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Review

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 regulate of N-methyl-D-aspartic acid receptor (NMDAR) 2B subunit phosphorylation by various transmembrane receptors, e.g., G-protein coupled receptors (GPCRs), EphB 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 eff ;ects 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

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Table 1
Fundamental characteristics of Src-family protein tyrosine kinases.

	Expression and distribution	Function	Upstream activation Mechanism	Downstream effectors	Reference
Src	CNS (arcuate nucleus) neurons, platelets, osteoclasts	Tumor development, progression, resistance to therapeutic agents, neuronal development, synaptic plasticity	PDGF, other growth factors, EGF, IL-1β, EphrinB2, GPCR, intracellular calcium	NMDAR, MOR	[6,7], [12], [17] [56],
Fyn	FynB: CNS; FynT: cells of hematopoietic origin	Cell growth, survival, adhesion, myelination, cytoskeletal remodeling, motility, axon guidance, synaptic function, platelet activation, TCR signaling	GPCR	NMDAR, AMPAR, Nav1.2, Nav1.5, Nav1.7, BCAR1, PKB/Akt, FAK HGF/MET	[12,13], [17,18] [34],
Yes	CNS	Neuronal development, synaptic plasticity, anti-tubulin agents	CDK1	PI3K/AKT	[9,10] [12],
Lyn	CNS, spinally derived microglia cells	Neuronal development, synaptic plasticity, LPS-initiated signaling	Antigens, CD14	P2X$_4$R, PI3K	[6] [32],
Lck	CNS, T cells, NK cells	Neuronal development, synaptic plasticity, TCR signaling, neurite outgrowth, cytokine production	PKC	TCR complex, PKC, PI3K, ITK	[6] [11,12],
Hck	hematopoietic cells	Integrin signaling, actin rearrangement, LPS-initiated signaling	Integrin signaling in polymorphonuclear leukocytes, CD14,m onocyte chemotaxis, EGF	PI3K	[7], [12] [32],
Yrk	Neuron, epithelial cells, monocyte/ macrophages,	Cell migration, differentiation	GPCR	Chemokines production	[8]

AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-subtype glutamate receptor; BCAR1: breast cancer anti-estrogen resistance 1; CNS: central nervous system; CD14: Lipopolysaccharide receptor; CDK1: cyclin-dependent kinase 1; EGF: epidermal growth factor; FAK: focal adhesion kinase; GPCR: G-protein coupled receptor; HGF/MET: hepatocyte growth factor/hepatocyte growth factor receptor; IL-1β: interleukin-1 beta; ITK:interleukin-2 tyrosine kinase; LPS: lipopolysaccharide; MOR: μ-opioid receptors; NMDAR: N-methyl-D-aspartate receptor; NK cells: natural killer cells; PDGF: Platelet derived growth factor; PI3K: Phosphoinositide 3-kinase; PKB/Akt: protein kinase B; PKC: protein kinase C; P2X$_4$R: P2X$_4$ receptor; TCR signaling: T cell receptor signaling.

