

MCP-1: A potential candidate for pain management

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Abstract: Chronic pain is a debilitating condition resulted from the damage or dysfunction of the nervous system. By now, treatments for chronic pain are accompanied by a great deal of undesirable side-effects which contribute to limited efficacy for pain relieving. Thus, new efficient therapeutic approaches for remedy of painful neuropathies are needed. Chemokine signaling has been recently reported to play a crucial role in the regulation of neuroinflammatory responses and development of chronic pain processing. In particular, monocyte chemoattractant protein 1 (MCP-1), has been shown to be involved in diverse pain conditions via its receptor chemokines C-C motif receptor 2 (CCR2). In this review, we discussed studies published recently demonstrated that the crucial role of MCP-1/ CCR2 axis in chronic pain in different pathological pain models. Insights into the role of MCP-1/ CCR2 signaling pathway in pain processing will identify novel targets for therapeutic intervention of chronic pain.

Keywords: Monocyte chemoattractant protein 1; Neuropathic pain; Inflammatory pain; Cancer pain; Chronic pain

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1. Introduction

Chronic pain is a pathological condition that lasts for more than 3 to 6 months[1], afflicting more than 20% of people around the world, and should be paid greater attention[2]. Injuries that lead to damages or dysfunction of the somatosensory nervous system both contribute to the occurrence of chronic pain, including peripheral or central nerve injury, inflammation and cancer-induced bone metastasis[3-5]. Current treatments for chronic pain meet with severe side effects and other problems, which offer limited effect to relieve pain for patients. Consequently, development of novel therapeutic approaches to the treatment of chronic pain is in dire need.

Chemokines are small heparin-binding proteins that control the trafficking of leukocytes to sites of inflammation or injury[6]. There are four subfamilies of chemokines, C, CC, CXC and CX3C, according to cysteine's number on N-terminal region[7]. Chemokines can be secreted by multifarious cell types in the central nervous system (CNS) including microglia, astrocytes and neurons[7], and the peripheral nervous system such as schwann cells[8] and the infiltrating monocytes/macrophages[9]. Recently, studies reported that chemokines is correlated with diverse chronic pain conditions clinically. Particularly, increasing evidence showed that chemokine MCP-1, the most thoroughly characterized CC chemokine[6], plays a crucial role in different pain condition by activating its receptor CCR2[10-13]. Here, we discussed preclinical evidences for the role of MCP-1/CCR2 signaling pathway in pain processing. Targeting MCP-1/CCR2 signaling may provide a novel approach to the treatment of chronic pain syndromes

2. The biological functions of MCP-1

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the C-C chemokine family that plays a critical role in the recruitment and activation of monocytes during acute inflammation. There are four members of MCP family: MCP-1, -2, -3, and -4. MCP-1 is the first discovered human CC chemokine that located on chromosome 17 (chr.17, q11.2)[14]. MCP-1 bears a high degree of homology with the murine JE gene, which was originally identified in mitogen-stimulated non-neoplastic fibroblasts[15]. There are a variety of mediators induce the gene expression of MCP-1, including platelet-derived growth factor, interleukins IL-1 and IL-4, TNF- α , VEGF, bacterial lipopolysaccharide, and interferon γ [14]. Besides, MCP-1 is produced by many cell types including fibroblasts epithelial, endothelial cells, smooth muscle, mesangial cells, astrocytes, monocytes and microglial cells[16,17]. Among these, monocyte is the primary source of MCP-1.

In addition to recruit monocytes, memory T-cells, and dendritic cells to sites of tissue injury and infection[18], MCP-1 could directly up-regulate monocyte cytokine production and cytostatic activity[17]. Since MCPs have a broad cell spectrum, their receptors are expressed on various leukocyte types. Although it recognizes several receptors, MCP-1 mediates its effects mainly through its receptor CCR2, a member of the group of G-protein-coupled receptors that contain seven transmembrane spanning domains[19].

