

Review

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Epigenetic regulation of CC-chemokine ligand 2 in nonresolving inflammation

Abstract: Inflammation mediated by the crosstalk between leukocytes and resident tissue cells is crucial for the maintenance of homeostasis. Because chemokine ligands and receptors, which recruit a variety of leukocytes, are widely distributed among tissues, it is important to understand the mechanisms regulating inflammatory disease. Chemokines such as CC-chemokine ligand 2 (CCL2) amplify and maintain inflammation through chemokine-cytokine networks after the recruitment of circulating leukocytes. Chemokine-dependent nonresolving inflammation occurs in the peripheral and central nervous systems, and underlies several intractable diseases, including cancer and neuropathic pain. The chronic upregulation of chemokines is often mediated by epigenetic mechanisms consisting of DNA methylation, histone modification, and nucleosome positioning. In particular, histone acetylation and methylation have been shown to play important roles in the upregulation of chemokine expression. In addition to CCL2, several other chemokines strongly contribute to neuropathic pain through epigenetic induction. Consequently, targeting epigenetic changes may have therapeutic potential for nonresolving inflammatory diseases such as neuropathic pain. Further research into the epigenetics of inflammatory diseases should promote the development of novel and effective treatment strategies for intractable inflammatory diseases.

Keywords: chemokine; histone; inflammation; macrophage; neuropathic pain.

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List of abbreviations: ACA, anacardic acid; CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CNS, central nervous system; GRO, growth-related oncogene; H3K27me³, trimethylated histone H3 lysine 27; H3K4me³, trimethylated histone H3 lysine 4; H3K9me³, trimethylated histone H3 lysine 9; H3K9Ac, acetylated histone H3 lysine 9; H4Ac, acetylated histone H4; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDM, histone demethylase; HMT, histone methyltransferase; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; NF- κ B, nuclear factor- κ B; PNS, peripheral nervous system; RANTES, regulated upon activation, normal T-cell expressed and secreted; SLC, secondary lymphoid tissue chemokine.

Introduction

Inflammation due to the crosstalk between leukocytes and tissue resident cells is integral to the maintenance of homeostasis (1, 2). After infection and injury, leukocytes derived from blood vessels participate in tissue repair after the clearance of pathogens and debris. During this process, chemokines, which are small chemotactic cytokines, play a fundamental role in the recruitment of leukocytes, such as monocytes/macrophages, neutrophils, and lymphocytes, into the tissue (3, 4). Chemokines are grouped into four families based on the sequence of their cysteine residues: CC, CXC, CX₃C, and XC (5). Although most chemokines directly induce the chemotaxis of leukocytes through binding to specific seven-transmembrane G-protein-coupled receptors, the relation between chemokine ligands and their receptors is highly complex. Indeed, most chemokine ligands bind to multiple chemokine receptors (6, 7). Because chemokine ligands and receptors have a wide tissue distribution, a thorough knowledge of chemokine systems is important for understanding the mechanisms underlying inflammatory diseases (8). Under normal conditions, inflammation generally resolves after tissue repair to avoid excessive and unwanted tissue damage. However, nonresolving

inflammation leads to functional tissue and organ failure in pathological conditions (9, 10). Herein, we highlight the features of chemokine-dependent chronic diseases associated with nonresolving inflammation and the epigenetic regulation of chemokine expression.