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 bone metastasis. Moreover, the role of SFKs in the development of 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-sciatically 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 NF-κB, 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,
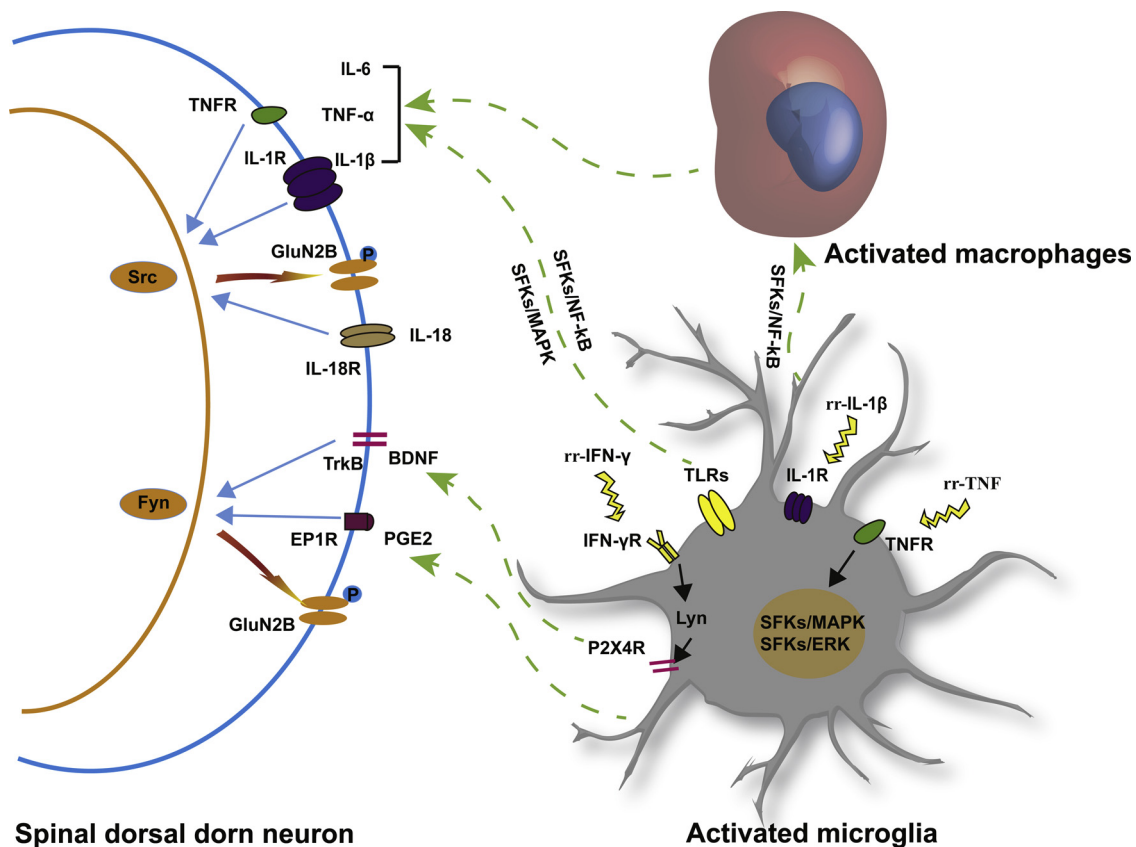
Fig. 1. The role of SFKs in regulating microglial functions and the immune response. SFKs are considered crucial activators of TLR, playing a crucial role in regulating the activation of NF-κB and the overexpression of pro-inflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α), which can amplify neuronal excitability and facilitate nociceptive transmission through interactions between microglia and other cell types in the spinal cord. Various intracellular signalling cascades, including the SFK/MAPK and SFK/ERK pathways, are known to play a crucial role in mediating the functions of microglial 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-18: interleukin-18; 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.

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) [18,39].

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].
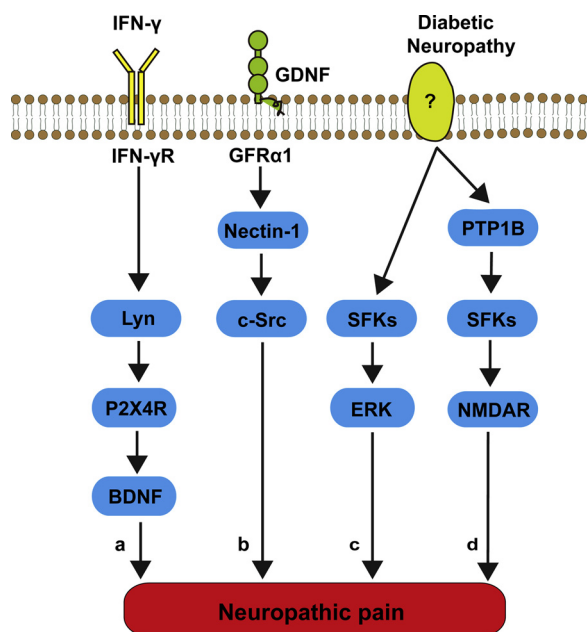
Fig. 2. Schematic representation of the potential mechanisms by which SFKs are involved in neuropathic pain. a) The peri-sciatic administration of recombinant rat IFN-γ can produce long-lasting pain hypersensitivity via activation of Lyn/P2X4R. b) GDNF can directly or indirectly regulate nectin-1/c-src signalling, thereby mediating the synaptic remodelling process in the spinal cord. c) The SFK/ERK signalling pathway is implicated in the process of microglial 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; GFRα1: glial cell line-derived neurotrophic factor receptor α 1; 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.