3. MCP-1 and chronic pain

3.1. MCP-1 and neuropathic pain

Neuropathic pain (NP) is a devastating disease

with no currently accepted treatment. In addition to neurons, glial cells in the central nervous system were also demonstrated to play a crucial role in the initiation and maintenance of pain hypersensitivity [20]. An increasing number of evidence has demonstrated that glial cells including astrocytes and microglia produced various mediators such as pro-inflammatory cytokines and chemokines[21, 22], which could interact with neurons and thus regulate pain transmission under pathological conditions[23-25]. Chemokines may have additional roles to play in pain. In particular, chemokine MCP-1 has been suggested to play a key role in the neuroinflammation and central sensitization after peripheral nerve injury[26,27]. The expression of MCP-1 and its major receptor CCR2 was up-regulated after peripheral nerve injury (PNI)[28-31]. Injury to peripheral nerves triggers MCP-1 release

from neurons and macrophages in the dorsal root ganglion (DRG)[30], the spinal cord[32] and the nucleus accumbens (NAc)[33], and glial cells in spinal cord have been also reported to induce the release of MCP-1[34,35]. MCP-1 may react with CCR2 in neurons and macrophages in DRG to enhance the excitability of neurons and infiltration of macrophages into DRG[30]. MCP-1, acted as a neuromodulator, was then transported anterogradely towards both the periphery[36] and the dorsal horn of the spinal cord, and played an important role in inducing microglia activation and mechanical allodynia [20,28]. Moreover, the inflammatory cytokines and chemokines released by the activated microglia not only contribute to neuropathic pain but also result in delayed astrocyte activation, which is crucial in sustaining long-lasting hypersensitivity[37] (Figure 1).

