The Role of Spinal Cord CX3CL1/CX3CR1 Signalling in Chronic Pain

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Abstract

Purpose of Review Chronic pain is a distressing condition that is ineffectively treated at present. In order to develop novel, more efficacious analgesics for chronic pain, a better understanding of the underlying mechanisms is required. Despite chronic pain initially being considered as a neurocentric process, the role of communication between immune cells and neurons has been shown to be essential to the modulation of chronic pain. In the spinal cord, chemokine-mediated communication between microglia and neurons has been shown to play a crucial mechanistic role in preclinical chronic pain.

Recent Findings Here, we present convincing evidence specifically for the role of the neuronal chemokine, fractalkine and its receptor CX₃CR1, which is expressed by microglia, in mediating neuronal/microglia crosstalk in the spinal cord in the context of preclinical pain behaviour.

Summary In light of the compelling preclinical evidence and emerging clinical evidence, we consider the promising therapeutic potential of manipulating this signalling partnership for the treatment of chronic pain.

Keywords Fractalkine · CX₃CR1 · Cathepsin S · Microglia · Pain

Introduction

Pain has an immense impact on the daily activities of those afflicted and dramatically reduces their quality of life. Whilst acute pain is a functional and necessary process that serves to protect, chronic pain is maladaptive – persisting beyond the injury or disease that triggered the initial onset of pain and thus outlasting its protective role. Chronic pain can develop following infection, disease or injury and can be characterised by heightened sensitivity to noxious stimuli (hyperalgesia), pain in response to innocuous stimuli (allodynia) and/or spontaneous pain [1]. At present, clinically available treatments for chronic pain have limited efficacy and can cause a multitude of undesirable side effects that further reduce patient quality of life. Advances in our understanding of the underlying mechanisms of chronic pain and thus the ability to develop

innovative therapies that are more efficacious and possess an improved side effect profile is therefore critical.

Historically, chronic pain was largely attributed to a purely neuronal response to injury, with the involvement of non-neuronal cells being relatively overlooked. However, in the last 15 years or so, the importance of the role of communication between immune cells and neurons has been appreciated both peripherally, at the site of injury, and centrally [2]. In the spinal cord dorsal horn, microglia, which are the tissue-resident macrophages of the central nervous system, have received much attention in the context of mechanisms underlying preclinical chronic pain behaviour [3, 4]. Microglia have been shown to mediate preclinical chronic pain through their communication with neurons using a variety of signalling pathways. For instance, injury-associated activation of the purinergic receptor P2X4 in microglia results in the release of brain-derived neurotrophic factor (BDNF), which activates TrkB receptors expressed by neurons and which in turn inhibits neuronal chloride channels, thus resulting in disinhibition and thence pain [5, 6]. Another crucial mediator of the communication between microglia and neurons in the spinal cord in the context of preclinical chronic pain is chemokine signalling [7]. In this review, we will focus specifically on the mechanistic role of communication between microglia and neurons in the spinal cord that is mediated by the neuronal

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chemokine fractalkine (CX₃CL1) and its receptor CX₃CR1 and how this may shape the development of novel therapeutic approaches for chronic pain.

CX₃CL1/Fractalkine and CX₃CR1 Expression in the Spinal Cord

Fractalkine (FKN), or CX₃CL1, is the only member of the CX₃C subfamily of chemokines, which, in the spinal cord, is predominantly expressed on the cell surface of neurons [8]. FKN is an atypical chemokine as, unlike the majority of chemokines, it is expressed constitutively [9]. FKN exists as both a membrane-bound form, which is approximately 100 kDa in size, and a soluble form (sFKN), which is 80 kDa in size, and thus is uncharacteristically large compared with other chemokines, which do not typically exceed 17 kDa. The specific forms of FKN possess distinct functions. Full-length, membrane-bound FKN possesses an adhesion function in the vascular immune system, whilst sFKN functions as a chemoattractant for monocytes, natural killer cells, B cells and T cells and is essential for the transendothelial migration of monocytes that express the FKN receptor, CX₃CR1 [10, 11]. The cleavage of FKN is mediated by two classes of proteases, namely, metalloproteases (ADAM10 and 17), which are expressed predominantly by endothelial cells [12–14] and the cysteine protease cathepsin S (CatS), which is released by microglia in the spinal cord [9]. Whilst the constitutive cleavage of FKN specifically is regulated by ADAM10 [12], induced cleavage under adverse conditions is mediated by both ADAM17 and CatS [9, 14]. Importantly, the ADAMs and CatS target FKN at different cleavage sites, and thus depending on the protease that is activated, different forms of sFKN are generated, which are likely to possess subtly different functionalities [15].