Brain-derived neurotrophic factor (BDNF), a classical neurotrophic factor released by spinal microglia, is a major driver that 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, it was shown that treatment with BDNF increases NMDAR currents in cultured DRG neurons and that this effect is reversed by a BDNF scavenger, a TrkB receptor antagonist and SFK inhibitors (PP2 and SU6656) [44]. Despite abundant evidence of the crucial role of NMDAR activation in the spinal cord in the development of neuropathic pain, little is known about the supraspinal mechanisms associated with the activation of NMDARs. It has been suggested that the activation of SFKs/NMDARs in the insular cortex may have a potential role in the regulation of pain transmission [15]. In this study, it was shown that the insular cortex shows changes in synaptic plasticity, that are associated with increases in long-term potentiation (LTP) in the amount of NMDARs after common peroneal nerve (CPN) ligation. Moreover, the activation of adenylyl cyclase subtype 1 (AC1)/PKA/Src was reported to be involved in the modulation of synaptic NMDARs 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 the 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 associated mechanism may be direct or indirect regulation of nectin-1/c-src signalling [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 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 naïve 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 eff;ects 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-arr $2^{-/-}$ mice exhibit upregulate MOR-mediated basal nociception and reverse morphine tolerance. Meanwhile, the inhibition of c-Src in DRG β-arr $2^{+/+}$ 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
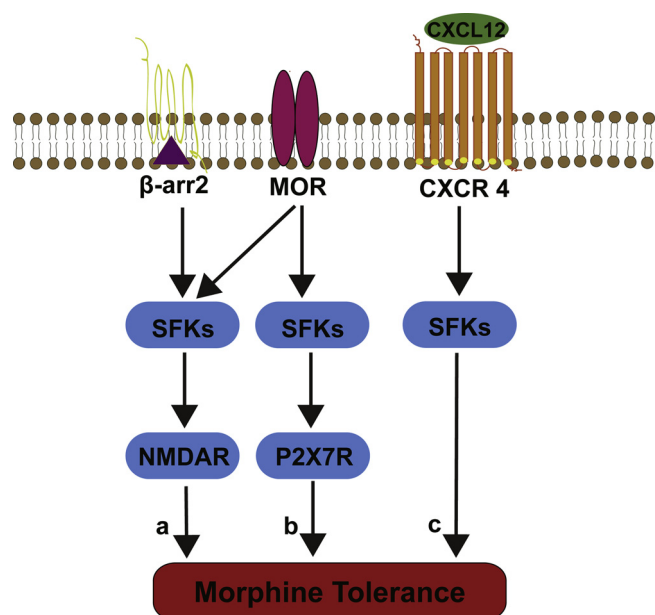
Fig. 3. Schematic representation of the potential mechanisms by which SFKs are involved in morphine tolerance. A large number of studies have shown that several signals, such as μ receptors, β-Arrestin2 and CXCR4, converge on SFKs, and their inhibition reverses morphine tolerance. a) *β*-Arrestin2, a protein that recruits c-Src to the MOR, is critical for the development of morphine tolerance through the phosphorylation of NMDAR. b) SFK activation by MOR is a key mechanism that contributes to the potentiation of P2X7R function by morphine. c) SFK signalling pathway may be a critical downstream signal of CXCR4 durring morphine tolerance. *β*-arr2: *β*-Arrestin2; CXCL12: chemokine CXC motif ligand 12; CXCR4: cognate G protein-coupled receptor 4; MOR: μ-opioid receptor; NMDAR: N-methyl-D-aspartate receptor; P2X7R: P2X7 receptor; SFKs: Src-family protein tyrosine kinases.

that NMDARs have a well-developed role in neural plasticity and various pain states [18,51]. Multiple groups have also demonstrated the functional cross-regulation between MORs and NMDARs. Opioids, such as morphine, can disrupt the interaction between MORs and NMDARs and activate NMDARs via PKC/Src, which contributes to the development of morphine tolerance [58].

Activated microglia have also been involved in the regulation of morphine tolerance. Furthermore, the injection of minocycline (a selective microglial 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 has been specifically implicated in the initiation and development of morphine tolerance [53]. In addition, Leduc-Pessah et al. found that morphine potentiates P2X7R-mediated Ca^{2+} responses in spinal microglia [52]. Moreover, increased P2X7R function is reversed by the SFKs inhibitor PP2 in cultured microglia. The researchers found that Src is a potential intracellular mediator of P2X7Rs and that its activation by morphine likely participates in the development of morphine tolerance. Chemokines, a superfamily of small proteins, play a crucial role in immune and neuromodulatory functions [60,61]. Recently, several studies reported that the activation of cognate G protein-coupled receptor (CXCR4) by exogenous chemokine CXC motif ligand 12 (CXCL12) can activate the SFK signalling pathway in sensory neurons in the DRG and microglia in the spinal cord [53,62]. Thus, the activation of SFKs induced 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 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 draw 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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References