Characteristics of CCL2

CC-chemokines are the largest family of chemokines, and the roles of several members in inflammation have been well characterized (5, 8). Notably, there are numerous reports showing that CC-chemokine ligand 2 (CCL2), also known as monocyte chemoattractant protein-1 (MCP-1), is a major chemotactic and activating factor for monocytes/macrophages and neutrophils expressing its primary cognate receptor, CC-chemokine receptor 2 (CCR2) (3, 11). In peripheral organs, CCL2 is derived from diverse cell types, including monocytes, lymphocytes, neutrophils, fibroblasts, dendritic cells, endothelial cells, and epithelial cells (12). Monocytes differentiate into macrophages through the CCL2/CCR2 pathway after tissue infiltration. The primary purpose of this process is recovery from disease or injury (1, 4). However, in some instances, the CCL2/CCR2 pathway triggers chronic inflammatory diseases associated with non-resolving inflammation, including neuropathic pain, rheumatoid arthritis, asthma, and cancer (10, 13). Infiltrating macrophages recruited by CCL2 are activated in peripheral tissues and produce inflammatory molecules, facilitating cell death or functional disturbance (9, 14, 15). CCL2 is also produced in the central nervous system (CNS) by microglia and astrocytes (16–18). CCL2 is required for innate immunity in the CNS, where microglia and astrocytes are the primary immune cells (19, 20). However, it has been reported that CCL2 disrupts the blood-brain barrier and induces the infiltration of circulating leukocytes into the site of inflammation (21, 22). Leukocytes can worsen inflammatory diseases, such as multiple sclerosis, through crosstalk with resident neurons or glial cells (16). Recent findings show that the CCL2/CCR2 pathway is involved in psychosis and psychic dependence to addictive drugs (17, 23, 24). Consequently, CCL2 is a potential therapeutic target for several disorders associated with nonresolving inflammation.

Role of CCL2 in inflammatory diseases

Owing to the recruitment of leukocytes and activation of tissue cells, CCL2 contributes to various inflammatory

diseases (25, 26). Notably, the expression of CCL2 has been demonstrated in several types of cancer, and it is produced by tumor cells, tissue cells, and bone marrow-derived cells (27, 28). CCL2 facilitates the proliferation and metastasis of tumor cells through the activation of CCR2 signaling (11). CCL2 promotes the angiogenesis and accumulation of tumor-associated macrophages, which are largely correlated with cancer progression (29, 30). In rheumatoid arthritis patients, CCL2 levels are elevated in macrophages and fibroblasts in synovial tissues (31). CCL2 plays key roles in the recruitment of monocytes/macrophages into the tissue, and worsens the state of disease. These findings are confirmed by the evidence that inhibition of CCL2 can attenuate the severity of rheumatoid arthritis (32). Also, in a rodent experimental arthritis model, inhibition of CCL2 ameliorates tissue damage and dysfunction due to the reduction of macrophage infiltration (33). There is some evidence showing the contribution to another inflammatory disease, including asthma and colitis (34–36). Furthermore, the CCL2/CCR2 pathway participates in metabolic diseases associated with macrophage-mediated inflammation (37). Indeed, CCL2 and CCR2 are highly expressed in adipose tissue of obese mammals (37, 38), and these upregulations are paralleled by the increment of macrophage infiltration (39), indicating that CCL2 is crucial for obesity. In addition, plasma CCL2 levels are often elevated in the obese condition, and cause several unwanted effects such as insulin resistance and type 2 diabetes (40, 41). CCL2 has been characterized as a key factor of atherosclerosis related to diabetes. Circulating CCL2 attracts monocytes/macrophages into the arterial wall, and induces the development of atherosclerosis (42).

CCL2 has a significant role in nonresolving inflammation in not only peripheral tissues but also the CNS (25, 43). After ischemic brain injury, CCL2 levels are increased in serum and cerebrospinal fluid (44, 45). Then, CCL2 triggers prolonged neuronal damage and dysfunction owing to the accumulation of inflammatory macrophages and neutrophil. Accumulation of circulating leukocytes into the CNS hardly occurs under the physiological state because of the presence of the blood-brain barrier (21). Thereafter, neuroinflammation after the penetration of leukocytes into the CNS can diminish neuronal functions. In multiple sclerosis, a chronic autoimmune disease, CCL2 levels are elevated in reactive astrocytes and infiltrating macrophages (46, 47). The promoting role of CCL2/CCR2 pathway in multiple sclerosis has been clarified using an experimental autoimmune encephalomyelitis animal model (48). Similarly, elevated CCL2 in serum and cerebrospinal fluid is observed in Alzheimer's disease (49), which is a typical neurodegenerative disease. Several lines of

evidence indicate that CCL2 worsens Alzheimer's disease through amyloid deposition and microglial accumulation, leading to nonresolving inflammation in the CNS (43, 50).

