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

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Abstract

Despite growing knowledge of the mechanisms of chronic pain, it remains a major challenge facing clinical practice. 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 critically central to various transmembrane receptors e.g. G-protein coupled receptor (GPCR), EphB receptor (EphBR), increased intracellular calcium, epidermal growth factor (EGF) and other growth factors that regulate the phosphorylation of N-methyl-D-aspartic acid receptor (NMDAR) 2B subunit, thus contributing to the development of chronic pain. SFKs have also been regarded as an important point of convergence of intracellular signaling components that regulate microglia functions and the immune response. Additionally, intrathecal administration of SFKs inhibitors significantly alleviates mechanical allodynia in different chronic pain models. Thus, here we reviewed the current evidence of 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 on the development of morphine tolerance has also been discussed. Management of SFKs therefore emerged as a potential therapeutic target for the treatment of chronic pain in terms of safety and efficacy.

Key words

Chronic pain; Src-family protein tyrosine kinases; N-methyl-D-aspartic acid receptor; Microglia.

Abbreviations

AC1: adenylyl cyclase subtype 1; ARC: arcuate nucleus; BDNF: brain derived neurotrophic factor; CCI: chronic constrictive injury; CFA: complete Freund's adjuvant; CML: chronic myeloid leukemia; CNS: central nervous system; COX-2: cyclooxygenase-2; CPN: common peroneal nerve; CXCL12: chemokine CXC motif ligand 12; CXCR4: cognate G protein-coupled receptor; DRG: dorsal root ganglia; EphBR: EphB receptor; EGF: epidermal growth factor; ERK: extracellular signal-related kinase; GDNF: glial cell line-derived neurotrophic factor; GPCR: G-protein coupled receptor; IFN-γ: interferon-γ; IFN-γR: IFN-γ receptor; IL-1β: interleukin-1β; iNOS: inducible nitric oxide synthase; LTP: long-term potentiation; MAPK: mitogen-activated protein kinases; MOR: μ-Opioid receptor; NF-κB: nuclear factor-κB; NMDAR: N-methyl-D-aspartic acid receptor; PKA: protein kinase A; PKC: protein kinase C; PNI: peripheral nerve injury; PTP1B: protein tyrosine phosphatase 1B; P2X4R: P2X4 receptor; P2X7Rs: P2X7 receptors; SCI: spinal cord injury; SFKs: Src-family protein tyrosine kinases; SH2: Src homology 2; SH3: Src homology 3; STZ: streptozotocin; TLR: toll-like receptor; TNF–α: tumor necrosis factor–α.

1. Introduction

Chronic pain is a major public health issue, which is generally categorized into cancer pain and chronic non-cancer pain including inflammatory pain, neuropathic pain, and idiopathic/dysfunctional pain [1]. The current clinical therapeutics for chronic pain are ineffective because of limited eff ects or due to several 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 in this family: 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 SFKs members: Src, Fyn, Lck, Yes and Lyn are ubiquitously expressed in the central nervous system (CNS) [6, 9-11]. While, Blk, Hck and Fgr are only expressed in specific tissues [7, 12] (Table 1). The activation of SFKs is strictly regulated by the phosphorylation and dephosphorylation of tyrosine residues [6, 13]. Recently, a number of studies demonstrated that aberrant SFKs activity may be a key element in the development of chronic pain. Thus, here we reviewed the current evidence of 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 on the development of morphine tolerance has also been discussed.

