therapeutic target for the management of chronic pain [36,56,63]. Furthermore, increased SFK activity is also associated with the processes of bone resorption, tumor growth, and metastasis in vitro and in vivo [19]. Recent advances in our understanding of the role of SFKs in preclinical studies have laid a foundation for the clinical application of SFK inhibitors (such as dasatinib and saracatinib) for the treatment of tumorigenesis, bone metastasis and chronic pain [19,64]. Emerging clinical data suggest that SFK inhibitors have the potential to inhibit cancer-related bone resorption and metastasis [65,66]. However, few clinical trials have studied SFK inhibitors for the treatment of chronic pain. Although the clinical value of SFK inhibitors for the treatment of chronic pain has not yet been clearly determined, preclinical studies of SFKs will ultimately provide the proper groundwork for drug development and clinical trials for pain therapies. Therefore, future extensive exploratory studies and clinical trials should be performed with more selective and clinically relevant drugs targeting SFKs.

Author contributions

Hui Yang, Da-Wei Ye, Yu-Ke Tian, and Ya-Qun Zhou designed the research study.

Meng-Meng Ge prepared the manuscript.

Meng-Meng Ge, and Ya-Qun Zhou drew the figures in the manuscript.

Hui Yang, Ya-Qun Zhou, and Xue-Bi Tian revised the content of the manuscript.

Hui Yang, Yu-Ke Tian, and Xue-Bi Tian provided the fund acquisition.

Declaration of Competing Interests

All authors have no competing interests.

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