Role of CCL2 in neuropathic pain

Accumulating evidence indicates that CCL2 plays a major role in neuropathic pain associated with neuroinflammation (13, 51). Neuropathic pain, characterized by long-lasting allodynia, hyperalgesia, and spontaneous pain, is produced after damage to the nervous system by injury, diabetes, cancer, or chemotherapy (52, 53). Because the symptoms of neuropathic pain are normally resistant to standard analgesics (54), novel therapeutic strategies are urgently needed. After peripheral nerve injury, macrophages, neutrophils, and lymphocytes accumulate in the injured region (53, 55, 56). Similar to other inflammatory conditions, the accumulation of macrophages depends, at least in part, on the CCL2/CCR2 pathway. Inhibition of CCL2 or CCR2, produced by macrophages and resident cells such as Schwann cells, suppresses neuropathic pain (57–59). CCL2 is also secreted by primary afferent neurons and spinal astrocytes after nerve injury (60–62). CCL2 delivered into the spinal dorsal horn causes abnormal discharge of secondary neurons and decreases the pain threshold through CCR2 activation (63). Furthermore, spinal microglia are activated by the CCL2/CCR2 pathway (64), and activated microglia play a pivotal role in spinal sensitization of pain transmission leading to neuropathic pain (65, 66). As a result, CCL2 is considered a key effector of neuropathic pain in the peripheral nervous system (PNS) as well as in the CNS.

Other chemokines involved in neuropathic pain

Among the several chemokines contributing to neuropathic pain, CCL2 was the first to be identified and is the best characterized. Recent reports suggest that other chemokines also significantly participate in neuropathic pain in the PNS and CNS (56, 67, 68). In the PNS, CCL3 (macrophage inflammatory protein-1 α ; MIP-1 α) (69), CCL4 (MIP-1 β) (70), CCL5 (regulated upon activation, normal T-cell expressed and secreted; RANTES) (71), CXCL2 (growth-related oncogene β ; GRO β) (72, 73), and CX₃CL1 (fractalkine) (74) were identified as key mediators of neuropathic pain. These chemokines substantially facilitate

the development of peripheral sensitization and mediate neuropathic pain through the recruitment of leukocytes and the cytokine-chemokine network (53, 67). In comparison, CCL1 (I-309) (75), CCL3 (76, 77), CCL7 (monocyte chemoattractant protein-3; MCP-3) (78), CCL21 (secondary lymphoid tissue chemokine; SLC) (79), CXCL1 (GRO β) (80), and CX₃CL1 (81, 82) were found to be responsible for the central sensitization associated with neuropathic pain after the activation of glial cells. Nevertheless, the detailed molecular mechanisms underlying neuropathic pain remain unclear. A comprehensive understanding of the role of chemokines is required to develop effective therapeutic strategies for neuropathic pain. The respective roles of chemokines leading to neuropathic pain are presented in Table 1.

