Harmonizing hope: navigating the osteoarthritis melody through the CCL2/CCR2 axis for innovative therapeutic avenues

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Osteoarthritis (OA) is characterized by a complex interplay of molecular signals orchestrated by the CCL2/CCR2 axis. The pathogenesis of OA has been revealed to be influenced by a multifaceted effect of CCL2/CCR2 signaling on inflammation, cartilage degradation, and joint homeostasis. The CCL2/CCR2 axis promotes immune cell recruitment and tips the balance toward degeneration by influencing chondrocyte behavior. Insights into these intricate pathways will offer novel therapeutic approaches, paving the way for targeted interventions that may redefine OA management in the future. This review article explores the molecular symphony through the lens of the CCL2/CCR2 axis, providing a harmonious blend of current knowledge and future directions on OA treatment. Furthermore, in this study, through a meticulous review of recent research, the key players and molecular mechanisms that amplify the catabolic cascade within the joint microenvironment are identified, and therapeutic approaches to targeting the CCL2/CCR axis are discussed.

1 Introduction

Osteoarthritis (OA) is characterized by the progression of articular cartilage degradation, inflammation, and changes in joint homeostasis that make osteoarthritis a significant global health burden (1). OA is associated chiefly with weight-bearing joints like the knees, hips, and spine, but it can also affect hands (2). It is associated with symptoms such as joint pain, swelling, and stiffness, with several risk factors including aging, obesity, injuries to joints, genetic predispositions, and certain metabolic conditions (3). Treatment strategies involve symptom management, lifestyle modifications, physical therapy, and, in severe cases, surgical interventions based on medical histories and physical examinations (4–6). Evidence revealed that the immune system and its components play a pivotal role in the pathogenesis of OA because immune cells are activated in OA, contributing to cartilage

degradation and joint inflammation (7, 8). Inflammatory mediators are released in OA, contributing to cartilage degradation and joint inflammation, previously considered a non-inflammatory disease (9). A complex interplay of molecular signals is orchestrated by the chemokine (C-C motif) ligand 2/chemokine (C-C motif) receptor 2 (CCL2/CCR2) axis in the pathogenesis of OA (10). As part of the intricate symphony of OA progression, this multifaceted signaling pathway profoundly impacts inflammation, cartilage degradation, and joint homeostasis (11). Inflammation cascades in the joint microenvironment are perpetuated by the CCL2/CCR2 axis because it promotes immune cell recruitment (12). As a result of this orchestrated immune response, the balance tips towards degeneration, influencing the behavior of chondrocytes and exacerbating the joint's catabolic process (13). OA is multifactorial, involving mechanical stress, genetic factors, and the immune system. A personalized treatment plan emphasizes lifestyle modifications, weight management, and physical therapy to address inflammation and structural changes (14). Moreover, nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroid injections, and disease-modifying osteoarthritis drugs (DMOADs) can manage the immune response (15). Emerging biological therapies also target specific inflammatory pathways and have promising clinical outcomes in patients with OA (16).

Accordingly, this review offers an in-depth overview of the molecular symphony orchestrated by the CCL2/CCR2 axis in OA, providing valuable insights that could pave the way for innovative and targeted interventions in the future, thereby redefining the management of this prevalent musculoskeletal disorder.

2 Understanding osteoarthritis

2.1 The onset: tracing the origins of osteoarthritis

Cartilage, which covers the ends of the bones of the joints, gradually breaks down in degenerative joint diseases such as OA (17). As cartilage wears away, bones rub against one another, causing joint pain, stiffness, and reduced mobility (18). As a result of prolonged inactivity or excessive use, OA causes joint pain, swelling, and stiffness. Several risk factors influence the development of OA. As cartilage naturally deteriorates with age, OA may lead to a reduced range of motion, joint instability, and the development of bone spurs over time. Therefore, aging can be another significant risk factor for OA development (5, 19). The development of OA is also associated with obesity since excess weight causes joint stress, with joint injuries and trauma that can accelerate cartilage breakdown (20). Genetic factors, joint overuse, and certain metabolic conditions can cause OA (21). As part of the diagnosis of OA, a physician will assess a patient's medical history, perform a physical examination, and conduct imaging studies (22). A clinical evaluation aims to determine whether there are characteristic symptoms of joint pain and exclude other possible causes. Symptoms of OA can be managed, and joint function improved through lifestyle modifications, physical therapy, pain medications, and surgical interventions such as joint replacement in

severe cases (23). The quality of life of individuals with OA must be improved through early diagnosis and holistic management.

Studies also emphasized the substantial role of trauma in the etiology of OA, particularly focusing on post-traumatic osteoarthritis (PTOA) (24, 25). It highlights that trauma can be responsible for a considerable proportion of OA cases, with U.S. clinical statistics indicating that PTOA represents 12–42% of OA cases (26). Regarding the prevalence and impact of PTOA, noting that knee injuries, mainly those involving the anterior cruciate ligament (ACL) and meniscal damage, considerably increase the risk of developing OA. PTOA has a distinct molecular pathophysiology compared to age-associated OA, which may explain the poor translation of preclinical outcomes to clinical trials. Therefore, there is a requirement for targeted therapeutic interventions and the development of predictive biomarkers to prevent and manage PTOA, emphasizing that understanding trauma-induced OA molecular mechanisms is essential for obtaining promising clinical outcomes (26).

2.2 The joint ensemble: cell types and their roles in osteoarthritis progression

Chondrocytes, the cells that reside in cartilage, play a crucial role in maintaining its structural integrity. Every cell in the joint plays a role in the progression of OA (27). The chondrocytes in OA undergo phenotypic changes and produce enzymes that contribute to cartilage destruction, resulting in an imbalance between cartilage degradation and repair (28). Synoviocytes, found in the synovium surrounding joints, become activated in OA, releasing inflammatory cytokines and enzymes that exacerbate joint inflammation and contribute to disease progression (29). Moreover, synovial inflammation promotes the production of joint fluid, which causes swelling and pain in the joint (30). Osteoblasts and osteoclasts play an imperative role in bone remodeling (31). Osteophytes and bone spurs are often present in OA due to excessive bone formation (32). Multiple studies in both experimental models and human OA have revealed an elevation in subchondral bone turnover, including bone resorption and formation, during the initiation and progression of the disease (33–36). Typically, bone resorption and formation are coupled processes, but in certain conditions, such as postmenopausal osteoporosis, they can become imbalanced. This imbalance is also detected in patients with osteoporosis or progressive OA, emphasizing a potential uncoupling of these processes in OA. Expressly, in human hip OA, increased bone resorption and formation have been noted in the subchondral bone beneath mild, moderate, and severe cartilage lesions (37). However, an increase in bone volume was only observed beneath moderate and severe lesions. These observations indicate an increase in the frequency of osteoclast in areas with subchondral osteosclerosis in advanced knee OA. Moreover, recent experimental OA models have further reported this uncoupled bone remodeling, disclosing that altered mechanical loading triggers subchondral bone resorption, followed by enhanced recruitment of osteoprogenitors and formation of osteoid islets in marrow spaces (35, 38, 39). Given

the uncoupled nature of bone remodeling in OA, inhibiting bone resorption may benefit both the early (osteopenia) and advanced (osteosclerosis) stages of OA (40, 41). Several types of immune cells, such as macrophages and T lymphocytes, contribute to joint inflammation (42). While traditionally associated with autoimmune responses, T lymphocytes are increasingly recognized for their involvement in the inflammatory processes of OA. Macrophages release pro-inflammatory mediators and enzymes, further accelerating cartilage degradation (43). It is also known that OA also affects the capsule of the joint as well as ligaments and tendons, which are comprised of fibroblasts (44). Fibroblasts produce extracellular matrix (ECM) proteins, and their dysregulation may impact joint stability and function (45). In addition, it has been shown that a non-immune cell, fibroblast-like synoviocytes (FLSs), reside in the synovium's inner layer. They are specialized mesenchymal cells that play an essential role in collagen homeostasis of the articular joint, provide ECM materials to cartilage, and contribute to joint destruction through multiple mechanisms (46). The intricate interplay between these types of cells is crucial for understanding how OA progresses. The current research focuses on identifying critical molecular pathways and therapeutic targets within this joint ensemble. In order to provide more effective treatments for individuals affected by OA, researchers intend to develop targeted interventions that alleviate symptoms and modify the disease course by elucidating the role of these cells in OA pathophysiology.