2. Possible mechanisms of SFKs in pain processing

Convincing evidence has shown that activation of the spinal N-methyl-D-aspartic acid receptor (NMDAR) is implicated in neuronal sensitization in chronic pain [14, 15]. An array of transmembrane receptors (e.g. G-protein coupled receptor (GPCR), EphB receptor (EphBR), increase intracellular calcium and the epidermal growth factor (EGF)) which might cause robust SFKs activation within the NMDAR in the spinal cord that leads to pain hypersensitivity [7, 16]. Of five SFKs members expressed in the

CNS, Src and Fyn are known to catalyze NMDARs through phosphorylating GluN2B at Tyr1472 [17, 18]. Intrathecal application of broad-spectrum SFKs 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 signaling components that regulate the immune response and microglial functions [6, 20, 21] (Figure. 1). 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-sciatic administration of interleukin (IL)-1β or tumor necrosis factor (TNF)-α could be up-stream signals of SFKs activation in both physiological and pathological conditions [21, 25]. SFKs are considered crucial activators of toll-like receptors (TLR), that play an important role in regulating the activation of nuclear factor (NF)-κB and over-expression of pro-inflammatory cytokine (e.g. IL-1β, TNF-α, IL-6) [5, 26]. The positive feedback mediated by an autocrine mechanism contributes to the development of chronic pain [25]. In addition, intrathecal administration of SFKs inhibitors may reduce the expression of these cytokines. Mitogen-activated protein kinases (MAPKs), activated mainly in the microglia, have been shown to be associated with the pathogenesis of chronic pain [20]. SFKs are vital intermediates for various signaling pathways leading to MAPKs and the downstream molecular extracellular signal-related kinase (ERK) activation that mediate pathological pain status [27-29]. As Li et al. demonstrated, peri-sciatic administration of recombinant TNF-α into adult an rat's sciatic nerve may trigger a positive feedback in the spinal cord, and ultimately induce the over-expression of cytokines after SFKs/MAPK activation [27]. These cytokines may control the direction of plastic changes at C-fiber synapses, contributing to

peripheral sensitization in the spinal cord [30]. Moreover, pretreatment with SFKs inhibitor PP2 reversed MAPK activation in the spinal microglia while mechanical allodynia was induced by the 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 in the innate immune response, triggered by physical injury or infection caused by bacteria, viruses, and fungi [26]. Inflammatory pain is associated with maladaptive plastic changes and activation of immune cells in the peripheral or central nociceptive networks [31]. Transcription factors, such as NF-κB and a variety of inflammation genes, including inducible nitric oxide synthase (iNOS), TNF-α and cyclooxygenase (COX)-2 are involved in the inflammatory process [32]. As Igwe et al. showed, treatment with wt-NF-κB double stranded oligodeoxynucleotides, suppressed c-Src and CFA-induced COX-2 expression in the dorsal root ganglia (DRG) neurons, suggesting that Src activation is involved in NF-κB activation [33]. A very recent study also found phosphorylation levels of Src in upstream regulatory molecules 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 strategy for the treatment of inflammatory pain.

Previous studies have shown that phosphorylation of NMDAR 2B subunit by Src or Fyn served as a key step to enhance NMDAR 2B subunit function in the spinal cord after intradermal injection of CFA [16, 34]. Various transmembrane receptors such as GPCR/protein kinase A (PKA), GPCR/protein kinase C (PKC), EphBR are involved in

the mechanisms [16, 35, 36]. Furthermore, intrathecal administration of 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 Xu et al. reported, CFA-treatment enhanced spontaneous firings of arcuate nucleus (ARC) neurons, which were suppressed by NMDAR antagonist Ro25-6981 and Src inhibitor PP2 [14]. This finding suggests that the 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 CNS [6]. One hallmark is the enhanced sensitivity to noxious stimuli (hyperalgesia) as well as abnormal pain behavior to innocuous stimuli (tactile allodynia) [37, 38]. Despite increasing knowledge of the mechanisms of chronic pain, neuropathic pain remains a major challenge in clinical practice [1]. Various animal models such as PNI, diabetes, spinal cord injury (SCI) and chemotherapy-induced pain have been established to study the mechanism of neuropathic pain of different etiologies [1, 38]. Recently, a growing body of evidence indicated that SFKs plays a critical role in neuropathic pain caused by PNI and diabetes [18, 39] (Figure. 2).