Epigenetic regulation of gene expression

A detailed understanding of the mechanisms regulating gene expression is crucial for elucidating the complex role of chemokines and cytokines in inflammation (83, 84). Gene expression is tightly regulated in the physiological and pathological states (85, 86). The nucleosome, a fundamental unit of chromatin structure, is composed of genomic DNA and core histones, including H2A, H2B, H3, and H4 (87). The chromatin structure is plastic and dynamically changes through nucleosome modification (88), an epigenetic process that regulates chemokine and cytokine gene expression. Epigenetic alterations are categorized into three types: DNA methylation, histone modification, and nucleosome positioning (86, 88). DNA methylation often occurs in C-G-rich sequences, known as CpG islands, in promoter regions, and silences gene expression (89). DNA methylation mediated by DNA methyltransferase participates in physiological functions such as genomic imprinting (90). Histone modifications are closely related to chromatin dynamics and regulate the transition between transcriptionally active euchromatin and inactive heterochromatin. Histone modifications, such as acetylation, methylation, phosphorylation, and ubiquitination, mainly occur in the histone tail of core histones (87, 91, 92). In this review, we focus on the impact of acetylation and methylation on chemokine expression. Nucleosome positioning influences the accessibility of several transcriptional proteins and regulators on DNA target sequences (93), and is affected by DNA methylation and histone modification. Thus, epigenetic mechanisms critically regulate gene expression in both

Table 1 Epigenetic induction of chemokines contributing to neuropathic pain.

Molecule	Histone modification	Region	Reference
CCL1 (I-309)		Spinal cord	(75)
CCL2 (MCP-1)	H3K9Ac ↑; H3K4me ³ ↑	Peripheral nerve Spinal cord	(58, 59, 109) (61, 62, 64)
CCL3 (MIP-1α)	H3K9Ac ↑; H3K4me ³ ↑	Peripheral nerve Spinal cord	(69, 109) (76, 77)
CCL4 (MIP-1β)		Peripheral nerve	(70)
CCL5 (RANTES)		Peripheral nerve	(71)
CCL7 (MCP-3)	H3K27me ³ ↓	Spinal cord	(78)
CCL21 (SLC)		Spinal cord	(79)
CXCL1 (GROα)		Spinal cord	(80)
CXCL2 (GROβ)	H3K9Ac ↑	Peripheral nerve	(72, 73)
CX3CL1 (fractalkine)		Peripheral nerve Spinal cord	(74) (81, 82)

Several chemokines are, at least in part, upregulated after histone modification and contribute to neuropathic pain in both the PNS and CNS.

the physiological and pathological states. There are several reports indicating that epigenetic modification of chemokine and cytokine gene expression is associated with intractable diseases characterized by nonresolving inflammation (94, 95).

Histone acetylation and CCL2

Histone acetylation is regulated by two enzymes, histone acetyltransferase (HAT) and histone deacetylase (HDAC) (91, 96). Acetylation occurs on lysine residues in the histone tail, and the degree of acetylation can dictate the chromatin structure. The hyperacetylation of histones by HAT induces the euchromatin state, enhancing transcription. In contrast, the heterochromatin state, produced by the hypoacetylation of histones, silences transcription (85, 86, 97). Several studies have demonstrated that these histone modifications modulate the expression of CCL2, a critical regulator of inflammatory diseases, in inflammatory cells (98–102). It is well known that CCL2 is upregulated in activated macrophages and fibroblasts by inflammatory stimuli (12). Transcription factors, such as nuclear factor-κB (NF-κB), Sp1, and c-Jun, contribute to the upregulation of CCL2 induced by histone acetylation (99, 101). Therefore, transcription of CCL2 in these cells is directly and indirectly enhanced under inflammatory conditions.

Pharmacological and genetic approaches have shown that expression levels and activities of HAT and HDAC influence the expression of CCL2. Activation of HAT, such as by p300/CREB binding protein, often increases CCL2 expression by promoting histone acetylation (99). The

expression levels of HDAC family members, which are modulated by inflammatory molecules, intricately regulate the expression of CCL2 (98, 100–102). For example, HDAC3 can activate intracellular NF-κB and c-Jun signaling, resulting in the upregulation of CCL2, whereas HDAC2 decreases CCL2 expression through histone deacetylation in mast cells (101). The hyperacetylation of histones robustly enhances the transcription of CCL2 in most cells (103). In the brain after injury, CCL2 expression is upregulated through histone acetylation, and this upregulation can be prevented by inhibiting HDAC (104). In Muller glia, upregulation of CCL2 depends on both histone H3 acetylation and NF-κB activation (105).