2.3 Immune system and osteoarthritis

Traditional views of OA as a non-inflammatory form of arthritis have been replaced with recognizing that the immune system plays an essential role in its pathogenesis (47). Immune responses contribute to the inflammation and tissue destruction observed in affected joints, even though OA is primarily characterized by cartilage breakdown and bone changes. OA is caused by the activation of immune cells, which release enzymes and cytokines that contribute to cartilage degradation and inflammation of joints (48). Moreover, OA is characterized by low-grade chronic inflammation in the synovium, which contributes to pain and joint damage and perpetuates the progression of the disease (49). Based on available knowledge, in autoimmune diseases, a wide range of cell types, such as macrophages, neutrophils, NK cells, dendritic cells (DCs), and lymphocytes, are involved in innate and adaptive immune responses (50). Activation of the innate immune system is started upon the ligation of pattern recognition receptors (PRRs) to damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLR), RIG-1-like receptors (RLR), and NOD-like receptors (NLR) are prominent immune sensors involved in this process, stimulating nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, upregulating the expression of interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), and inducing cartilage catabolism (51–53). It has been widely reported that TLR-2 and TLR-4 are upregulated in OA lesions

compared to unaffected OA cartilage and normal cartilage. Additionally, endogenous signals like hyaluronan and fibronectin may activate TLRs 2 and 4 in OA (54). As another essential component of the innate immune system, the complement system, which consists of components C1-C9 and cofactors, is found in an increased abundance of synovial fluid from OA patients. Inflammatory molecules and membrane attack complex (MAC) formation are affected by genetic deficiencies in complement components C5, C6, and CD59a, indicating that complement plays a pivotal role in the progression of OA (55). In OA, cartilage degradation exposes PRRs, leading to persistent innate immune system activation (48).

It has been revealed that the key components of the adaptive immune system are T cells, divided into cytotoxic T cells (CTLs) and T helper (Th) cells. Th cells are categorized into Th1, Th2, Th9, Th17, and Th22 cells (56). Furthermore, B cells are crucial in developing an adaptive immune response through antibody production (humoral immunity). Still, the T cells play an important role in maximizing the protection against intracellular pathogens and tumors (cellular immunity) (57). Depending on the secretion of cytokines, Th2 cells may influence macrophage polarization toward regenerative M2 or inflammatory M1 phenotypes (58). Conventionally, Th cells are involved in microbe elimination; however, these lymphocytes can also participate in inflammatory phenomena and tissue repair processes by inducing macrophage differentiation in response to tissue-derived mediators in an IL-4-dependent manner (59). There is an emerging area of research into the role of T cells in OA progression (48). Evidence reveals that patients with OA have higher T and B cells than healthy controls (60). In OA patients, synovial fluid and tissue have elevated levels of Th1, Th9, Th17 cells, and CTLs, releasing catabolic cytokines like IL-2, IFN-γ, and TNF-α (61). Further research is needed to understand the specific role of T cells in osteoarthritis progression, even though they are present and contribute to cartilage matrix destruction.

2.4 Role of pathogens in osteoarthritis

Pathogens and infections may play a role in OA development via mechanisms involving chronic low-grade inflammation and immune system activation. Evidence revealed that innate immune system-mediated inflammation in OA can be exacerbated by microbiota imbalances and synovial infections, activating toll-like receptors (TLRs) and the complement system, resulting in the degradation of cartilage and synovitis (62, 63). Moreover, gut microbiota can influence OA progression by inducing systemic inflammation, as shown in pre-clinical studies where microbiota isolated from metabolically compromised mice accelerated OA in these animals (64). Furthermore, specific microbes in the joint synovia have been accompanied by hyperinflammation, contributing to OA pathogenesis (65). As discussed, TLRs are a critical component of the innate immune system and play a remarkable role in the pathogenesis of OA. These receptors recognize PAMPs and DAMPs, triggering inflammatory responses. Specifically, TLRs such as TLR2 and TLR4 have been

involved in OA via mediating the expression of chemokines like CCL2 (66). Following infections and activation by PAMPs, TLRs trigger signaling pathways that produce inflammatory mediators, including CCL2. Regarding the role of CCL2 in recruiting monocytes and macrophages to sites of inflammation, its increased levels have been detected in the synovial fluid of OA patients. The CCL2/CCR2 axis is critical in recruiting monocytes/macrophages to the OA joint, resulting in synovitis and cartilage destruction. Accordingly, blocking this pathway considerably reduced inflammation and tissue damage in experimental OA models (67). On the other hand, TLR activation by microbial products or endogenous ligands produces pro-inflammatory mediators and MMPs, degrading the cartilage matrix and provoking inflammation. Studies have reported that TLR2 and TLR4 activation in chondrocytes and synoviocytes upregulates the expression of MMPs, further contributing to joint degradation (68, 69).

Increased levels of lipopolysaccharides (LPS), as an important TLR4 ligand, can occur following impaired hepatic clearance, high dietary fat intake, dysregulation of the endocannabinoid system, decreased intestinal motility, and reduced physical activity (70). Beyond LPS, additional metabolites produced by gut microbiota, capable of promoting OA, might penetrate the circulation via a compromised intestinal barrier. Notably, recent studies have identified microbial DNA, including that from common gut microorganisms, in osteoarthritic cartilage and synovial tissue (71, 72). This evidence suggests that increased intestinal permeability may facilitate the translocation of gut microbes to joint tissues (73).

Collectively, activation of the TLRs and the subsequent expression of CCL2 following infections can play a crucial role in mediating the inflammatory response in OA, leading to synovitis, cartilage destruction, and disease progression. Targeting these pathways holds the potential for developing therapeutic approaches to reduce inflammation and tissue damage in OA.

2.5 Treatment of osteoarthritis

NSAIDs are commonly used to reduce pain and inflammation associated with OA to alleviate symptoms and slow down its progression (74). A disease-modifying osteoarthritis drug (DMOAD) is a treatment for osteoarthritis aimed at altering the underlying disease process and may have immunomodulatory effects on the immune system (75). Additionally, corticosteroid administration can provide targeted relief in OA (76). The interest in biological therapies that target specific inflammatory pathways involved in OA has increased in recent years (77–79). Specific cytokines, chemokines, or other immune mediators often trigger joint inflammatory responses. Several anti-inflammatory drugs and inhibitors, such as monoclonal antibodies and small molecules, can target and block these ligands or their receptors to reduce inflammation (80–82). OA is a multifactorial disease, and several other factors, such as mechanical stress and genetic predisposition, contribute to its pathogenesis (83). Even though the immune system is known to play a role, several other potential therapeutic targets are involved in the pathogenesis of OA. Various approaches are often used in customized treatment plans to address joint inflammation and structural alterations in OA.

3 CCL2 and CCR2: the protagonists

Chemokines and their corresponding receptors are essential in the intricate dance of immune responses. The inflammation and immune regulation narrative is intricately shaped by CCL2 and its receptor CCR2, two key orchestrators among these players.

3.1 CCL2: master conductor of inflammation

In the symphony of immune responses, CCL2 is the master conductor, also termed monocyte chemoattractant protein-1 (MCP-1) (84). The gene coding for CCL2, a 13 kDa glycoprotein consisting of 76 amino acids, is located on chromosome 17 at position q11.2 (85). Among the G protein-coupled receptors (GPCR) family members, CCL2 is a potent chemoattractant primarily for monocytes, NK cells, and memory T cells (86). Monocytes, astrocytic cells, endothelial cells, fibroblasts, keratinocytes, mesangial cells, smooth muscle cells, microglia, and neurons can release CCL2 (87, 88). Following the ligation of CCL2 to CCR2, immune cells infiltrate inflammation sites due to these molecules' ability to recruit and migrate monocytes, T lymphocytes, and DCs (89). Initiating and perpetuating inflammatory cascades depend on its indispensable role in coordinating immune cell trafficking. CCL2 levels are elevated in various pathological conditions, including atherosclerosis, rheumatoid arthritis (RA), OA, diabetes, psoriasis, cancer, and infectious diseases, highlighting its importance in disease pathogenesis (90–94).