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 pathologically altered nociceptive transmission requires the interaction between microglia and other cell types [40]. After PNI, resting microglia are transformed into the activated state through a series of molecular changes. Tsuda et al. indicated that stimulation of the IFN-γ receptor (IFN-γR) in a naïve rat coverts spinal microglia into activated cells and produces long-lasting pain hypersensitivity.

Conversely, ablating IFN-γR significantly impairs nerve injury-evoked microglia activation and mechanical allodynia. They also found that IFN-γ-stimulated spinal microglia which could upregulate Lyn and the P2X4 receptor (P2X4R) [38]. Furthermore, Lyn deficient mice also showed suppressed microglia activation in the spinal cord, indicating that Lyn is involved in the molecular changes underlying microglia activation [6]. Activated spinal microglia also released various proinflammatory cytokines, chemokines and neurotrophic factors that regulate pain transmission [41]. Brain-derived neurotrophic factor (BDNF), a classical neurotrophic factor released by spinal microglia, is a major driver which disrupts the balance between synaptic excitation and inhibition by mediating phosphorylation of the GluN2B-NMDAR via Fyn after PNI [42, 43]. Moreover, In a vitro experiment, treatment with BDNF increased the NMDAR currents in cultured DRG neurons, and this effect was reversed by a BDNF scavenger, a TrkB receptor antagonist and SFKs inhibitors (PP2 or SU6656) [44]. Despite abundant evidence demonstrating the crucial role of NMDAR activation in the spinal cord in the development of neuropathic pain, the supraspinal mechanisms linked to the activation of NMDAR is little known. It is suggested that activation of SFKs/NMDAR in the insular cortex may have a potential role in the regulation of pain transmission [15]. In this study, they established that the insular cortex changes in synaptic plasticity, and is associated with the increase in long-term potentiation (LTP) in the amount of NMDAR after ligation of the common peroneal nerve (CPN). Moreover, activation of adenylyl cyclase subtype 1 (AC1)/PKA/Src was reported to be involved in the modulation of synaptic NMDAR in the insular cortex.

Glial cell line-derived neurotrophic factor (GDNF), a member of the transforming growth factor-β superfamily, has been shown to be involved in various biological processes, such as development, survival, and maintenance of neurons in the CNS [45].

Treatment with GDNF can significantly relieve chronic constrictive injury (CCI)-induced mechanical allodynia, and the involved mechanism may be to directly or indirectly regulate nectin-1/c-src signaling [46]. These findings might provide new targets for the treatment of neuropathic pain.

3.2.2 SFKs and Diabetic Neuropathy

Neuropathy, one of the most common complications of diabetes, remains an unmet clinical problem [47]. It is often resistant to current analgesics due to largely unknown cellular and molecular mechanisms in diabetic neuropathy. 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 PTP inhibitor repressed Src activity and reversed mechanical allodynia in STZ-injected rats. These findings demonstrate that Src/GluN2B signaling represents a vital pathway for PTP1B to exaggerate painful responses. The present studies also confirmed activation of spinal microglia in STZ-injected rats, not only by the alterations in morphology, but also by activation of intracellular signaling that is involved in microglia functions. As Tsuda et al. showed, SFK/ERK signaling pathway is implicated in the process of microglia activation caused by STZ injection [28]. Moreover, intrathecal administration of U0126, an inhibitor of ERK activation, produced a striking alleviation of tactile allodynia in diabetic rats.