[1] Y.Q. Zhou, Z. Liu, Z.H. Liu, S.P. Chen, M. Li, A. Shahveranov, D.W. Ye, Y.K. Tian, Interleukin-6: an emerging regulator of pathological pain, J. Neuroinflammation 13 (1) (2016) 141, https://doi.org/10.1186/s12974-016-0607-6.
[2] Y.Q. Zhou, D.Q. Liu, S.P. Chen, J. Sun, X.R. Zhou, F. Luo, Y.K. Tian, D.W. Ye, Cellular and molecular mechanisms of calcium/calmodulin-dependent protein kinase II in chronic pain, J. Pharmacol. Exp. Ther. 363 (2) (2017) 176–183, https://doi.org/10.1124/jpet.117.243048.
[3] C. Lu, Y. Liu, B. Sun, Y. Sun, B. Hou, Y. Zhang, Z. Ma, X. Gu, Intrathecal injection of JWH-015 attenuates bone cancer pain via time-dependent modification of pro-inflammatory cytokines expression and astrocytes activity in spinal cord, Inflammation 38 (5) (2015) 1880–1890, https://doi.org/10.1007/s10753-015-0168-3.
[4] S. Liu, Y.P. Liu, Y. Lv, J.L. Yao, D.M. Yue, M.Y. Zhang, D.Y. Qi, G.J. Liu, IL-18 contributes to bone cancer pain by regulating glia cells and neuron interaction, J. Pain 19 (2) (2018) 186–195, https://doi.org/10.1016/j.jpain.2017.10.003.
[5] Y. Huang, Y. Li, X. Zhong, Y. Hu, P. Liu, Y. Zhao, Z. Deng, X. Liu, S. Liu, Y. Zhong, Src-family kinases activation in spinal microglia contributes to central sensitization and chronic pain after lumbar disc herniation, Mol. Pain 13 (2017) 1744806917733637, , https://doi.org/10.1177/1744806917733637.
[6] M. Tsuda, H. Tozaki-Saitoh, T. Masuda, E. Toyomitsu, T. Tezuka, T. Yamamoto, K. Inoue, Lyn tyrosine kinase is required for P2X(4) receptor upregulation and neuropathic pain after peripheral nerve injury, Glia 56 (1) (2008) 50–58, https://doi.org/10.1002/glia.20591.
[7] D.G. Jeong, W.K. Park, S. Park, Artemin activates axonal growth via SFK and ERK-dependent signalling pathways in mature dorsal root ganglia neurons, Cell Biochem. Funct. 26 (2) (2008) 210–220, https://doi.org/10.1002/cbf.1436.
[8] M. Martins-Green, J.L. Bixby, T. Yamamoto, T. Graf, M. Sudol, Tissue specific expression of Yrk kinase: implications for differentiation and inflammation, Int. J.

Biochem. Cell Biol. 32 (3) (2000) 351–364.

[9] Z. Wang, X. Chen, M.Z. Zhong, S. Yang, J. Zhou, D.L. Klinkebiel, A.R. Karpf, Y. Chen, J. Dong, Cyclin-dependent kinase 1-mediated phosphorylation of YES links mitotic arrest and apoptosis during antitubulin chemotherapy, Cell. Signal. 52 (2018) 137–146, https://doi.org/10.1016/j.cellsig.2018.09.007.

[10] Y. Jin, M. Huang, Y. Wang, C. Yi, Y. Deng, Y. Chen, L. Jiang, J. Wang, Q. Shen, R. Liu, Q. Xi, c-Yes enhances tumor migration and invasion via PI3K/AKT pathway in epithelial ovarian cancer, Exp. Mol. Pathol. 101 (1) (2016) 50–57, https://doi.org/10.1016/j.yexmp.2016.06.002.

[11] P. Kumar Singh, A. Kashyap, O. Silakari, Exploration of the therapeutic aspects of Lck: a kinase target in inflammatory mediated pathological conditions, Biomed. Pharmacother. 108 (2018) 1565–1571, https://doi.org/10.1016/j.biopha.2018.10.002.

[12] D. Elias, H.J. Ditzel, Fyn is an important molecule in cancer pathogenesis and drug resistance, Pharmacol. Res. 100 (2015) 250–254, https://doi.org/10.1016/j.phrs.2015.08.010.

[13] Y. Li, T. Zhu, H. Yang, S.D. Dib-Hajj, S.G. Waxman, Y. Yu, T.-L. Xu, X. Cheng, Nav1.7 is phosphorylated by Fyn tyrosine kinase which modulates channel expression and gating in a cell type-dependent manner, Mol. Pain 14 (2018), https://doi.org/10.1177/1744806918782229 1744806918782229-1744806918782229.

[14] L. Xu, Y. Pan, Q. Zhu, S. Gong, J. Tao, G.Y. Xu, X. Jiang, Arcuate Src activation-induced phosphorylation of NR2B NMDA subunit contributes to inflammatory pain in rats, J. Neurophysiol. 108 (11) (2012) 3024–3033, https://doi.org/10.1152/jn.01047.2011.

[15] S. Qiu, T. Chen, K. Koga, Y.Y. Guo, H. Xu, Q. Song, J.J. Wang, G. Descalzi, B.K. Kaang, J.H. Luo, M. Zhuo, M.G. Zhao, An increase in synaptic NMDA receptors in the insular cortex contributes to neuropathic pain, Sci. Signal. 6 (275) (2013), https://doi.org/10.1126/scisignal.2003778 ra34.