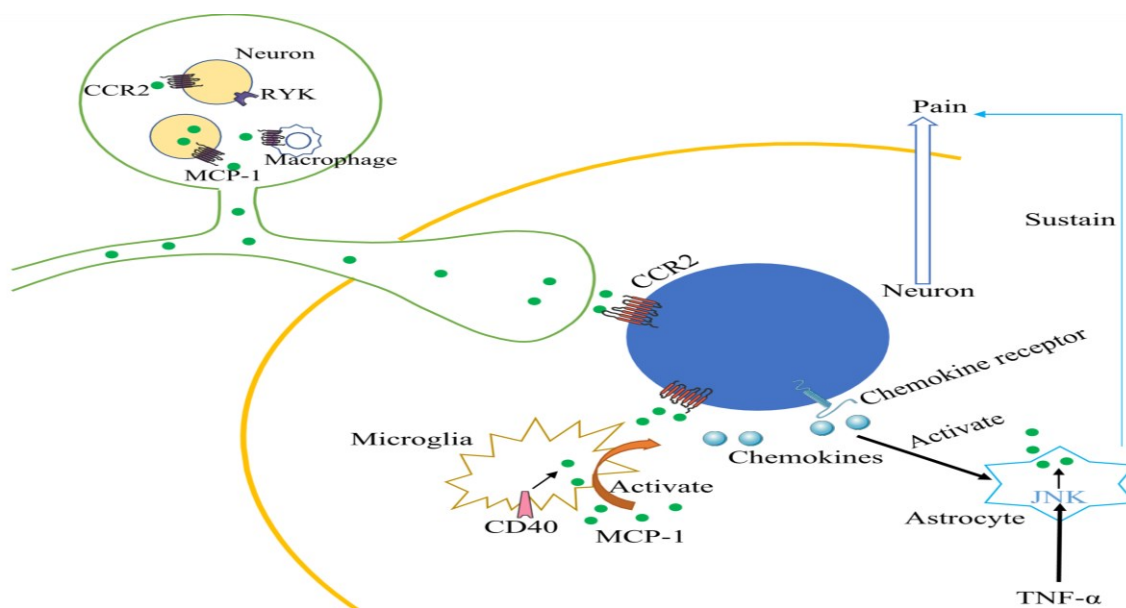


Figure 1. The regulatory mechanisms of MCP-1/CCR2 signal in neuropathic pain. In the DRG, injury to peripheral nerves induces an increase in expression level of MCP-1 and CCR2 in neurons and macrophages. MCP-1 may react with CCR2 in neurons and macrophages to enhance the excitability of neurons and infiltration of macrophages to DRG. MCP-1, acted as a neuromodulator, was then transports anterogradely towards both the periphery and the dorsal horn of the spinal cord, and plays an important role in increasing the neurons excitability and inducing microglia activation. The activated microglia produces and release diverse pro-inflammatory cytokines and chemokines, which communicated with neurons by acting on chemokine receptors that contribute to the development of neuropathic pain. In addition, chemokines released by the activated microglia also result in delayed astrocyte activation, which plays a crucial role in sustaining long-lasting hypersensitivity. Moreover, TNF- α intrathecally evoked JNK-dependent mechanical allodynia and MCP-1 increase in the spinal astrocytes. Besides, the expression of Ryk receptors in DRG neurons and CD40 in spinal microglia are also increased after nerve injury, which play an important role in the pathogenesis of neuropathic pain.

Evidences have demonstrated that the production of MCP-1 can be affected by many factors. Tanshinone IIA, an important component of a traditional Chinese drug, Danshen, was reported to provide with effective immuno-suppressive activities[38]. Administration of Tanshinone IIA intraperitoneally reduced the mechanical allodynia

induced by SNL through inhibiting SNL-mediated astrocytic activation, which is via suppressing the JNK/MCP-1 pathway[38]. Moreover, evidence also showed that production of MCP-1 was TNF- α /JNK pathway dependent[34]. Administration of TNF- α intrathecally evoked JNK-dependent mechanical allodynia and MCP-1 increase in astrocytes (Figure

1). TNF- α -induced increase of MCP-1 was suppressed by the JNK inhibitors SP600125 in a dose dependently manner. Additionally, CD40, a cell surface receptor, have been found to be produced by activated microglia[39]. The numbers of CD40+ microglia were markedly up-regulated in the lumbar spinal cord dorsal horn in a model of neuropathic pain[35]. Microglia promoted the expression of CD40, which played a critical role in the maintenance of L5Tx-induced mechanical hypersensitivity[40]. Spinal cord MCP-1 production was one of pathways that mediated CD40-induced behavioral hypersensitivity[35] (Figure 1). Finally, Wnts (wingless and Int) and its specific receptor Ryk have been reported to play an important role in the pathogenesis of neuropathic pain. Both expression of Wnt and Ryk receptors was increased in the dorsal horn after SNL, and Wnt1 was also increased in activated astrocytes[41]. Blocking of the Wnt/Ryk signaling by Wnt inhibitor, IWP-2, or Anti-Ryk antibodies reduced SNL-evoked mechanical allodynia but not thermal hyperalgesia (Figure 1). The analgesic effect of inactivation of Wnt1/Ryk signaling may contribute to the suppression of Wnt1-induced excitatory synaptic transmission and

subsequent reduce the expression of MCP-1 in the dorsal root ganglia neurons[41].

An increasingly researches has shown that neutralization the activity of MCP-1 or blockade of the CCR2 receptor may provide a new therapy for the treatment of chronic pain. Intrathecal injection of AZ889, a novel CCR2 antagonist, induced dose-dependent analgesia in CCI rats[42]. Intracisternal administration of RS504393, a CCR2 antagonist, markedly attenuated the inferior alveolar nerve and mental nerve transection (IAMNT)-induced heat hyperalgesia[29] and PNI-induced neuropathic pain [28,29,33,43]. Notably, in addition to activation of microglia resident to the CNS, PNI also promoted infiltration of the hematogenous macrophage/monocyte into the spinal cord, proliferate, and differentiate into microglia[20]. Expression of CCR2 either in resident microglia or BMDM is sufficient for the development of neuropathic pain. Suppressing only one of them thus could not effective inhibit nerve injury induced neuropathic pain[20]. The therapeutic effect of drugs that target at MCP-1/CCR2 axis in chronic pain is presented in Table 1.