Chemokine signalling is typically promiscuous; however, FKN is an exception. FKN/CX₃CR1 signalling displays high fidelity, with FKN only being able to activate CX₃CR1, and in turn, CX₃CR1 is exclusively activated by FKN. CX₃CR1 was initially identified in both rats and humans over 20 years ago [16, 17] and in the spinal cord and is exclusively expressed by microglia [18, 19]. The high fidelity of FKN/CX₃CR1 signalling as well as the exclusive expression pattern of both ligand and receptor in the spinal cord makes this signalling partnership a potentially ideal therapeutic target in any context as non-specific effects are less likely to occur and the treatment approach can be highly focussed.

Spinal Cord FKN/CX₃CR1 Signalling in Preclinical Models of Chronic Pain

A key site in the spinal cord at which modulation of nociceptive signalling occurs is the first synapse of the nociceptive pathway between terminals of primary afferent fibres and

dorsal horn neurons. Heightened activity of glial cells in the spinal cord and hence changes in their communication with neurons are well-established consequences of damage to peripheral nerves in a variety of preclinical chronic pain models [20]. The expression patterns of FKN and CX₃CR1 in the spinal cord – neuronal and microglial, respectively [8, 21, 22] – make this signalling pair an intuitive candidate for the mediation of changes in neuronal-microglial crosstalk. Indeed, both sFKN and CX₃CR1 are elevated following peripheral nerve injury, whilst disruption of their signalling, either pharmacological or genetic, has anti-allodynic effects [2].

Pronociceptive effects are associated specifically with cleaved, or soluble, FKN as opposed to the membrane-bound form. Indeed, following peripheral nerve injury, whilst the expression of membrane-bound FKN remains relatively unchanged, the expression of sFKN in the CSF increases significantly [9, 23]. It is likely that increases in sFKN play a causative role in pain behaviour as opposed to being an epiphenomenon as sFKN specifically results in both thermal and mechanical hypersensitivity when intrathecally administered [9]. The pronociceptive effect of sFKN is exerted via activation of CX₃CR1, which in turn induces intracellular phosphorylation of microglial p38 MAPK [23–25] resulting in pro-inflammatory mediator release [26]. In addition to an increase in sFKN, the expression of CX₃CR1 in the spinal cord is also elevated following peripheral nerve injury in the majority of preclinical models [27, 28]. This overall increase in CX₃CR1 expression however could be either a result of an increase in receptor expression within microglia or an increase in microglial number, which would be accompanied by an increase in overall expression of CX₃CR1 in the spinal cord. Nonetheless, an increase in both sFKN and CX₃CR1 and thus heightened spinal FKN/CX₃CR1 signalling is a consistent feature of preclinical chronic pain models.

Manipulation of FKN/CX₃CR1 signalling through the use of genetic deletion or neutralising antibodies has enabled us to establish the importance of spinal FKN/CX₃CR1 signalling in the context of preclinical pain behaviour. Mice deficient in CX₃CR1, for example, fail to develop mechanical hypersensitivity associated with several models of chronic pain. For instance, in models of both peripheral nerve injury and HIV-associated pain, CX₃CR1-deficient mice display reduced hypersensitivity [24, 29]. Such a reduction in hypersensitivity is likely to be due to changes in microglial signalling as deletion of the CX₃CR1 receptor has been found to reduce microglial-associated pro-inflammatory responses following peripheral nerve and spinal cord injury [24, 30]. Furthermore, intrathecal delivery of a CX₃CR1-neutralising antibody both delays the onset of pain and attenuates established allodynia in a model of bone cancer pain [31, 32]. Critically, acute pain is unchanged in CX₃CR1-deficient mice [24]. Therefore, whilst FKN/CX₃CR1 signalling plays a crucial role in mediating chronic pain, it does not play a role in acute pain, which is