[16] H.B. Yang, X. Yang, J. Cao, S. Li, J.N. Liu, Z.W. Suo, H.B. Cui, Z. Guo, X.D. Hu, cAMP-dependent protein kinase activated Fyn in spinal dorsal horn to regulate NMDA receptor function during inflammatory pain, J. Neurochem. 116 (1) (2011) 93–104, https://doi.org/10.1111/j.1471-4159.2010.07088.x.

[17] E. Sato, Y. Takano, Y. Kuno, M. Takano, I. Sato, Involvement of spinal tyrosine kinase in inflammatory and N-methyl-d-aspartate-induced hyperalgesia in rats, Eur. J. Pharmacol. 468 (3) (2003) 191–198, https://doi.org/10.1016/s0014-2999(03)01718-7.

[18] T. Abe, S. Matsumura, T. Katano, T. Mabuchi, K. Takagi, L. Xu, A. Yamamoto, K. Hattori, T. Yagi, M. Watanabe, T. Nakazawa, T. Yamamoto, M. Mishina, Y. Nakai, S. Ito, Fyn kinase-mediated phosphorylation of NMDA receptor NR2B subunit at Tyr1472 is essential for maintenance of neuropathic pain, Eur. J. Neurosci. 22 (6) (2005) 1445–1454, https://doi.org/10.1111/j.1460-9568.2005.04340.x.

[19] C.K. Appel, S. Gallego-Pedersen, L. Andersen, S. Blancheflor Kristensen, M. Ding, S. Falk, M. Sayilekshmy, C. Gabel-Jensen, A.M. Heegaard, The Src family kinase inhibitor dasatinib delays pain-related behaviour and conserves bone in a rat model of cancer-induced bone pain, Sci. Rep. 7 (1) (2017) 4792, https://doi.org/10.1038/s41598-017-05029-1.

[20] Y.H. Tan, K. Li, X.Y. Chen, Y. Cao, A.R. Light, K.Y. Fu, Activation of Src family kinases in spinal microglia contributes to formalin-induced persistent pain state through p38 pathway, J. Pain 13 (10) (2012) 1008–1015, https://doi.org/10.1016/j.jpain.2012.07.010.

[21] X.H. Wei, T. Yang, Q. Wu, W.J. Xin, J.L. Wu, Y.Q. Wang, Y. Zang, J. Wang, Y.Y. Li, X.G. Liu, Peri-sciatic administration of recombinant rat IL-1beta induces mechanical allodynia by activation of src-family kinases in spinal microglia in rats, Exp. Neurol. 234 (2) (2012) 389–397, https://doi.org/10.1016/j.expneurol.2012.01.001.

[22] D. Schomberg, J.K. Olson, Immune responses of microglia in the spinal cord: contribution to pain states, Exp. Neurol. 234 (2) (2012) 262–270, https://doi.org/10.1016/j.expneurol.2011.12.021.

[23] S.-P. Chen, J. Sun, Y.-Q. Zhou, F. Cao, C. Braun, F. Luo, D.-W. Ye, Y.-K. Tian, Sinomenine attenuates cancer-induced bone pain via suppressing microglial JAK2/STAT3 and neuronal CAMKII/CREB cascades in rat models, Mol. Pain 14 (2018) 1744806918793232.

[24] M.-M. Ge, S.-P. Chen, Y.-Q. Zhou, Z. Li, X.-B. Tian, F. Gao, A. Manyande, Y.-K. Tian, H. Yang, The therapeutic potential of GABA in neuron-glia interactions of cancer-induced bone pain, Eur. J. Pharmacol. 858 (2019) 172475, , https://doi.org/10.1016/j.ejphar.2019.172475.

[25] X.-H. Wei, Y. Zang, C.-Y. Wu, J.-T. Xu, W.-J. Xin, X.-G. Liu, Peri-sciatic administration of recombinant rat TNF-α induces mechanical allodynia via upregulation of TNF-α in dorsal root ganglia and in spinal dorsal horn: the role of NF-kappa B pathway, Exp. Neurol. 205 (2) (2007) 471–484, https://doi.org/10.1016/j.expneurol.2007.03.012.

[26] J.S. Yu, J.H. Kim, S. Lee, K. Jung, K.H. Kim, J.Y. Cho, Src/Syk-targeted anti-inflammatory actions of triterpenoidal saponins from gac (Momordica cochinchinensis) seeds, Am. J. Chin. Med. 45 (3) (2017) 459–473, https://doi.org/10.1142/S0192415X17500288.