Inflammatory stimuli such as IL-1β and TNF-α, as well as microbial products (e.g., Lipopolysaccharide), can increase the expression of CCL2 via various signaling pathways (95, 96). Key pathways include the nuclear factor kappa B (NF-κB) and MAPK pathways, activating transcription factors that bind to the CCL2 promoter, enhancing transcription (97). Extracellular matrix protein cellular communication network factor 1 (CCN1) or Cyr61 has been recognized as a remarkable regulator of CCL2 expression (98). CCN1 can interact with integrins and heparan sulfate proteoglycans to activate NF-κB, increasing CCL2 production (99). This CCN1-related mechanism is critical, as targeting CCN1 could suggest a potential therapeutic tactic for modulating CCL2 levels and subsequent inflammatory responses (100). The induction of CCL2 expression in response to inflammatory stimuli further emphasizes its dynamic role in amplifying immune responses. Upon arrival on the battlefield, this multifaceted chemokine modulates the functions of immune effectors by attracting them. CCL2 expression is regulated by transcriptional, post-transcriptional, and post-translational mechanisms, as shown by studies (101, 102). Understanding CCL2's role in orchestrating immune responses and its potential as a therapeutic target for various inflammatory conditions requires a deep understanding of its regulatory intricacies.

3.2 CCR2 takes the spotlight: its impact on cellular migration and activation

CCR2 plays a crucial role in cellular migration and activation as the designated receptor for CCL2 (103). The CCR2 amino acid sequence exhibits a high degree of identity between species and is a member of the GPCR superfamily (104). Despite sharing conserved cysteine residues with other members of the CCR family, CCR2 has minimal homology with them. CCR2 comprises seven transmembrane domains containing a putative N-linked glycosylation at amino acid 14, a sulfotyrosine at amino acid 26, a phosphotyrosine by Janus kinase 2 (JAK2) at amino acid residue 139, and a disulfide bond at amino acid 113 ↔ 190. Human CCR2 contains multiple domains, including α-helices and β-strands (86). CCR2 expression on the surface of monocytes, macrophages, and specific subsets of T lymphocytes acts as a molecular compass, directing immune cells toward the inflammatory sites with high concentrations of CCL2 (105). Cell migration toward the site of injury or infection is facilitated when CCR2 triggers intracellular signaling cascades upon engagement with CCL2 (84).

Activation of CCR2 has been found to modulate immune cell function beyond its canonical role in chemotaxis. The protein plays a role in regulating macrophage polarization, which affects the balance between pro-inflammatory M1 and anti-inflammatory M2 phenotypes. It has been revealed that the CCL2/CCR2 axis is involved in determining the extent of macrophage polarization because CCL2 enhances the LPS-induced production of IL-10 as an anti-inflammatory and inducer of M2 macrophage polarization. At the same time, CCL2 blockade upregulates the expression of M1 polarization-associated genes and cytokines and downregulates the expression of M2-associated markers in human macrophages (106). In addition, $Ccr2^-$ deficient bone marrow-derived murine macrophages demonstrated an M1-skewed polarization profile at the transcriptomic level and significantly increased proinflammatory cytokine expression when LPS was applied. As a result, the CCL2/CCR2 axis influences macrophage polarization by regulating genes relevant to polarization and proinflammatory cytokines (106). According to the available studies, infiltrating immune cells in an inflammatory milieu is associated with overexpression of CCL2 and CCR2. It has been reported that CCR2-driven activation of NF-κB is involved in regulating DC and Langerhans cell maturation (107). In addition, evidence indicates that CCR2-mediated signaling may modulate T-cell differentiation and activation, influencing adaptive immune responses. Studies conducted *in vitro* and *in vivo* have demonstrated that CCR2 directly influences the production of inflammatory cytokines in T cells. In the absence of CCR2 in T cells, the progression of colitis slows down. Additionally, the lack of CCR2 in T cells triggers a process that increases the number of Foxp3^{+} regulatory T cells (Tregs) and reduces the levels of Th17 cells in living organisms. This suggests that CCR2 is crucial in regulating the immune response by balancing the ratio of effector T cells to Tregs (108). Moreover, disorders characterized by dysregulated inflammation may benefit from therapeutic intervention through the intricate regulatory networks governing their expression and activation (109). Therefore, the collaboration between CCL2 and CCR2 in orchestrating immune responses tells an exciting story of molecular interactions influencing inflammation's course. Understanding the nuances of their roles could lead to the development of targeted therapeutic strategies aimed at fine-tuning immune responses in different pathological conditions.

While the review's main focus is the CCL2/CCR2 axis, it is advantageous to briefly mention other pivotal pathways responsible for OA pathogenesis, influencing chondrocytes and other cell types. Depending on the context, the Wnt/β-catenin signaling pathway has been revealed to play a dual role, both provoking and suppressing chondrocyte activity (110). NF-κB signaling is another essential pathway that mediates inflammation and catabolic responses, including upregulating MMPs and inducible iNOS (54). The TGF-β pathway, as a double-edged sword, can either stimulate or inhibit cartilage degradation based on the specific receptors and involved downstream signaling (111). Furthermore, AMP-activated protein kinase (AMPK) signaling has been recognized to regulate energy homeostasis and inflammatory responses in chondrocytes (112). The role of fibroblast growth factor 2 (FGF2) signaling in the pathogenesis of OA via modulation of Wnt/β-catenin and other signaling pathways emphasizes its potential therapeutic relevance (113). Hypoxia-inducible factors (HIFs) contribute to chondrocyte survival under low oxygen states typical in OA joints (111). These signaling pathways collectively play expressive roles in OA pathogenesis and are imperative considerations alongside the CCL2/CCR2 axis.

4 The intricate dance: CCL2/CCR2 axis in osteoarthritis

The CCL2/CCR2 axis is a pivotal player in the inflammatory complex orchestration. This section aims to provide a comprehensive overview of the current knowledge about the CCL2/CCR2 axis in OA, emphasizing the cellular choreography accompanying inflammation and the delicate balance that governs harmony and discord in this dance (Figure 1).