an essential protective response. This further suggests that targeting FKN/CX₃CR1 signalling would be a particularly useful approach for treating chronic pain as it would not dampen crucial acute pain responses.

Whilst genetic deficiencies and neutralising antibodies enable us to identify potential mechanistic roles and serve as a proof on concept, such studies need to be interpreted with caution in terms of their clinical applicability. Silencing or knocking-down a gene, for example, is not only an unrealistic therapeutic approach but can have compensatory effects that alter other underlying mechanistic pathways. For instance, in CX₃CR1-deficient mice, monocytes express elevated levels of CCR₂ – a receptor for the chemokine CCL₂, which also has a crucial role in the underlying mechanisms of chronic pain [33]. Whilst this does not necessarily mean that similar compensatory effects occur in microglia on CX₃CR1-deficient mice, the possibility cannot be ruled out. It is therefore essential to utilise pharmacological manipulation in addition to genetic studies. The potential of CX₃CR1 antagonists as therapeutic tools has been studied more extensively in contexts other than chronic pain [34, 35]. Their efficacy in terms of reducing preclinical chronic pain has yet to be comprehensively addressed. CX₃CR1 antagonists however are likely to show some therapeutic promise given the evidence for the importance of the role of FKN/CX₃CR1 signalling, the anti-allodynic effects of neutralising antibodies and the fact that such inhibitors possess anti-inflammatory effects [36]. One caveat however is that CX₃CR1 inhibition would not differentiate between homeostatic and pathological FKN/sFKN signalling and so their use and dosage would need to be carefully considered.

Indeed, in order to most effectively develop efficacious therapies that possess minimal side effects, it is crucial to target the precise source of sFKN/CX₃CR1 signalling that constitutes the underlying mechanism. As mentioned above, the current consensus is that neurons are the source of FKN, whilst microglia exclusively express CX₃CR1 in the spinal cord. Recently however, alternative CX₃CR1-expressing immune cells have received attention in the context of underlying mechanisms of preclinical chronic pain. Peripherally circulating monocytes, which also express CX₃CR1, have been suggested to infiltrate into the spinal cord and also provide a potential source of spinal FKN/CX₃CR1 signalling [37•]. This however is not a consistent finding between preclinical chronic pain models. For instance, in a nerve injury model of chronic pain, whilst CX₃CR1-expressing monocytes are crucial for the initiation of pain, working synergistically with microglia to fulfil this role [38], their infiltration into the spinal cord is not apparent. In a chemotherapy model of neuropathic pain however, specifically the vincristine model, evidence suggests that peripherally circulating monocytes are likely to infiltrate into the spinal cord [38], bringing with them not only a means for FKN-CX₃CR1 signalling, but a source of CatS,

which elevates pro-nociceptive sFKN expression. It is therefore apparent that whilst FKN/CX₃CR1 signalling in the spinal cord plays a crucial role in the underlying mechanisms of preclinical chronic pain, the precise source of such signalling may vary between models and should therefore be carefully considered in the design of potential therapies targeting this signalling partnership.