[27] Y.Y. Li, X.H. Wei, Z.H. Lu, J.S. Chen, Q.D. Huang, Q.J. Gong, Src/p38 MAPK pathway in spinal microglia is involved in mechanical allodynia induced by peri-sciatic administration of recombinant rat TNF-alpha, Brain Res. Bull. 96 (2013) 54–61, https://doi.org/10.1016/j.brainresbull.2013.05.002.

[28] M. Tsuda, H. Ueno, A. Kataoka, H. Tozaki-Saitoh, K. Inoue, Activation of dorsal horn microglia contributes to diabetes-induced tactile allodynia via extracellular signal-regulated protein kinase signaling, Glia 56 (4) (2008) 378–386, https://doi.org/10.1002/glia.20623.

[29] Y. Kawasaki, T. Kohno, Z.Y. Zhuang, G.J. Brenner, H. Wang, C. Van Der Meer, K. Befort, C.J. Woolf, R.R. Ji, Ionotropic and metabotropic receptors, protein kinase A, protein kinase C, and Src contribute to C-fiber-induced ERK activation and cAMP response element-binding protein phosphorylation in dorsal horn neurons, leading

[30] to central sensitization, J. Neurosci. 24 (38) (2004) 8310–8321, https://doi.org/10.1523/JNEUROSCI.2396-04.2004.

[31] Y. Zhong, L.J. Zhou, W.J. Ren, W.J. Xin, Y.Y. Li, T. Zhang, X.G. Liu, The direction of synaptic plasticity mediated by C-fibers in spinal dorsal horn is decided by Src-family kinases in microglia: the role of tumor necrosis factor-alpha, Brain Behav. Immun. 24 (6) (2010) 874–880, https://doi.org/10.1016/j.bbi.2010.01.007.

[31] X.J. Liu, J.R. Gingrich, M. Vargas-Caballero, Y.N. Dong, A. Sengar, S. Beggs, S.H. Wang, H.K. Ding, P.W. Frankland, M.W. Salter, Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex, Nat. Med. 14 (12) (2008) 1325–1332, https://doi.org/10.1038/nm.1883.

[32] F. Meng, C.A. Lowell, Lipopolysaccharide (LPS)-induced macrophage activation and signal transduction in the absence of Src-family kinases Hck, Fgr, and Lyn, J. Exp. Med. 185 (9) (1997) 1661–1670.

[33] O.J. Igwe, Modulation of peripheral inflammation in sensory ganglia by nuclear factor (kappa)B decoy oligodeoxynucleotide: involvement of SRC kinase pathway, Neurosci. Lett. 381 (1–2) (2005) 114–119, https://doi.org/10.1016/j.neulet.2005.02.020.

[34] Y.N. Liu, X. Yang, Z.W. Suo, Y.M. Xu, X.D. Hu, Fyn kinase-regulated NMDA receptor- and AMPA receptor-dependent pain sensitization in spinal dorsal horn of mice, Eur. J. Pain 18 (8) (2014) 1120–1128, https://doi.org/10.1002/j.1532-2149.2014.00455.x.

[35] W. Guo, S. Zou, Y. Guan, T. Ikeda, M. Tal, R. Dubner, K. Ren, Tyrosine phosphorylation of the NR2B subunit of the NMDA receptor in the spinal cord during the development and maintenance of inflammatory hyperalgesia, J. Neurosci. 22 (14) (2002) 6208–6217 https://doi.org/10.20026599.

[36] S. Slack, A. Battaglia, V. Cibert-Goton, I. Gavazzi, EphrinB2 induces tyrosine phosphorylation of NR2B via Src-family kinases during inflammatory hyperalgesia, Neuroscience 156 (1) (2008) 175–183, https://doi.org/10.1016/j.neuroscience.2008.07.023.

[37] R.R. Ji, Z.Z. Xu, G. Strichartz, C.N. Serhan, Emerging roles of resolvins in the resolution of inflammation and pain, Trends Neurosci. 34 (11) (2011) 599–609, https://doi.org/10.1016/j.tins.2011.08.005.

[38] M. Tsuda, T. Masuda, J. Kitano, H. Shimoyama, H. Tozaki-Saitoh, K. Inoue, IFN-gamma receptor signaling mediates spinal microglia activation driving neuropathic pain, Proc. Natl. Acad. Sci. U. S. A. 106 (19) (2009) 8032–8037, https://doi.org/10.1073/pnas.0810420106.

[39] J.Y. Zhao, L. Yang, H.H. Bai, J.P. Liu, Z.W. Suo, X. Yang, X.D. Hu, Inhibition of protein tyrosine phosphatase 1B in spinal cord dorsal horn of rats attenuated diabetic neuropathic pain, Eur. J. Pharmacol. 827 (2018) 189–197, https://doi.org/10.1016/j.ejphar.2018.03.012.