3.3 SFKs and Cancer Pain

Treating cancer pain remains a clinical challenge, and the current analgesics may be inadequate, therefore, there is a great need for new treatment strategies [49]. The mechanism of cancer pain may contain components of both neuropathic and inflammatory pain but also has its own distinctive characteristics [50]. In this review, we focus on the role of SFKs in pain caused by bone metastasis. There are several factors including damage to the surrounding nerves and tissue, release of inflammatory mediators, injury to sensory nerve fiber terminals and increase in bone degradation which contribute to the development of bone cancer pain [19]. Src, a non-receptor protein tyrosine kinase, is involved in several processes such as cancer growth, angiogenesis and metastasis, leading to bone cancer pain [19]. Src is widely expressed in osteoclasts, platelets, and neurons [51]. In regards to pain pathologies, it has been widely demonstrated that activation of Src contributes to bone cancer pain through phosphorylation of the NMDAR. As Liu et al. showed, spinal administration of recombinant IL-18 in naïve rats could induce pain hypersensitivity, as well as activation of GluN2B [4]. Furthermore, Src inhibitor PP1 remarkably inhibited 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 the Src inhibitor could reduce pain hypersensitivity in bone cancer pain rats, and that it is associated with both reducing NMDAR activity and inhibiting bone resorption [51].

3.4 SFKs and Morphine Tolerance

Tolerance to the opioids analgesic effects is a major clinical issue in chronic pain treatment due to poor recognition of its core mechanisms [52]. In the present studies, it has been demonstrated that morphological changes of opioid receptors, activation of NMDAR were implicated in the development of opioids tolerance [53]. μ-Opioid receptor (MOR) mediates both the beneficial and the adverse effects of opioids [54, 55]. It is worth noting that β-Arrestin2, a protein that recruits c-Src to the MOR, is critical for the development of morphine tolerance [56]. Additionally, β-arr $2^{-/-}$ mice can up-regulate MOR-mediated basal nociception and reverse morphine tolerance. Meanwhile, inhibition of c-Src in DRG β-arr $2^{+/+}$ neurons increases the expression of MOR and abolishes opioid-induced desensitization in vitro [57]. Therefore, c-Src, recruited by β-arrestin2, is required for the development of morphine tolerance. It is largely known that NMDARs have a well-developed role in neural plasticity and various pain states [18, 51]. Multiple groups also demonstrated the functional cross-regulation between MOR and NMDARs. Opioids, such as morphine, can disrupt the interaction between MOR and NMDARs and activate NMDARs via PKC/Src, which contribute to the development of morphine tolerance [58].

Activated microglia have also been involved in the regulation of morphine tolerance. Furthermore, injection of minocycline (selective microglia inhibitor) has been reported to reverse the development of morphine tolerance [59]. Although the mechanisms by which microglia regulates morphine tolerance are largely unknown, the activation of P2X7 receptors (P2X7Rs) and MAPK have been specifically involved in the initiation and development of morphine tolerance [53]. In addition, Leduc-Pessah et al. found that morphine potentiates P2X7Rs-mediated Ca^{2+} responses in spinal microglia [52]. Moreover, the increased P2X7Rs function was reversed by the SFKs inhibitor PP2 in

cultured microglia. They identify that Src is a potential intracellular mediator of P2X7Rs and its activation by morphine likely participated in the development of morphine tolerance. Chemokines, a superfamily of small proteins, plays a crucial role in immune and neuro-modulation functions [60, 61]. Recently, several studies reported that activation of cognate G protein-coupled receptor (CXCR4) by exogenous chemokine CXC motif ligand 12 (CXCL12) could activate the SFKs signaling pathway in sensory neurons DRG and microglia in the spinal cord [53, 62]. Thus, the microglia SFKs activation by the administration of CXCL12, is also consistent with the regulatory role of microglia in morphine tolerance.