4.1 Cellular choreography: how CCL2/CCR2 axis orchestrates inflammation

The expression of CCL2 in chondrocytes, osteoblasts, and synovial cells is associated with bone metabolism and OA. In the synovium of patients with OA, CCR2^{+} cells are prevalent (114). The CCL2/CCR2 axis has been demonstrated to regulate inflammation in OA and other pathological conditions (115). Some previous studies showed that this axis orchestrates an intricate cellular choreography during OA pathogenesis (66, 116, 117). In OA, the dynamic interplay between CCL2 and CCR2 is mediated by synovial tissues, chondrocytes, and infiltrating immune system
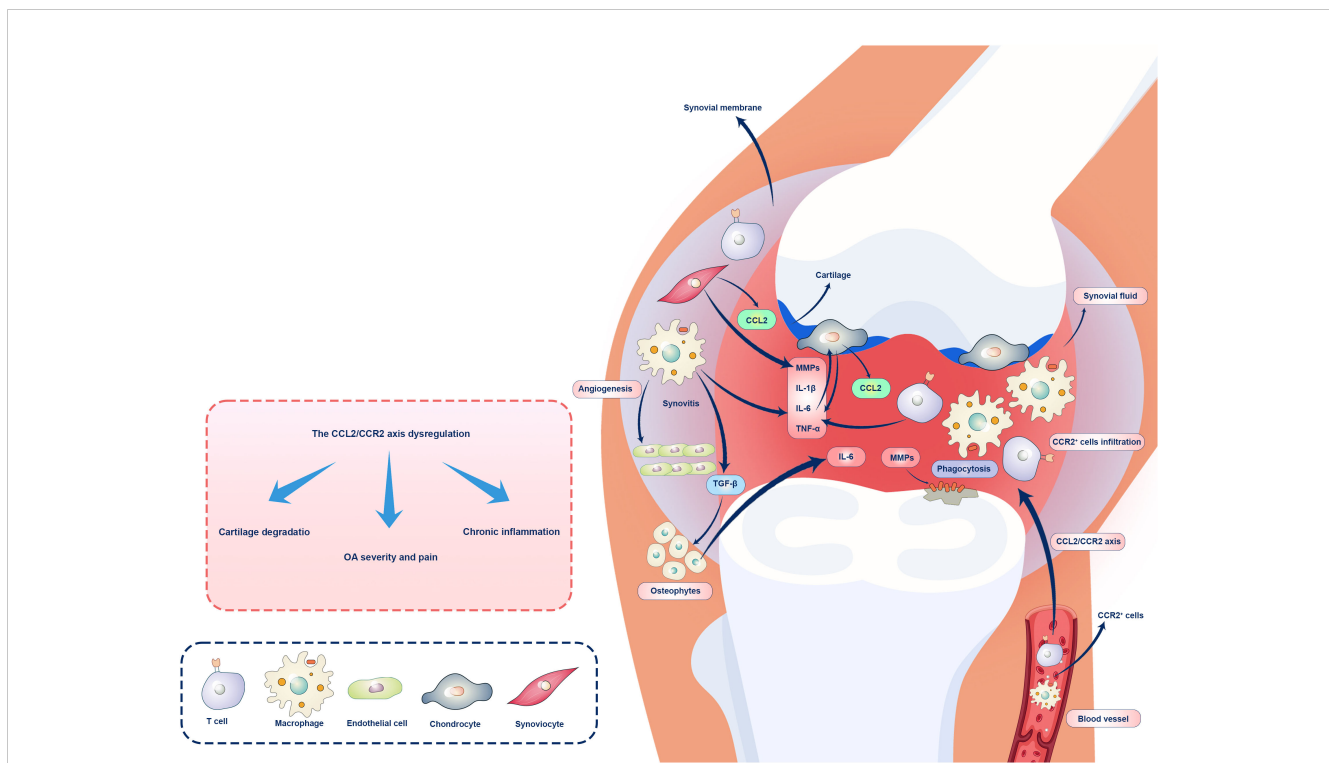
FIGURE 1

Role of the CCL2/CCR2 axis OA immunopathogenesis. Overexpression of CCL2 in chondrocytes and synovial cells is associated with CCR2^{+} macrophages and T cell recruitments and infiltration in the synovium, chronic inflammation, and OA. CCL2/CCR2 axis regulates inflammation in OA and other pathological conditions, and activation of synovial fibroblasts by pro-inflammatory cytokines leads to CCL2 release and recruitment of CCR2^{+} monocytes and macrophages. Macrophages play a role in preserving inflammation and inducing cartilage damage via cytokine production (IL-1β, IL-6, TNF-α) and MMP activation. Chondrocytes upregulate CCL2 expression in response to mechanical stress and inflammatory signals, leading to cartilage degeneration. CCR2 facilitates cartilage and bone damage independently of synovial macrophage infiltration. Ultimately, the dysregulation of the CCL2/CCR2 axis can lead to joint inflammation and tissue damage.

cells. In the synovium, pro-inflammatory cytokines such as IL-1β and TNF-α are responsible for activating synovial fibroblast, releasing CCL2, and recruiting CCR2^{+} monocytes and macrophages (118). Infiltrating macrophages contributes to developing a pro-inflammatory milieu and sustaining inflammation within the joint (119). In addition to their role in innate immunity and tissue remodeling, macrophages produce cytokines and activate matrix metalloproteinases (MMPs), damaging cartilage. Depleting macrophages in mouse models of OA reduced cartilage degeneration and osteophytosis, demonstrating their role in pathologic bone formation (120). A study reported that S100A8 and S100A9 proteins are mainly produced by GM-CSF–differentiated macrophages in the synovium, with lesser production by M-CSF–differentiated macrophages and not by fibroblasts (121). S100A9 stimulation of OA synovial tissue led to increased production of IL-1β, IL-6, IL-8, and TNF-α, as well as upregulation of MMPs. Further experiments revealed that these effects were mainly due to the stimulation of GM-CSF–differentiated macrophages and, to a lesser extent, M-CSF-differentiated macrophages, while fibroblasts remained unaffected by S100A9. Additionally, S100A9 activated canonical Wnt signaling in GM-CSF-differentiated macrophages but not M-CSF-differentiated ones. Using the S100A9 inhibitor paquinimod on OA synovium reduced the activation of this signaling pathway

(121, 122). CCL2/CCR2 dance is also actively performed by chondrocytes, the primary cells of articular cartilage. The expression of CCL2 is increased in chondrocytes in response to mechanical stress and inflammatory signals, resulting in a positive feedback loop that promotes inflammation and cartilage degeneration (123). This process is further exacerbated by the infiltration of CCR2^{+} immune cells into the cartilage, resulting in a progressive loss of cartilage integrity (66).

However, an investigation explored the role of CCR2 in the pathogenesis of injury-induced OA. Utilizing a murine model of injury-induced OA through DMM, CCR2 was systemically blocked using the antagonist RS504393 at various stages of disease progression. Joint degeneration was assessed by evaluating cartilage (including metrics such as cartilage loss, chondrocyte hypertrophy, and MMP-13 expression) and bone lesions (such as bone sclerosis and osteophyte formation) with and without CCR2 inhibition. Pain-related behaviors were also studied by measuring weight distribution between normal and arthritic hind paws using the IITS incapacitance meter. This study showed that the timing of CCR2 antagonist administration was crucial for mitigating joint damage. Early intervention with the CCR2 antagonist within the first four weeks post-injury significantly reduced OA-related cartilage and bone damage. In contrast, later interventions proved less effective. Furthermore, early to moderate CCR2 blockade (1–4

weeks and 4–8 weeks post-surgery, respectively) resulted in decreased pain measures, with sustained pain improvement even after cessation of treatment. These results indicate the potential of early CCR2 antagonism to not only decelerate the progression of post-injury OA but also to alleviate associated pain symptoms, suggesting a therapeutic window for optimizing the efficacy of CCR2-targeted treatments in OA management (124).

It has been demonstrated that CCL2 treatment activated ERK and p38 via CCR2 in healthy chondrocytes. CCL2 treatment of healthy chondrocytes for short (6h) or long (7–72h) periods resulted in upregulating *CCR2, MMP-1, MMP-3, MMP-13*, and tissue inhibitor of matrix metalloproteinase 1 (*TIMP1*). CCL2-mediated upregulation of CCR2 and cartilage catabolic markers was mediated by ERK and p38 signaling in healthy and OA chondrocytes (125). Further, the CCL2/CCR2 axis influences how different types of cells interact in the joint microenvironment. CCR2$^+$ osteoclast precursors are recruited by CCL2, resulting in bone resorption and the characteristic subchondral changes observed in patients with OA (126).

A study explored whether the specific inactivation of the *Ccr2* gene in osteoblasts at various stages of PTOA can improve joint structure, bone parameters, and pain outcomes (126). This study used a tamoxifen-inducible system to inactivate *Ccr2* in collagen1α-expressing cells, resulting in osteoblasts deficient in *Ccr2* (CCR2-Col1αKO). The PTOA model was induced in both CCR2-Col1αKO and control (*CCR2$^{+/+}$*) mice using the medial meniscus (DMM) method destabilization. Recombination was triggered before or after the DMM procedure to compare early versus late inactivation effects. Joint damage was assessed at two, four, eight, and twelve weeks post-DMM using various metrics, including articular cartilage structure (ACS), Safranin-O staining, histomorphometry, osteophyte size and maturity, subchondral bone thickness, and synovial hyperplasia. Pain levels, both spontaneous and evoked, were monitored for up to 20 weeks. Results demonstrated that early inactivation of *Ccr2* in osteoblasts delayed the progression of articular cartilage damage and matrix degradation compared to *CCR2$^{+/+}$* controls, and it also decreased DMM-induced bone thickening. The formation and maturation of osteophytes were only slightly influenced. Conversely, late inactivation of collagen1α-*Ccr2* resulted in less pronounced improvements. Furthermore, *Ccr2* deletion in osteoblasts improved static pain measures, though evoked pain remained unchanged. This study demonstrates that *Ccr2* expression in osteoblasts plays a critical role in the progression of PTOA and associated pain by impacting both cartilage and bone tissues (126).