Histone methylation and CCL2

Methylation of lysine and arginine residues in core histones, which is regulated by histone methyltransferase (HMT) and histone demethylase (HDM), affects gene expression in a manner distinct from acetylation (92). For histone H3, trimethylation of K4 (H3K4me³) induces euchromatin, whereas H3K9me³ and H3K27me³ promote heterochromatin formation (83, 106). Recent reports have clarified how the frequencies of histone modifications influence gene activity in human antigen-presenting cells, i.e., monocytes, macrophages, and dendritic cells (107). Approximately 70% of histone H3 associated with transcriptionally active genes is modified, and H3K4me³ and acetylated H3 (H3Ac) account for most of the modifications in these cells. In contrast, H3K27me³ and unmodified histone H3 are associated with most of the inactive genes in these cell types (107). Thus, we predict that acetylation

and methylation of histone H3 both play pivotal roles in regulating the expression of chemokines, including CCL2. A previous report showed that upregulation of CCL2, linked to hypermethylation of H3K4 and hypomethylation of H3K27, is associated with kidney damage in diabetes (108). In addition, we recently demonstrated that CCL2 expression is upregulated in macrophages after peripheral nerve injury in a model of neuropathic pain, and is linked to a shift toward euchromatin, based on H3K4me³ and H3K9Ac modifications (109) (Figure 1).

Epigenetic regulation of inflammatory diseases

Accumulating research suggests that epigenetic mechanisms play a critical role in the pathogenesis of intractable diseases associated with nonresolving inflammation (88, 94, 110). Indeed, the expression of various inflammatory chemokines, including CCL2, and cytokines such as interleukin-1 β and tumor necrosis factor- α are controlled by epigenetic processes (111, 112). Cancer, in particular, has a strong epigenetic component. Several reports indicate that DNA methylation, histone modification (acetylation

and methylation), and nucleosome positioning are altered in cancer (86, 88, 89, 100). Similar epigenetic changes contribute to rheumatoid arthritis (95), asthma (113), colitis (114), and type 1 diabetes (115), which are mediated by inflammatory chemokines and cytokines. Furthermore, epigenetic alterations also underlie neurological and neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis (88), and neuropathic pain (112). Pharmacological modulation of histone acetylation using specific HAT and HDAC inhibitors can control the severity of these diseases (91).

Epigenetic regulation of neuropathic pain

Epigenetic alterations play critical roles in neuropathic pain through the regulation of pain-related genes. We previously demonstrated that upregulation of CCL2 in the injured peripheral nerve is mediated by histone acetylation and methylation (109). H3K9Ac and H3K4me³ in the CCL2 promoter region are elevated in macrophages accumulating around the injured nerve. Similar to CCL2, upregulation of CCL3 is also mediated by H3K9Ac and