4. Conclusions

In this review, we summarized the cellular and molecular mechanisms of SFKs in the initiation and development of chronic pain and morphine tolerance (Figures. 3). SFKs are critically central to various cellular signaling that promote pain hypersensitivity, suggesting that aberrant SFKs activity may be a potential therapeutic tool for the management of chronic pain [36, 56, 63]. Furthermore, increased SFKs activity is also associated with the process of bone resorption, tumor growth, and metastasis in the vitro and vivo studies [19]. Recent advances in understanding the role of SFKs in preclinical studies have laid a foundation for the clinical application of its inhibitors (such as dasatinib or saracatinib) in the treatment of tumorigenesis, bone metastasis and chronic pain [19, 64]. Emerging clinical data support that SFKs inhibitors have the potential to inhibit cancer-related bone resorption and metastasis [65, 66]. However, there are few clinical trials that target SFKs inhibitors for the treatment of chronic pain. Although the clinical value of SFKs inhibitors in chronic pain have not yet been clearly defined, these preclinical studies for SFKs will ultimately provide the proper groundwork for pain therapy in drug development and clinical trials. Therefore,

future extensive exploration and ongoing clinical trials should be performed with more selective and clinically relevant drugs targeting SFKs.

Declaration of Competing Interest

All authors have no competing interests.

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Figure legends

Figure 1. The role of SFKs in regulating microglia functions and the immune response. SFKs are considered crucial activators of TLR, playing a crucial role in regulating the activation of NF-κB and over-expression of pro-inflammatory cytokine (e.g. IL-1β, IL-6, TNF-α), which can amplify neuronal excitability and facilitate nociceptive transmission through the interaction between microglia and other cell types in the spinal cord. Various intracellular signaling cascades including SFKs/MAPK and SFKs/ERK are known to play a crucial role in mediating functions of microglia activity. BDNF: brain derived neurotrophic factor; EP-1R: prostaglandin receptor; ERK: extracellular signal-related kinase; IL-1β: interleukin-1β; IL-1R: interleukin-1 receptor; IL-6: interleukin-6; IL-18R: interleukin-18 receptor; MAPK: mitogen-activated protein kinases; NF-κB: nuclear factor-κB; PGE2: prostaglandin 2; P2X4R: P2X4 receptor; SFKs: Src-family protein tyrosine kinases; TNF-α: tumor necrosis factor-α; TNF-αR: tumor necrosis factor-α receptor; TLR: toll-like receptor.

Figure 2. Schematic representation of potential mechanisms by which SFKs are involved in neuropathic pain. a) Peri-sciatic administration of recombinant rats IFN-γ could produce long-lasting pain hypersensitivity via activation of Lyn/P2X4R. b) GDNF can directly or indirectly regulate nectin-1/c-src signaling thereby mediating the synaptic remolding process in the spinal cord. c) SFK/ERK signaling pathway is implicated in the process of microglia activation in STZ-injected rats. d) PTP1B can stimulate Src and enhance the tyrosine phosphorylation of NMDAR in the spinal cord, contributing to the development of diabetic neuropathy. BDNF: brain derived neurotrophic factor; ERK: extracellular signal-related kinase; GDNF: glial cell line-derived neurotrophic factor; IFN-γ: interferon-γ; IFN-γR: IFN-γ receptor; NMDAR: N-

methyl-D-aspartate receptor; PTP1B: Protein tyrosine phosphatase 1B; P2X4R: P2X4 receptor; SFKs: Src-family protein tyrosine kinases; STZ: streptozotocin.

Figure 3. Schematic representation of potential mechanisms by which SFKs is involved in morphine tolerance. A large number of studies have shown that several signals such as μ receptors, β-Arrestin2 and CXCR4 converged on SFKs, and their inhibition reversed morphine tolerance. a) β-Arrestin2, a protein that recruits c-Src to the μ receptor, is critical for the development of morphine tolerance through the phosphorylation of NMDAR. b) SFKs activation by μ receptors as a key mechanism contributes to morphine potentiation of P2X7R function. c) SFKs signaling pathway may be a critical downstream signal of CXCR4 under morphine tolerance situation. β-arr: beta-Arrestin2; CXCL12: chemokine CXC motif ligand 12; CXCR4: cognate G protein-coupled receptor; MOR: mu opioid receptors; NMDAR: N-methyl-D-aspartate receptor; P2X7R: P2X7 receptor; SFKs: Src-family protein tyrosine kinases.