Table 1. The therapeutic effect of drugs that targets at MCP-1/CCR2 axis in chronic pain

| Drugs | Drug types | Suggested mechanism | Types of pain | Chronic pain model | Ref. |
|-------------------|---------------------------------|--|------------------|--|------|
| Tanshinone A | II A traditional chines drug | Inhibited astrocytes activation via suppressing JNK/MCP-1 pathway | Neuropathic pain | Spinal nerve ligation (SIVL) | [38] |
| SP600125 | JNK inhibitor | Inhibited spinal nerve ligation(SIVL)-induced production of MCP-1 in astrocyte | Neuropathic pain | Spinal nerve ligation (SIVL) | [34] |
| IWP-2 | Wnt inhibitor | Inhibited the crosstalk between astrocytes and neurons by blocking | Neuropathic pain | Spinal nerve ligation (SIVL) | [41] |
| Anti-Ryk antibody | Ryk receptors inhibitor | MCP-1 expression and cytokine transmission | Neuropathic pain | Spinal nerve ligation (SIVL) | [41] |
| Az889 | CCR2 antagonist | Inhibited MCP-1 mediated hyperalgesia | Neuropathic pain | Chronic nerve constriction injury (CCI) | [42] |
| RS504393 | CCR2 antagonist | Inhibited MCP-1/mediated hyperalgesia | Neuropathic pain | The inferior alveolar nerve and mental nerve | [29] |

| | | | | | transection | |
|------------------|--|--|------------------------|--|-------------|------|
| RS504393 | CCR2 antagonist | Inhibited the function of NMDA and tetanic stimulation induced long-term potentiation (LTP) | Inflammatory pain | Complete Freund's adjuvant (CFA)-induced inflammatory pain | | [11] |
| TLK48462 | CCR2/CCR5 antagonist | Inhibited MCP-1/CCL4-mediated pain | Inflammatory pain | Formalin-induced inflammatory pain | | [79] |
| RS504393 | CCR2 antagonist | Inhibited astroglial activation | Bone cancer pain model | Bone pain | | [12] |
| RS102895 | CCR2 antagonist | Downregulated NMDAR/nNOS signal transduction pathway, upregulated the expression of Toll-interleukin-1 receptor member | Bone cancer pain model | Bone pain | | [64] |
| Levo-corydalmine | A traditional Chinese drug | SIGIRR Inhibited glial cells activation by downregulating the expression of MCP-1/CCR2 | Bone cancer pain model | Bone pain | | [65] |
| Rolipram | Aselective phosphodiesterase 4 inhibitor | Inhibited MCP-1/JNK pathway in astrocyte | Bone cancer pain model | Bone pain | | [67] |

3.2. MCP-1 and inflammatory pain

3.2.1. In CFA and formalin-induced inflammatory pain

Acute inflammation is a physiological process that plays an important role in protecting against invading pathogens and in facilitating tissue remodeling and repair. Contrarily, chronic inflammation is a destructive response which renders tissue injury and pain[44]. Tissue inflammation induced the production and release of a multifarious of chemical mediators, which reacted with nociceptive nerve to lower neuronal excitation threshold and contributed to the development of inflammatory pain[45]. In recent year, MCP-1/CCL signal has also been reported to be involved in inflammatory pain in several animal model of inflammatory pain. For example, IL-6, MCP-1 and CXCL2 were markedly up-regulated and were suggested to associated with pain intensity at 3 h in oral surgery outpatients, the onset of acute pain, indicating that the important role of the increased expression of IL-6 and MCP-1/CCL2 gene in acute inflammation and inflammatory pain[46]. CCR2 antagonist, RS504393, could reverse the heat hyperalgesia induced by