Taken together, due to the intricate choreography orchestrated by the CCL2/CCR2 axis, OA progression is a multifaceted phenomenon.

4.2 Harmony or discord: balance and imbalance in the CCL2/CCR2 dance

Dysregulation of the CCL2/CCR2 dance can lead to pathology, crucial to maintaining a delicate balance. It has been suggested that

disruptions in the equilibrium between CCL2 and CCR2 are responsible for the severity and progression of OA (127). A CCL2/CCR2 axis-mediated mechanism is thought to mediate the pain of OA, suggesting that increased CCL2 levels during OA may indicate altered bone and cartilage metabolism, causing pain and destruction of joints (114). There is evidence that elevated CCL2 levels in synovial fluid and serum are related to OA severity and pain (128, 129). As a result of dysregulated CCL2 production in response to inflammatory stimuli, chronic inflammation is perpetuated, leading to relentless cartilage degradation. A positive correlation has been reported between synovial fluid CCL2 concentrations, the Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain ratings, WOMAC function scores, and total WOMAC scores (128). This association remained considerable even after adjusting for potential confounding factors through multivariate linear regression analysis. Additionally, the correlation between synovial fluid and serum CCL2 concentrations with the K-L grading system has been evaluated, and no significant difference was found, suggesting that CCL2 concentrations associated with radiographic changes in knee OA patients were not linked to symptomatic severity (128). These findings suggest that CCL2 synovial fluid levels could be a novel biochemical marker reflecting pain severity and functioning as a pain mediator in knee OA patients. These outcomes may pave the way for new, targeted therapeutic approaches to alleviate pain and its associated symptoms in patients with knee OA.

Excessive activation of inflammatory pathways due to dysregulated CCR2 signaling can lead to joint inflammation and tissue damage. In contrast, inadequate activation of CCR2 may result in a lack of immune cells recruited to repair tissues and resolve inflammation (130). Furthermore, the interplay between the CCL2/CCR2 axis and other signaling pathways adds another layer of complexity to the dance. Cross-talk with Transforming Growth Factor-beta (TGF-β), NF-κB, and MAPK pathways modulate the inflammatory response in OA (131). Consequently, Disease progression can be positively or negatively affected depending on how these interactions are balanced.

A potential role for TGFα-EGFR signaling in OA progression has been identified, particularly in its untested role *in vivo* (132). Except for endothelin A, the downstream effectors of TGFα signaling in cartilage are poorly understood. It has been shown that EGFR signaling induces the expression of CCL2 in osteoblasts, and CCL2 is elevated in human OA cartilage, which promotes proteoglycan loss (127). When CCL2 is elevated, its expression is further driven by a positive feedback loop that prevents mesenchymal progenitor cells from differentiating along chondrogenic lines, inhibiting natural joint repair processes (133). It was revealed that exposure to CCL2 prompts synovial multipotent progenitor cells (sMPCs) pro-inflammatory activation (increasing the expression of IL-6 in sMPCs) while impeding their ability to undergo chondrogenesis *in vitro*, the process by which these cells mature into cartilage. Furthermore, exposure to CCL2 at levels typically found in OA knee joint synovial fluid induces significant alterations in the gene expression profile of

sMPCs. Additionally, prolonged exposure to CCL2 results in the heightened expression of CCL2 itself within sMPCs, setting up a positive feedback loop from which these cells struggle to disengage. Consequently, elevated CCL2 can attract sMPCs to the site of injury but also disrupt the normal transcriptional regulation of these cells, hampering their progression toward chondrogenesis (133). These findings present promising avenues for further exploring the mechanisms underlying the observed lack of intrinsic repair following articular cartilage injury and/or arthritis.

The interaction between TGF-α and CCL2 plays a critical role in the pathogenesis of OA (134, 135). A study elucidated that TGF-α stimulates the expression of CCL2 through various intracellular signaling pathways, including MEK/ERK, p38 MAPK, and phosphoinositide 3-kinases (PI3Ks) in chondrocytes (136). TGF-α-induced CCL2 expression subsequently promotes the production of MMPs, specifically MMP-3, which contributes to the degradation of type II collagen and aggrecan, essential components of cartilage extracellular matrix. Pharmacologic inhibition of either TGF-α or CCL2 signaling has been revealed to reduce cartilage degradation and disease progression in a rat model of posttraumatic OA, suggesting that the TGF-α/CCL2 axis is a significant pathway in OA pathophysiology (136). These findings indicate that targeting the TGF-α/CCL2 signaling pathway could be a promising therapeutic approach to mitigate cartilage destruction in OA.

Polymorphisms, while not directly involved in the pathogenesis of OA, can considerably increase the risk of developing the disease. These genetic variations can impact the biological pathways associated with OA, thereby acting as imperative prognostic biomarkers (137). By identifying individuals with specific polymorphisms, it is possible to predict the probability of OA onset and progression, enabling more personalized approaches to prevention and management. In this context, a study found that specific variations in the CCL2 gene, rs1024611 and rs4586, significantly increased the risk of developing OA, emphasizing the central role played by the CCL2/CCR2 axis in disease susceptibility (138). After further analysis, it was determined that the rs1024611 polymorphism was associated with increased risk for males and non-drinkers. In contrast, the rs4586 polymorphism was associated with heightened risk for smokers and drinkers. As a result of the rs4586 polymorphism, individuals with the CC genotype showed significantly higher levels of CCL2 compared to those with TT genotypes. As a result, variation in CCL2, particularly rs1024611 and rs4586, is associated with OA susceptibility and could serve as early diagnostic markers (138). Another study revealed that certain genetic variations within the CCL2 gene might be linked to an increased risk of developing knee OA (139). Specifically, the rs2857657 variant (G) allele appears more common in female knee OA cases than in individuals without OA. Additionally, identifying a haplotype (H5) exclusively in the control group implies a potential protective effect against knee OA (139). These findings entail that CCL2-associated genetic factors could contribute to the susceptibility to knee OA.

Collectively, CCL2 appears to be a crucial downstream mediator in the cartilage degradation process, including the MEK/ERK pathway, according to the study.

5 Clinical implications and therapeutic perspectives

With the emergence of targeted therapies for various chronic inflammatory diseases, such as RA and OA, focusing on the CCL2/CCR2 axis and its clinical implications can offer new perspectives on how to manage these common and debilitating joint diseases (Figure 2). The clinical implications and therapeutic perspectives of these emerging strategies reveal their potential to reshape the treatment landscape for OA. Treatment of OA requires a personalized approach due to the heterogeneity and diversity of the disease (140). By targeting the CCL2/CCR2 axis, interventions can be tailored to patients' underlying inflammatory profiles. Developing biomarker-driven therapeutic approaches that identify patients with elevated CCL2/CCR2 signaling could pave the way for more effective and personalized treatments (141). Diagnostic tools could be developed to stratify patients by investigating the genetic and molecular signatures associated with the dysregulation of CCL2/CCR2 in OA. Precision medicine facilitates OA management because clinicians can identify individuals more likely to respond positively to CCL2/CCR2-targeted treatments (142, 143). As the current treatment for OA focuses on symptom relief, pain management, and joint function improvements, targeting the CCL2/CCR2 axis may offer a dual benefit of alleviating symptoms and possibly modifying the disease's course (144, 145). These therapies may alter OA's natural history by suppressing the inflammatory cascade within the joint. Disease modification can reduce OA progression, offering great hope to patients (146). As a result of this paradigm shift, OA therapeutics have gained significant advancements from purely symptomatic relief to interventions with disease-modifying capabilities. For instance, the role of microRNA-183 (miR-183) in OA pain and its underlying mechanisms was investigated (147). Clinical samples from OA patients and a surgically induced OA pain mouse model were used. Expression levels of miR-183 and various factors linked to OA pain, including TGFα, CCL2, IL-1β, IL-6, TNF-α, transient receptor potential vanilloid subtype-1 (TRPV1), and voltage-gated sodium channels Nav1.3, Nav1.7, and Nav1.8) were measured. The results showed downregulation of miR-183 in both human OA tissue samples and the OA mouse model. Overexpression of miR-183 in mice inhibited the expression of proinflammatory cytokines and pain-related factors in dorsal root ganglia (DRG), alleviating OA pain. Moreover, miR-183 suppressed macrophage infiltration in DRG, directly targeting TGFα (147). These outcomes indicate that miR-183 can ameliorate OA pain by inhibiting the TGFα-CCL2/CCR2 signaling axis, emphasizing its potential as a therapeutic target for OA treatment.