[40] S. Marinelli, B. Basilico, M.C. Marrone, D. Ragozzino, Microglia-neuron crosstalk: signaling mechanism and control of synaptic transmission, Semin. Cell Dev. Biol. (2019), https://doi.org/10.1016/j.semcdb.2019.05.017.

[41] L.J. Zhou, T. Yang, X. Wei, Y. Liu, W.J. Xin, Y. Chen, R.P. Pang, Y. Zang, Y.Y. Li, X.G. Liu, Brain-derived neurotrophic factor contributes to spinal long-term potentiation and mechanical hypersensitivity by activation of spinal microglia in rat, Brain Behav. Immun. 25 (2) (2011) 322–334, https://doi.org/10.1016/j.bbi.2010.09.025.

[42] X. Ding, J. Cai, S. Li, X.D. Liu, Y. Wan, G.G. Xing, BDNF contributes to the development of neuropathic pain by induction of spinal long-term potentiation via SHP2 associated GluN2B-containing NMDA receptors activation in rats with spinal nerve ligation, Neurobiol. Dis. 73 (2015) 428–451, https://doi.org/10.1016/j.nbd.2014.10.025.

[43] M.E. Hildebrand, J. Xu, A. Dedek, Y. Li, A.S. Sengar, S. Beggs, P.J. Lombroso, M.W. Salter, Potentiation of synaptic GluN2B NMDAR currents by fyn kinase is gated through BDNF-Mediated disinhibition in spinal pain processing, Cell Rep. 17 (10) (2016) 2753–2765, https://doi.org/10.1016/j.celrep.2016.11.024.

[44] W. Chen, W. Walwyn, H.S. Ennes, H. Kim, J.A. McRoberts, J.C. Marvizon, BDNF released during neuropathic pain potentiates NMDA receptors in primary afferent terminals, Eur. J. Neurosci. 39 (9) (2014) 1439–1454, https://doi.org/10.1111/ejn.12516.

[45] T.J. Boucher, K. Okuse, D.L.H. Bennett, J.B. Munson, J.N. Wood, S.B. McMahon, Potent analgesic effects of GDNF in neuropathic pain states, Science 290 (5489) (2000) 124, https://doi.org/10.1126/science.290.5489.124.

[46] Y.Y. Gao, X.Y. Hong, H.J. Wang, Role of Nectin-1/c-Src signaling in the analgesic effect of GDNF on a rat model of chronic constrictive injury, J. Mol. Neurosci. 60 (2) (2016) 258–266, https://doi.org/10.1007/s12031-016-0792-x.

[47] S.R. Chen, G. Samoriski, H.L. Pan, Antinociceptive effects of chronic administration of uncompetitive NMDA receptor antagonists in a rat model of diabetic neuropathic pain, Neuropharmacology 57 (2) (2009) 121–126, https://doi.org/10.1016/j.neuropharm.2009.04.010.

[48] H.-P. Bai, P. Liu, Y.-M. Wu, W.-Y. Guo, Y.-X. Guo, X.-L. Wang, Activation of spinal GABAB receptors normalizes N-methyl-D-aspartate receptor in diabetic neuropathy, J. Neurol. Sci. 341 (1) (2014) 68–72, https://doi.org/10.1016/j.jns.2014.04.002.

[49] S. Liu, W.T. Liu, Y.P. Liu, H.L. Dong, M. Henkemeyer, L.Z. Xiong, X.J. Song, Blocking EphB1 receptor forward signaling in spinal cord relieves bone cancer pain and rescues analgesic effect of morphine treatment in rodents, Cancer Res. 71 (13) (2011) 4392–4402, https://doi.org/10.1158/0008-5472.CAN-10-3870.

[50] Y.Q. Zhou, Z. Liu, H.Q. Liu, D.Q. Liu, S.P. Chen, D.W. Ye, Y.K. Tian, Targeting glia for bone cancer pain, Expert Opin. Ther. Tar. (2016), https://doi.org/10.1080/14728222.2016.1214716.

[51] M. De Felice, D. Lambert, I. Holen, K.J. Escott, D. Andrew, Effects of Src-kinase inhibition in cancer-induced bone pain, Mol. Pain 12 (2016), https://doi.org/10.1177/1744806916643725.

[52] H. Leduc-Pessah, N.L. Weilinger, C.Y. Fan, N.E. Burma, R.J. Thompson, T. Trang, Site-specific regulation of P2X7 receptor function in microglia gates morphine

analgesic tolerance, J. Neurosci. 37 (42) (2017) 10154–10172, https://doi.org/10.1523/JNEUROSCI.0852-17.2017.