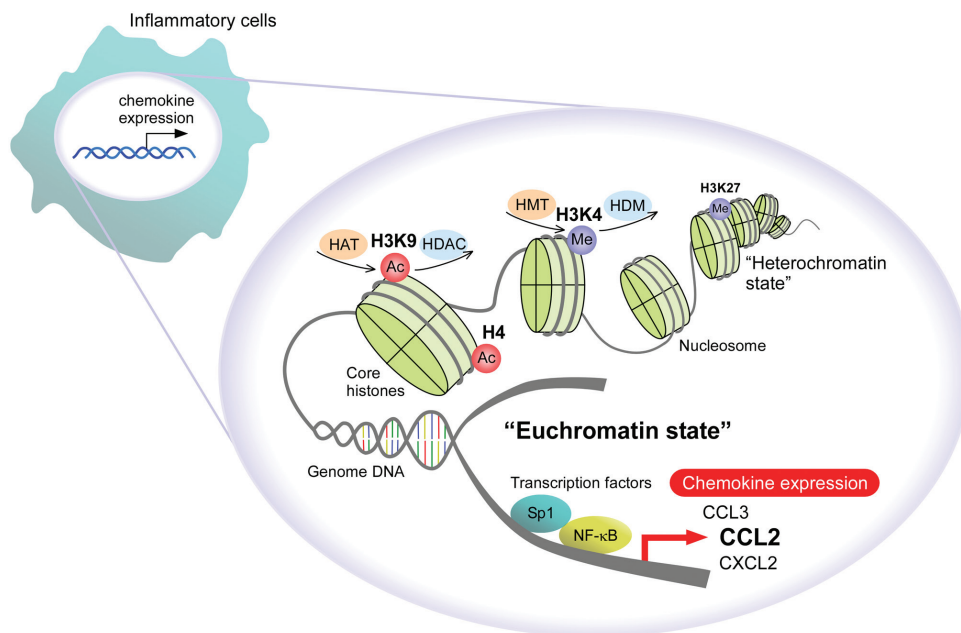


Figure 1 Epigenetic mechanisms regulating chemokine expression.

In the nuclei of inflammatory cells, histone acetylation (H3K9Ac, H4Ac) and methylation (H3K4me³) induce the euchromatin state, enhancing gene expression. In contrast, histone methylation (H3K9me³ or H3K27me³) promotes the heterochromatin state, repressing gene expression. These alterations are enzymatically regulated by HAT, HDAC, HMT, and HDM. Under the euchromatin state, transcription factors can easily access the promoter regions of inducible genes, such as chemokines.

H3K4me³ in macrophages (109). Upregulation of CCL2 and CCL3 is suppressed by a HAT inhibitor, anacardic acid (ACA) (116), indicating the direct epigenetic induction of these chemokines in the PNS after injury. Moreover, CXCL2 is epigenetically upregulated in accumulating leukocytes after nerve injury through H3K9Ac, as shown by the suppression of CXCL2 by ACA (73).

Epigenetic alterations contribute to both the upregulation and downregulation of several key factors involved in neuropathic pain. After nerve injury, neuron-restrictive silencer factor is upregulated in primary sensory neurons after histone H4 acetylation, leading to the chronic suppression of μ -opioid receptor and sodium channel expression, resulting in C-fiber dysfunction (117). In the spinal cord, CCL7 is drastically upregulated after peripheral nerve injury, and participates in neuropathic pain. It has been reported that the long-lasting induction of CCL7 is due to the concomitant downregulation of H3K27me³ (78). Taken together, these findings suggest that the epigenetic upregulation of chemokines, including CCL2, is a fundamental component of neuropathic pain associated with nonresolving neuroinflammation.

Summary and outlook

Chemokines and cytokines play a pivotal role in both physiological and pathological responses through the activation of target inflammatory cells. Notably, chemokines such as CCL2 amplify and maintain inflammation due to the activation of the chemokine-cytokine network after the recruitment of circulating leukocytes. Chemokine-dependent nonresolving inflammation occurs in both the PNS and CNS, and underlies several intractable diseases. Long-lasting upregulation of chemokines and cytokines is often mediated by epigenetic mechanisms such as histone acetylation (e.g., H3K9Ac) and methylation (e.g., H3K4me³). Therefore, targeting epigenetic alterations may have therapeutic potential for nonresolving inflammatory diseases, including neuropathic pain. Nevertheless, a number of processes in the epigenetic regulation of chemokines and cytokines remain unclear. For example, the factors that initiate and maintain the epigenetic changes are poorly understood. In addition, the cell-type-specific effects of epigenetic alterations among diverse cell types that contribute to the pathogenesis of intractable diseases are also unknown. Further studies are required to address these and other unresolved issues. A better understanding of the underlying epigenetic processes should promote the development of novel and

effective targeted therapeutic strategies for intractable inflammatory diseases.

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