injecting complete Freund's adjuvant (CFA) to the plantar surface of a hind paw[11]. MCP-1 may react directly with excitatory neurons expressing CCR2 in spinal cord to regulate central sensitization via enhancing NMDAR function. Inhibition of CCR2 by RS504393 reduced tetanic stimulation-induced long-term potentiation (LTP)[11], which has been closely associated with the genesis of chronic pain [47]. Also, using Dicer-substrate small interfering RNA (DsiRNA), capable to reach and penetrate sensory neurons, decreased the gene expression of both rat CCR2 (rCCR2) and MCP-1, and therefore reversed CFA-induced inflammatory pain. Furthermore, rCCR2 DsiRNAs may change the activation state of microglia in spinal cord without any density changes of glial cells[48]. In acute nociception CCL2 tg (mice overexpressed CCL2 under control of glial fibrillary acidic protein promoter (CCL2 tg)) mice, they exhibited obvious increased nociceptive behavior after thermal and chemical stimulus and greater thermal hyperalgesia and longer duration in CFA-induced inflammatory pain[49].

Formalin injection induces a biphasic response: the first phase (neuropathic pain) and the second phase (inflammatory pain)[50]. The first phase is due

to the peripheral stimulus-induced C-fibre activation[51] while the second phase seems to be involved in inflammation-induced central sensitization in the dorsal horn of the spinal cord[44]. TLK48462, a novel dual antagonist of CCR2/CCR5 antagonist, attenuated both neuropathic and inflammatory pain evoked by formalin injection, indicating the relevance of CCR2/CCR5 in both neuropathic and inflammatory pain. Moreover, TLK48462 also decreased carrageenan-evoked thermal or mechanical hyperalgesia, further verifying that the crucial role of CCR2/CCR5 in inflammation-induced pain reactions[52].

3.2.2. In pelvic pain

Pelvic pain is a hallmark symptom of chronic inflammatory condition in interstitial cystitis/ bladder pain syndrome (IC/BPS) and chronic pelvic pain syndrome (CPPS)[53,54]. It is currently thought that inflammatory mediators including cytokines and chemokines are the pathogenesis of pelvic pain[55]. The expression of MCP-1 was markedly increased in bladder tissues[53,55,56] and urine[53,55] and lumbosacral DRG cells[57] in IC/BPS rats. MCP-1 binding with CCR2, expressed in bladder tissues and mast cells (MCs) surfaces, promoted degranulation of MC and increased the release of histamine from MCs, which play an important role in the development of IC/BPS [55]. The numbers of activated mast cells was also increased in the bladder in a uroplakin peptide-evoked IC/PBS[56]. MCP-1 promoted mast cell accumulation in bladder via CCR2. Suppression the effects of mast cell by ranitidine, cetirizine, and cromolyn sodium or by deleting MCP-1 or Ccr2 gene markedly attenuated chronic tactile allodynia[56]. In contrary, MCP-1/CCR2 up-regulation in the urothelium and suburothelial nerve plexus of the urinary bladder has been reported to contribute to bladder inflammation, which results in pelvic pain and bladder dysfunction in acyclovir-induced cystitis model[57]. Moreover, a novel urothelial MCP-1 secretion mouse model (URO-MCP-1) was vulnerable to develop pelvic pain after administration of bladder irritants such as LPS[53].

In addition, the majority of prostatitis cases are classified as CPPS characterized by pelvic pain, voiding symptoms and varying degrees of inflammation[54]. Expression of MCP-1 and macrophage inflammatory protein (MIP-1 α /CCL3) of prostatic secretions (EPS) was increased in both CPPS IIIA and IIIB patients[54]. MCP-1 and MIP-1 α may initiate the early inflammatory process, which acts as effective diagnostic markers and provides a potential strategy for CPPS patients[54]. Interestingly, inhibition of MCP-1 and CCL3 by anti-MCP-1 and anti-CCL3 neutralizing antibodies or gene knock-out mice reduced pelvic pain development in autoimmune prostatitis (EAP), a

murine model of chronic prostatitis/chronic pelvic pain syndrome (CPPS), but only anti-CCL2 antibodies were effective therapeutically[58]. Besides, it is recently reported that TRPV1 and MCP-1/CCR2 pathways are involved in post-urinary tract infection (UTI)-induced chronic pain. TRPV1 played a role in establishment of *E. coli*-induced post-UTI chronic pain while MCP-1/CCR2 was crucial to maintenance of chronic pelvic pain[59].