Targeting FKN Cleavage and Transcription as a Therapeutic Approach for Neuropathic Pain

As well as targeting sFKN and CX₃CR1 actions directly, targeting their cellular processing may also provide another avenue for the development of therapies. Indeed, targeting the cleavage of FKN could be a highly effective approach as it would target pro-nociceptive sFKN without disrupting homeostatic functions of full length FKN. Consistent with an increase in sFKN, CatS expression and release increases in the event of peripheral nerve injury [9]. It is released from microglia in a P2X7-dependent manner [39] and, following injury, increases in microglia in the area of the dorsal horn that is innervated by the damaged primary afferent terminals. As it has the capacity to cleave FKN, it plays a crucial role in regulating pain behaviour in various preclinical models of chronic pain. Indeed, CatS maintains microglial activity in pain states [40], and both intrathecal [9] and systemic [41, 42] delivery of a CatS inhibitor successfully reverses established allodynia that was induced by peripheral nerve injury. The therapeutic potential of targeting CatS initially appeared to be most promising during the maintenance phase of chronic pain as opposed to the induction. For instance, CatS inhibition in the spinal cord was ineffective in reducing allodynia when administered during the initiation phase in a preclinical surgical model of neuropathic pain [9]. The lack of efficacy of CatS inhibitors during this time has been attributed to the relatively low level of extracellular (released) CatS that are present at earlier stages of such models [9]. However, the effectiveness of CatS inhibitors during the induction phases of preclinical pain could be dependent on the initial cause of injury/pain. Recent evidence indicates that either variable could account for lack of efficacy in the induction phase of pain. Indeed, targeting CatS in the spinal cord has shown promise during pain induction in the vincristine model of neuropathic pain. Specifically, administration of a centrally penetrant CatS inhibitor significantly reduces the severity of allodynia during early vincristine treatment, whereas administration of a peripherally restricted CatS inhibitor has no appreciable effect [37•]. In this case however, the elevation of CatS in the spinal cord is not a result of microglial activation and thus CatS release but is attributed to infiltrating monocytes from the periphery [37•]. Furthermore, despite systemic CatS

inhibition showing limited efficacy in reversing established pain in surgical models, in a recent study using the partial sciatic nerve ligation preclinical model, treatment with an orally active CatS inhibitor, MIV-247, significantly reversed surgery-induced allodynia within 3 h of administration [43]. This suggests that the specific antagonist used could also determine the effectiveness of CatS disruption in different locations, i.e. central versus peripheral.

As discussed above, the cleavage of FKN is mediated by proteases other than CatS. The role of ADAM-mediated cleavage of FKN in the context of chronic pain has not been studied extensively as the role of CatS at present. Recently however, mice containing a genetic knockdown of ADAM17 have been found to be less sensitive to noxious mechanical, thermal and cold sensitivity [44•]. It therefore appears that ADAM17 could play a key role in the underlying mechanisms of pain and the efficacy of its pharmacological inhibition warrants investigation. ADAM17 expression is induced under adverse conditions, whereas ADAM10 is constitutively expressed [12]. It is therefore perhaps intuitive that ADAM17-mediated cleavage is more likely to play a role in chronic pain. However, the role of ADAM10 in pro-

inflammatory conditions cannot necessarily be completely discounted. For instance, in stimulated human astrocytes in culture, ADAM10 has been reported to mediate the release of pronociceptive cytokines [45]. It is crucial to remember however that culture conditions do not necessarily reflect the *in vivo* situation and thus further investigation about the role of astrocyte-derived ADAM10 is necessary.

In addition to targeting cleavage of FKN, studies have also considered targeting upstream regulators of FKN transcription. In the spinal cord, for example, a transcriptional regulator of FKN, Stat3 (signal transducer and activator of transcription 3), appears to play a role in chronic pain through its regulation of FKN. Specifically, mechanical allodynia induced by intrathecal LPS administration is associated with activation of Stat3 and a concurrent increase in FKN expression. Allodynia is significantly reduced however by blockade of Stat3, which also serves to reduce expression of FKN [46]. Whilst this highlights the potential mechanistic role of Stat3-mediated transcription of FKN, the side effects of the manipulation of transcription factors are likely to be widespread and non-specific and would affect crucial homeostatic functions of other target proteins. Such therapies are therefore likely to

Figure 1 Potential therapeutic targets for fractalkine and CX₃CR1 expression in the dorsal horn of the spinal cord. Schematic representation of fractalkine/CX₃CR1 expression in the dorsal horn of the spinal cord. Fractalkine (FKN) is expressed exclusively by dorsal horn neurons, whilst CX₃CR1 is expressed exclusively by microglia. Potential therapeutic strategies could include pharmacological inhibition of CX₃CR1 signalling (A) or targeting the generation of soluble FKN through inhibition of cathepsin S (CatS) (B). Although less intensively studied, pharmacological inhibition of ADAMs could also provide a potential therapeutic strategy

have many undesirable side effects clinically, and a precise understanding of their mechanism of action given their widespread actions will be challenging to obtain.