To effectively navigate the complex pathology of OA, CCL2/CCR2-targeted therapies can be combined with other anti-inflammatory agents to achieve synergistic effects. Several signaling pathways are involved in the orchestration of inflammation in OA, and targeting these pathways simultaneously may lead to more profound therapeutic outcomes (148). In addition to nonsteroidal anti-inflammatory drugs
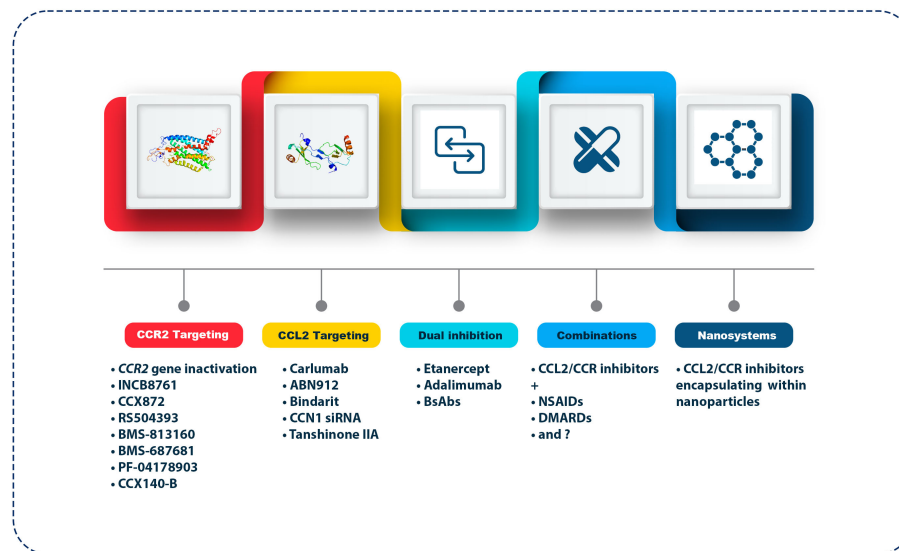
FIGURE 2
Therapeutic approaches targeting the CCL2/CCR2 axis in OA. The figure outlines various strategies targeting the CCL2/CCR2 axis for OA treatment. CCR2 targeting agents include gene inactivation and antagonists like INCB8761, CCX872, RS504393, BMS-813160, BMS-687681, PF-04178903, and CCX140-B, which block inflammatory cell recruitment. CCL2 targeting includes monoclonal antibodies (Carlumab, ABN912), Bindarit (an NF-κB pathway inhibitor), CCN1 siRNA, and Tanshinone IIA (a traditional medicine extract). Dual inhibition strategies combine CCL2/CCR2 inhibitors with TNF-α inhibitors (Etanercept, Adalimumab) or bi-specific antibodies. Combination therapies pair CCL2/CCR2 inhibitors with NSAIDs or DMARDs. Lastly, nanosystems utilize nanoparticles for targeted delivery of these inhibitors, enhancing bioavailability and reducing systemic side effects.

(NSAIDs) or disease-modifying anti-rheumatic drugs (DMARDs), combining CCR2 antagonists or anti-CCL2 antibodies with existing therapies could help manage OA in a multifaceted way (149–151). These combination therapies may enhance OA treatment efficacy while minimizing adverse effects, signifying a potential approach to the intricate interplay of inflammatory mediators in OA.

5.1 Tuning the orchestra: current and potential therapeutic approaches

5.1.1 CCR2 targeting

A prime target for interventions to regulate immune cell infiltration and inflammation within joints is CCR2, which is predominantly expressed on monocytes and macrophages' surfaces (152). Therefore, CCR2 targeting by various approaches can be a promising avenue in the search for OA therapeutics. A recent study evaluated sCCR2 E3 gene therapy against OA in an animal model induced by monosodium iodoacetate (MIA). The effects of *sCCR2 E3* gene expression were compared to control groups that received either full-length sCCR2 or an empty vector through intraarticular injection. It has been shown that *sCCR2* gene expression reduces pain severity, suggesting its potential to alleviate pain in OA. Furthermore, micro-computed tomographic analyses revealed a protective effect on bone integrity conferred by sCCR2 E3 activation based on enhanced bone resorption (152). These outcomes were further corroborated by histological assessments demonstrating anti-inflammatory properties and protective effects against cartilage damage in joints and intestines. These findings suggest that *sCCR2 E3* therapy might be an effective treatment for

OA, as it inhibits cartilage degradation, alleviates intestinal inflammation, and mitigates pain. Preclinical studies have demonstrated that small molecules that inhibit CCR2 disrupt the chemotaxis of inflammatory cells toward arthritic joints (145).

Another study examined the influence of *Ccr2* inactivation in aggrecan-expressing cells on PTOA progression, joint structures, synovial thickness, and pain (153). Through tamoxifen-inducible *Ccr2* inactivation in skeletally mature mice, the researchers detected that early deletion of *Ccr2* in aggrecan-expressing cells led to substantial improvements in articular cartilage structure, cartilage area and Safranin-O staining compared to mice without *Ccr2* deletion. Moreover, delayed subchondral bone thickness upsurge and delayed osteophyte maturation were noted following early recombination. While late aggrecan-*Ccr2* deletion exhibited some improvement in cartilage health, most data did not reach statistical significance. Remarkably, early *Ccr2* deletion enhanced weight-bearing capacity and mechanosensitivity, specifying improved pain measures compared to control mice (153). These outcomes propose that *Ccr2* inactivation in aggrecan-expressing cells alleviates the initiation, but not the progression, of PTOA, highlighting a potential therapeutic target for managing osteoarthritic pain and joint damage (Table 1).

INCB8761/PF-4136309, a potent CCR2 antagonist that blocks the binding of CCL2 to CCR2 receptors on monocytes, myeloid-derived suppressor cells (MDSCs), and macrophages, thereby inhibiting their migration and reducing inflammation with high selectivity, weak hERG activity, high free fraction in protein binding, and a promising *in vitro* and *in vivo* ADMET (ADME and toxicology) profile, was introduced by a study (156). A phase 1b clinical trial showed that following administration of PF-4136309 in

TABLE 1 Therapeutic approach targeting the CCL2/CCR2 axis in OA (Directly and indirectly).

Intervention	Outcome	Ref
Tamoxifen-inducible *Ccr2* inactivation	• Improvement of articular cartilage structure • Enhancement of cartilage area • Increase in Safranin-O staining • Delay in subchondral bone thickness increase • Delay in osteophyte maturation • Improvement in weight-bearing capacity • Enhancement of mechanosensitivity	(153)
RS504393 (CCR2 antagonist)	• Reduction in cartilage and bone damage • Diminished effectiveness with subsequent treatments • Decrease in pain measures • Enhancement of pain relief • Reduction of cartilage damage, synovitis, and osteophyte formation • Reduction of the number of F4/80-positive macrophages	(66, 124)
Anti-CCN1 siRNA and Tanshinone IIA	• Inhibition of CCN1 reduces senescence markers Inhibition of CCN1 decreases inflammatory markers • Inhibition of CCN1 reduces matrix degradation • Tanshinone IIA (TanIIA) reduces CCN1 expression in a dose- and time-dependent manner • TanIIA treatment decreases senescence and inflammatory markers • TanIIA preserves cartilage integrity in human cartilage explants and aged mice • Intra-articular injection of TanIIA inhibits CCN1-driven cartilage inflammaging and OA progression in mice TanIIA-treated mice show reduced cartilage damage and lower OARSI scores	(154)
RS-PLs nanosystem	• Increase in release of CCR2 inhibitor (RS504393) • Improvement of early and severe PTOA stages • Reduction of bone damage • Decrease in synovial hyperplasia • Prevention of extracellular matrix loss	(155)