[53] C. Rivat, S. Sebaihi, J. Van Steenwinckel, S. Fouquet, P. Kitabgi, M. Pohl, S. Melik Parsadaniantz, A. Reaux-Le Goazigo, Src family kinases involved in CXCL12-induced loss of acute morphine analgesia, Brain Behav. Immun. 38 (2014) 38–52, https://doi.org/10.1016/j.bbi.2013.11.010.

[54] J.T. Williams, S.L. Ingram, G. Henderson, C. Chavkin, M. von Zastrow, S. Schulz, T. Koch, C.J. Evans, M.J. Christie, Regulation of mu-opioid receptors: desensitization, phosphorylation, internalization, and tolerance, Pharmacol. Rev. 65 (1) (2013) 223–254, https://doi.org/10.1124/pr.112.005942.

[55] H.W.D. Matthes, R. Maldonado, F. Simonin, O. Valverde, S. Slowe, I. Kitchen, K. Befort, A. Dierich, M. Le Meur, P. Dollé, E. Tzavara, J. Hanoune, B.P. Roques, B.L. Kieffer, Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene, Nature 383 (6603) (1996) 819–823, https://doi.org/10.1038/383819a0.

[56] F.A. Bull, D.T. Baptista-Hon, C. Sneddon, L. Wright, W. Walwyn, T.G. Hales, Src kinase inhibition attenuates morphine tolerance without affecting reinforcement or psychomotor stimulation, Anesthesiology 127 (5) (2017) 878–889, https://doi.org/10.1097/ALN.0000000000001834.

[57] W. Walwyn, C.J. Evans, T.G. Hales, Beta-arrestin2 and c-Src regulate the constitutive activity and recycling of mu opioid receptors in dorsal root ganglion neurons, J. Neurosci. 27 (19) (2007) 5092–5104, https://doi.org/10.1523/JNEUROSCI.1157-07.2007.

[58] P. Sanchez-Blazquez, M. Rodriguez-Munoz, E. Berrocoso, J. Garzon, The plasticity of the association between mu-opioid receptor and glutamate ionotropic receptor N in opioid analgesic tolerance and neuropathic pain, Eur. J. Pharmacol. 716 (1–3) (2013) 94–105, https://doi.org/10.1016/j.ejphar.2013.01.066.

[59] Y.-Q. Zhou, D.-Q. Liu, S.-P. Chen, J. Sun, X.-M. Wang, Y.-K. Tian, W. Wu, D.-W. Ye, Minocycline as a promising therapeutic strategy for chronic pain, Pharmacol. Res. 134 (2018) 305–310, https://doi.org/10.1016/j.phrs.2018.07.002.

[60] A. Réaux-Le Goazigo, J. Van Steenwinckel, W. Rostène, S. Mélik Parsadaniantz, Current status of chemokines in the adult CNS, Prog. Neurobiol. 104 (2013) 67–92, https://doi.org/10.1016/j.pneurobio.2013.02.001.

[61] Y.Q. Zhou, D.Q. Liu, S.P. Chen, J. Sun, X.R. Zhou, C. Xing, D.W. Ye, Y.K. Tian, The role of CXCR3 in neurological diseases, Curr. Neuropharmacol. 17 (2) (2019) 142–150, https://doi.org/10.2174/1570159X15666171109161140.

[62] X. Chen, E.B. Geller, T.J. Rogers, M.W. Adler, Rapid heterologous desensitization of antinociceptive activity between mu or delta opioid receptors and chemokine receptors in rats, Drug Alcohol Depend. 88 (1) (2007) 36–41, https://doi.org/10.1016/j.drugalcdep.2006.09.010.

[63] H. Katsura, K. Obata, T. Mizushima, J. Sakurai, K. Kobayashi, H. Yamanaka, Y. Dai, T. Fukuoka, M. Sakagami, K. Noguchi, Activation of Src-family kinases in spinal microglia contributes to mechanical hypersensitivity after nerve injury, J. Neurosci. 26 (34) (2006) 8680, https://doi.org/10.1523/JNEUROSCI.1771-06.2006.

[64] F. Saad, A. Lipton, SRC kinase inhibition: targeting bone metastases and tumor growth in prostate and breast cancer, Cancer Treat. Rev. 36 (2) (2010) 177–184, https://doi.org/10.1016/j.ctrv.2009.11.005.

[65] A. Gnoni, I. Marech, N. Silvestris, A. Vacca, V. Lorusso, Dasatinib: an anti-tumour agent via Src inhibition, Curr. Drug Targets 12 (4) (2011) 563–578.

[66] L.C. Kim, L. Song, E.B. Haura, Src kinases as therapeutic targets for cancer, Nature reviews, Clin. Oncol. 6 (10) (2009) 587–595, https://doi.org/10.1038/nrclinonc.2009.129.