Sexual Dimorphism

In recent years, much interest has surrounded potential sex differences in the underlying mechanisms of pain given that pain/behavioural differences are often observed both clinically and preclinically. In preclinical models in which differences in pain behaviour between males and females have been commonly observed, for example, nerve injury and inflammatory pain [47], a corresponding sexual dimorphism in FKN/CX₃CR1 signalling in the spinal cord has yet to be established. Indeed, in some preclinical models in which the role of microglia in the spinal cord appears to be most crucial during the initiation of pain [47], differences in pain-like behaviour between males and females appear to be present during the maintenance phases [47]. At present it therefore appears unlikely that the role of FKN/CX₃CR1 signalling in the spinal cord is sexually dimorphic, although due to subtle differences in the role of FKN/CX₃CR1 in different models of pain, this cannot be completely ruled out.

FKN and CX₃CR1 in Clinical Neuropathic Pain

The role of FKN/CX₃CR1 signalling in the spinal cord is now well-established in the context of preclinical pain. However, it is crucial to also establish if this signalling partnership also plays a role clinically in order to fully justify the clinical use of therapies that target FKN/CX₃CR1. Studies investigating the levels of fractalkine in patients with chronic pain, for example, either at a tissue level or in the CSF, are few in number but nonetheless indicative that FKN/CX₃CR1 signalling does indeed play a role in chronic pain clinically. For instance, in fibromyalgia patients, high levels of FKN are measured in the CSF compared to controls [48••]. There is also isolated evidence to suggest that fractalkine expression is elevated at a tissue level in patients with chronic sciatic pain [49]. Specifically, the expression of FKN is elevated in the soft tissues of the nerve root and intervertebral disc in patients with severe chronic pain caused by lumbar disk herniation compared to those reporting milder pain. It is therefore a possibility that in some clinical cases of chronic pain, FKN/CX₃CR1 may regulate or be reflective of pain severity as opposed to regulating the occurrence of pain per se. This however would need to be more firmly established and also investigated in the context of patients experiencing chronic pain from other diseases/injuries.

Concluding Remarks

At present, the clinical treatment of chronic pain shows limited efficacy and is often accompanied by a host of undesirable side effects that further reduce patient quality of life. A greater understanding of underlying mechanisms of chronic pain and the consequential development of novel therapies is therefore required. Here, we have considered the role of communication between microglia and neurons in the spinal cord, specifically that which is mediated by fractalkine and its receptor CX₃CR1, expressed by neurons and microglia, respectively. We have considered both ligand and receptor as potential therapeutic targets as well as considering the potential of targeting cleavage of full length fractalkine into soluble fractalkine – the latter of which is more closely associated with chronic pain. At present, although pharmacological tools that target FKN/CX₃CR1 signalling and FKN cleavage are not used routinely in the clinic, in recent years, they have been recognised as valuable innovative approaches for a variety of pathological conditions. Therapies that target the generation or signalling of sFKN specifically, or those which target CX₃CR1 in crucial areas and timeframes (Fig. 1), could therefore form part of the next generation of analgesics for chronic pain conditions.

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Compliance with Ethical Standards

Conflict of Interest Dr. Malcangio, Dr. Montague-Cardoso and Dr. Mrozkova declare that they have no conflict of interest.

Human Studies/Informed Consent All reported studies/experiments with human or animal subjects performed by the authors were performed in accordance with all applicable ethical standards including the Helsinki Declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines.

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