patients with pancreatic ductal adenocarcinoma (PDAC), levels of $CD14^+ CCR2^+$ inflammatory monocytes reduced in the peripheral blood (157). Regarding the role of $CCR2^+ CD14^+$ inflammatory monocytes in the pathogenesis of OA, it is possible that using PF-4136309 in OA patients would be beneficial by reducing the frequency of these cells at the site of inflammation (158). Fluorouracil (5-FU) is a chemotherapy agent that, in addition to its cytotoxic effects on cancer cells, can induce immunogenic cell death, which enhances the anti-tumor immune response and modulates the TME (159). Moreover, CCX872-B, another CCR2 antagonist, disrupts the CCL2/CCR2 signaling pathway, reducing monocyte recruitment and subsequent tumor-promoting inflammation (160). The combination of FOLFIRINOX (5-FU, leucovorin, irinotecan, oxaliplatin) and CCX872-B showed promising results in patients with locally advanced or metastatic pancreatic cancer (161). The regimen was given to patients for up to 24 weeks, with progression-free survival being the primary endpoint. The secondary endpoints were tumor control and objective response rates. With a tumor control rate of 78% and an ORR of 37% among 50 patients enrolled, 35 completed the 12-week treatment. There was no significant increase in severe adverse events compared to historical data with FOLFIRINOX alone for CCX872-B. For patients with pancreatic cancer, the combination therapy demonstrated encouraging efficacy and acceptable safety profiles, suggesting potential benefits (161). Accordingly, CCX872 is hypothesized to dampen the inflammatory cascade by inhibiting inflammatory monocyte infiltration into the joint by interfering with CCR2 signaling in OA. While these mechanisms have shown promising outcomes in cancer studies, extrapolating them to OA requires caution due to the distinct pathophysiological contexts of cancer and OA. In OA, the primary focus is on chronic inflammation and cartilage degradation, suggesting that while CCR2/CCL2 inhibition could reduce inflammation, the direct effects and safety profiles need thorough investigation in OA-specific models.

Neuropathic pain and OA pain are two distinct forms of chronic pain, each with unique underlying mechanisms. Neuropathic pain arises from damage or dysfunction within the nervous system, either peripheral or central (162). This type of pain is characterized by peripheral sensitization, where injury to peripheral nerves (such as from diabetes, shingles, or trauma) leads to abnormal electrical signals and ectopic activity, causing spontaneous and aberrant nerve firing. Central sensitization also plays a critical role, involving increased excitability and synaptic efficacy in central neurons, often leading to pain amplification and persistent pain (163). In contrast, OA pain is primarily nociceptive, resulting from joint degeneration and the associated inflammatory processes (164). The mechanical wear and tear of cartilage, subchondral bone changes, and synovitis contribute to pain by activating nociceptors (165). Inflammatory mediators released during joint degeneration sensitize these nociceptors, resulting in the characteristic pain of OA (166). While neuropathic pain is primarily driven by neural dysfunction, OA pain is rooted in structural joint changes and inflammation, highlighting the distinct pathophysiological pathways underlying these chronic pain conditions. RS504393 is a specific CCR2 inhibitor that similarly blocks CCL2 binding, diminishing inflammatory cell infiltration and activity (66). To further substantiate the pathogenic role of CCL2/CCR2 signaling in the development of OA in mice was evaluated. It was demonstrated that RS-504393

significantly reduced cartilage damage, synovitis, and osteophyte formation. Additionally, the number of F4/80-positive macrophages was markedly lower in RS-504393-treated mice than those treated with the vehicle (66). These findings suggest that pharmacological inhibition of the CCL2/CCR2 chemokine axis effectively suppresses OA progression in mice, partly by decreasing synovial macrophage accumulation. In another study, RS504393 was administered to OA patients early or delayed to examine the effects on joint damage. According to the results, pharmacological blockade of CCR2 had differing effects on cartilage damage and bone damage based on the timing of administration (124). After the initial 4 weeks following injury, administering the RS504393 significantly reduced cartilage and bone damage associated with OA, but effectiveness decreased with subsequent treatments. In addition, pain-related behavioral studies found that blocking CCR2 signaling during the early (1–4 weeks after surgery) or moderate (4–8 weeks post-surgery) stages of OA led to decreased pain measures, with sustained improvement even after stopping treatment (124). Accordingly, an early intervention targeting CCR2 may slow the progression of post-injury OA and improve pain symptoms, suggesting that CCR2 antagonists could be highly effective in managing OA (Table 1). It seems that the mechanism of RS504393 is reducing synovial inflammation, inhibiting joint destruction, and ameliorating clinical symptoms via suppression of the CCL2/CCR2 axis signals (167, 168). In this context, a study used RS504393 to target the MCP-1/CCR2 axis, and the findings showed anti-inflammatory therapeutic potential in animal models of RA (168).

It has been found that changes in the signaling of chemokines and receptors within sensory neurons contribute to the maintenance of neuropathic pain behaviors (169). As a result, molecular mechanisms governing chemokine and receptor interactions in sensory neurons will likely play an essential role in sustaining neuropathic pain after an initial injury or insult (170). These changes may be triggered by various signaling pathways that sensitize sensory neurons, resulting in persisting neuropathic pain. It may be possible to develop novel therapeutic strategies for managing neuropathic pain conditions by understanding and targeting these alterations in chemokine/receptor signaling. It has been reported that (R)-4-Acetyl-1-(4-chloro-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (CCR2 RA [R]), a CCR2 antagonist, at 14 and 28 days after nerve injury significantly reduced bilateral nociceptive behavior (pain sensitivity). In this phase following nerve injury, the CCL2/CCR2 axis is essential in maintaining pain hypersensitivity (171). According to these findings, it is possible that using this CCR2 antagonist reduces pain in patients with OA. In addition to the mentioned CCR2 inhibitors, BMS-813160 (172), BMS-687681 (173), PF-04178903 (174), and CCX140-B (175), as other potent CCR2 inhibitors used in inflammatory-based disorders, may be beneficial in managing OA.

5.1.2 CCL2 targeting

Another approach to modulating the CCL2/CCR2 axis is targeting the chemokine. A monoclonal antibody (mAb), Carlumab, was previously known as CNTO888)human IgG1κ),

which possessed a high affinity and specificity for human CCL2 (176). Carlumab binds to CCL2, hindering its interaction with CCR2 and disrupting the chemotactic signal that drives immune cell recruitment. A preclinical study found systemic carlumab administration repressed prostate tumor growth, reducing CD68$^+$ macrophage infiltration and tumor microvascular density following monotherapy (177). Clinical outcomes of a phase 1 trial also revealed that carlumab was well tolerated at 15 mg/kg administered every two weeks in patients with solid tumors (178).

There are intriguing insights into the dynamics of treating cancer patients with Carlumab to target CCL2. After an infusion, carlumab temporarily suppresses free CCL2 levels, but these effects are transient, and levels resume or exceed baseline within a week (179). This contrasts with other mAbs like Omalizumab, where sustained suppression of target levels is observed. The inability of Carlumab to maintain prolonged suppression suggests potential compensatory mechanisms at play, possibly involving increased CCL2 production in response to inhibition. Moreover, the observation of elevated free CCL2 levels, particularly in patients receiving higher doses of Carlumab and associated with worse outcomes, raises questions about the role of CCL2 in disease pathogenesis and the potential overstimulation of compensatory mechanisms. Possible overstimulation of compensatory mechanisms, particularly in patients receiving high doses of Carlumab, is raised by elevated CCL2 levels, particularly in patients receiving higher doses (179, 180). Despite Carlumab's lack of efficacy in treating progressive idiopathic pulmonary fibrosis (IPF), these findings suggest that newer agents are needed to suppress CCL2-mediated signaling more effectively. This could provide hope for future therapeutic advances in dealing with related diseases (180). There are several vital reasons why clinical trials for ABN912 (another anti-CCL2 mAb) and Carlumab, intended to treat RA and cancer, have been unsatisfactory. Due to the transient neutralization of free-CCL2, a significant accumulation of total-CCL2 in the bloodstream contributed to the limited therapeutic efficacy (176). Pharmacokinetic/pharmacodynamic (PK/PD) modeling emphasized a substantial mismatch between the in vitro and in vivo binding kinetics, excluding the possibility of treatment-induced amplified CCL2 production as an explanation (179). A significant obstacle posed by CCL2 and Carlumab's target was the high turnover rate of these proteins (181). As a result, improving bioanalytical tools to measure free ligands in vivo was emphasized, as this could provide crucial insights into sustaining target inhibition over time (182). In murine models of metastatic breast cancer, it has been demonstrated that prolonged inhibition of free-CCL2 is essential for the clinical development of any CCL2-targeting agent and can mitigate metastasis formation after treatment cessation (183, 184).