3.3. MCP-1 and cancer pain

Cancer pain, especially bone cancer pain, is an important clinical issue arose in patients with advanced breast, prostate, and lung cancer, which seriously disrupt a patient's quality of life[60]. Cancer-induced pain is a mixed pain that combines both neuropathic, inflammatory, ischemic, and visceral mechanisms[61]. Current traditional analgesic ladder strategy is not effective and brings severe side-effects[62], which promotes to find novel analgesic targets and treatments. It is reported that the expression of MCP-1 and CCR2 was up-regulated in the spinal cord in bone cancer pain (BCP) rats[61,63]. However, evidence also showed that the expression of MCP-1 is increased in astrocytes and microglial cells, without up-regulation of CCR2 in tumor-bearing mice[12]. MCP-1 acted with CCR2, expressed in microglia, induced the spinal microglia activation, which contributed to central sensitization, and increased the crosstalk between neurons and glia. Suppression of MCP-1 or CCR2 by anti-MCP-1 neutralizing antibody or RS 504393 reduced the mechanical allodynia in BCP rats[12,63]. Besides, RS 102895, another CCR2 antagonist, has also been reported to suppress the pain induced by both mechanical and thermal stimulation in BCP rats. Analgesic effects of RS 102895 in BCP rats may be associated with the negative role of RS 102895 in NMDAR/nNOS signal transduction pathway and the positive role in Toll-interleukin-1 receptor member SIGIRR[64].

Many drugs that used for cancer pain treatment are targeted at MCP-1/CCR2 signaling. For example, Levo-corydalmine (L-CDL), an alkaloid isolated from roots of *Corydalis chaerophylla*[65], has been reported to play a crucial role in regulating MCP-1/CCR2 expression[66]. L-CDL inhibited the activation of glial cells and secretion of inflammatory factors through downregulating the expression of MCP-1/CCR2 in mRNA and protein levels. Suppression of MCP-1/CCR2 by L-CDL alleviated tumor compression-induced pain (TCIP), providing an alternative medication for TCIP[66]. Also, rolipram (ROL), a selective phosphodiesterase 4 inhibitor, has been showed to reduce mechanical allodynia and thermal hyperalgesia induced by BCP via suppressing MCP-1/JNK pathway in astrocytes[67]. In addition, ROL also decreased the levels of IL-1 β , IL-6 and TNF- α , and thus inhibited

the activation of astrocyte and microglial, producing an analgesic effect[67].

3.4. MCP-1 and other pain

3.4.1. In joint pain associated with osteoarthritis

Osteoarthritis is a common joint disorder accompanied by chronic pain. Inefficient pain management is the reason why an increasing number of patients with osteoarthritis undergo joint-replacement surgery[68]. Evidence showed that MCP-1/ CCR2 axis plays a central role in the development of chronic pain correlated to osteoarthritis[69]. The expression of MCP-1 and CCR2 in mRNA and protein levels was increased in innervating DRG in animal model of osteoarthritis induced by destabilization of the medial meniscus (DMM). MCP-1, binding with CCR2, could excite DRG nociceptive neuron directly as the concentrations of intraneuronal calcium is enhanced after MCP-1 stimulation[69]. Importantly, Ccr2-null mice failed to develop movement-provoked pain although the mechanical allodynia is not been affected. MCP-1/CCR2 pathway is only involved in initiating mechanical allodynia while the movement-provoked pain dependent on the infiltrated macrophage induced by MCP-1[69]. Moreover, the Ccl2/Ccr2 knockout mice inhibited selective inflammatory response genes in the joint such as arginase 1, prostaglandin synthase 2, nitric oxide synthase 2 and inhibin A, and exhibited a 4-5 week delay in the onset of pain-related behavior in mice model of osteoarthritis induced by DMM[13]. Regulating CCL2/CCR2 axis in the DRG or joint may relieve pain associated with osteoarthritis possibly by up-regulating the sensitivity of joint tissues[13].

3.4.2. In lumbar disc herniation (LDH)-associated pain

Lumbar disk herniation (LDH) is very common in young and middle-aged adults[70], and it is a major cause of low back pain (LBP) and sciatica[10]. LDH-associated pain is not only attribute to mechanic pressure but also a significant contribution by chemical factors[71]. Human intervertebral disc tissue has been reported to share the ability to produce the pro-inflammatory chemokines such as MCP-1 and interleukin-8[72]. The expression of insulin-like growth factor-1 (IGF-1) and MCP-1 were increased mainly in the nucleus pulposus cells[73]. IGF-1 could direct increase the activation of nuclear factor-k-gene binding (NF-kB) signaling while MCP-1 enhanced the expression of receptor activator of NF-kB (RANK), and enhanced cellular sensitivity to RANKL, led to increase osteoclastogenesis and activity, which aggravated vertebral erosions[73]. Targeting MCP-1 and IGF-1 may provide potential clinical significance in LDH

and LBP treatment. Moreover, CX3CL1 and MCP-1 has been demonstrated to play crucial roles in maintaining pain in LDH patients[74]. It is suggested that only local expressions of CX3CL1 and MCP-1, such as in the soft tissues around nerve root (STANR) instead of serum levels, were positively correlated with LDH-induced pain, indicating that LDH-associated inflammation or pain is a local response rather a systemic activity[74]. Interestingly, evidence from another study showed that the concentration of MCP-1 in blood samples of LBP patients is lower than healthy people, and MCP-1 was also lower in patients with acute non-persistent than in those with persistent LBP, reflecting a progressive adaptation mechanism of MCP-1 reduced the process of pain sensitization in LBP patients[75]. Also, the expression of MCP-1 and CCR2 was increased in the DRG and the spinal cord under LDH-induced pain condition[10]. Targeting CCL2/CCR2 signaling may be an efficiency therapy for patients with LDH-induced pain[10].

3.4.3. In postoperative pain

Ion channels play an indispensable role in pain signal initiation and conduction[76]. The role of potassium (K⁺) channels in pain has captured an increasing attention. ATP-sensitive potassium channel (KATP) family, one family of K⁺ channels, has been reported to share the ability to regulate membrane excitability and neurotransmitter release, and play a crucial role in regulating pain[77,78]. KATP channel subunits Kir6.1, SUR1 and SUR2 were markedly reduced while the expression of MCP-1 and p-JNK was significantly increased in the spinal cord after skin/muscle incision and retraction (SMIR) surgery[79]. Pinacidil (Pina) enhanced the activity of KATP channel while suppressed the expression of MCP-1 and p-JNK in vivo and vitro, which alleviated SMIR-induced mechanical allodynia, suggesting that up-regulation the activity of KATP channel could reduce pain via inhibiting JNK/ MCP-1 signaling pathway[79].

4. Conclusion

It is evident that MCP-1 has diverse functions and may be involved in the development of chronic pain. Both glial cells and neurons could be responsible for MCP-1 action within both the central and peripheral nervous system. A thorough understanding of the role of MCP-1 in pain processing is thus necessary for the design of therapeutics that aimed to target this process to alleviate pain. In this review, we have summarized recent findings associated with the role of MCP-1 in chronic pain. The expression of MCP-1 is increased in varying degree in diverse pain conditions, and plays a crucial role in the genesis of pain via CCR2. Despite the challenge of targeting therapies of chemokines, targeted chemokine signaling, including MCP-1/ CCR2 signaling

pathway should still be considered to improve the efficacy in inhibiting chronic pain processing.

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