An alternative way to inhibit CCL2 expression is to block the regulator of its expression. As an anti-inflammatory molecule, Bindarit (AF 2838 or 2-methyl-2-[[1-(phenylmethyl)-1Hindazol-3-yl] methoxy] propanoic acid) inhibits CCL2 synthesis and reduces CCL2 production by inhibiting the NF-κB pathway (185, 186). Several preclinical and clinical studies have shown that Bindarit, via suppressing the production of CCL2 and reducing the recruitment of monocytes and macrophages, is effective in

treating inflammatory-based disorders, such as diabetes-associated periodontitis (animal model study) (187), lupus nephritis (human study) (188), Alzheimer's disease (*in vitro* study) (189), and experimental autoimmune encephalomyelitis (EAE) (animal model study) (190). Tanshinone IIA extracts anti-inflammatory properties from the extensively utilized traditional Chinese medicine Danshen (*Salvia miltiorrhiza*) (191). Researchers conducted experiments using isolated primary human chondrocytes, cartilage explants, and a pre-clinical mice model to elucidate how CCN1 contributes to cartilage aging and inflammation (154). They found that CCN1 expression increases with age and is associated with cartilage degeneration. Overexpression of CCN1 promoted chondrocyte senescence and inflammation, while its inhibition via small interfering RNA (siRNA) or the administration of Tanshinone IIA (TanIIA) reduced inflammatory responses and preserved cartilage integrity (154). These findings indicate that targeting CCN1 signaling could be a potential therapeutic strategy for mitigating OA progression by reducing senescence-associated secretory phenotype (SASP) factors and preserving cartilage homeostasis. According to the CCN1 inducing CCL2 through FAK, PI3K/Akt, and NF-κB pathways in retinal vascular endothelial cells (192), inhibiting CCN1 can be beneficial in suppressing CCL2 and reducing the infiltration of monocytes and macrophages in the synovium and related inflammatory responses in OA (100) (Table 1).

An investigation revealed that CCN1 also plays a crucial role in RA pathogenesis. CCL2 is the critical chemokine that regulates the migration and infiltration of monocytes in RA. This study reported higher levels of CCN1 and CCL2 in synovial fluid from RA patients compared to non-RA controls. The MAPK signaling pathway mediates the CCN1-induced increase in CCL2 expression, and CCN1 negatively regulated miR-518a-5p expression via the MAPK cascade. In contrast, inhibition of CCN1 expression with lentiviral vectors expressing short hairpin RNA ameliorated articular swelling, cartilage erosion, and infiltration of monocytes in the ankle joints of mice with collagen-induced arthritis. Regarding the similar role of CCL2 in the pathogenesis of RA and OA, CCN1 inhibition could serve as a potential strategy via reducing the CCL2/CCR2 axis-mediated inflammation in these bone and cartilage disorders (98).

5.1.3 Dual inhibition strategies

To achieve a more comprehensive therapeutic effect, researchers are exploring dual inhibition strategies recognizing the intricate crosstalk between different signaling pathways within the joint microenvironment (66). Combining CCL2/CCR2-targeted agents with inhibitors of other inflammatory mediators, such as TNF-α or IL-1β, may offer synergistic benefits (193). An investigation on patients with RA showed that administrating anti-TNF-α antibodies (infliximab) downregulated CCL2 levels, while the concentrations of CCL3 and IL-8 remained unchanged (194). Another study assessed the effects of etanercept and adalimumab (TNF-α inhibitors) on the expression of CCL2 and evaluated potential intracellular mechanisms, such as regulation of epigenetics. The outcomes of this study also showed that etanercept

and adalimumab decreased the production of CCL2 in leukemic monocyte cell lines (THP-1 cells) and human primary monocytes (195). According to these outcomes, infliximab reduces CCL2 production *in vivo*, signifying a broader effect on inflammatory pathway activity. Moreover, regarding the limitations of anti-CCL2 monotherapies in chronic inflammatory disorders such as RA and OA, it is possible that combining anti-TNF-α and anti-CCL2 antibodies can be a potential approach in treating OA.

Another interesting therapeutic tactic in this context is using bi-specific antibodies (BsAbs), which can bind to two types of epitopes (196). The clinical assessment of bsAbs in autoimmune diseases is in progress, with both achievements (phase II trials of obexelimab in systemic lupus erythematosus) and failures (phase II trials of lutikizumab in OA and romilkimab in IPF) (197, 198). Lutikizumab (ABT-981) is a human dual variable domain Ig that can bind and inhibit IL-1α and IL-1β (199). According to the results of this trial, IL-1 inhibition was not an effective analgesic/antiinflammatory treatment in most patients with knee OA and associated synovitis, based on the limited improvement in WOMAC pain scores and lack of synovitis improvement with lutikizumab. Regarding these limitations and promising results from evaluating BsAbs in RA, designing novel BsAbs, targeting CCL2 and other inflammatory mediators such as TNF-α, IL-1β, and IL-17 may improve the effectiveness of immunotherapy in patients with OA in a synergistic fashion (200, 201). However, this approach needs more studies in the preclinical and clinical phases.

5.1.4 Nanoparticle-based therapies

Targeted therapies have become increasingly common in utilizing nanoparticles as delivery vehicles (202). Nanoparticles can help therapeutic agents become more bioavailable, prolong circulation, and enhance their accumulation in the joints of arthritis patients (203). Evidence demonstrated that by employing nanomaterials to treat OA, cargo such as small-molecular drugs, nucleic acids, and peptides/proteins can be transported to inhibit its progression (204, 205). Accordingly, the therapeutic potential of CCR2 antagonists or anti-CCL2 antibodies can be enhanced by encapsulating them within nanoparticles. By minimizing systemic side effects and maximizing local concentrations of therapeutic agents within the joint, nanoparticle-based therapies offer the advantage of controlled release.

In this context, an investigation revealed that the inhibitor was investigated for treating PTOA through a unique method of releasing RS504393 slowly and continuously into injured knees using biodegradable microplates (PLs) (155). In mice with DMM/sham surgery, RS-PLs (1 mg/kg) were administered intraarticularly one week after surgery, followed by subsequent administrations at 4- and 7 weeks following surgery. Drug-free PLs (DF-PLs) or saline injections were given as controls. Results indicated that RS-PLs facilitated sustained release of CCR2 inhibitors over several weeks, with approximately 20% of the drug still available after 21 days. Both early and severe PTOA stages were significantly improved with this extended release, which reduced bone damage, synovial hyperplasia, and extracellular matrix loss (Table 1). However, extracellular matrix loss was less effectively mitigated. The

BOX 1 Key points of clinical implications and therapeutic perspectives.

Key Points of Clinical Implications and Therapeutic Perspectives:

1. Targeting the CCL2/CCR2 Axis :
   - Emerging therapies focusing on the CCL2/CCR2 axis offer new perspectives for managing chronic inflammatory joint diseases like RA and OA.
   - Personalized treatment approaches targeting CCL2/CCR2 signaling can tailor interventions to patients' specific inflammatory profiles, potentially leading to more effective outcomes.

2. Biomarker-Driven Therapeutic Approaches:
   - Biomarker-driven therapeutic approaches identifying patients with elevated CCL2/CCR2 signaling could pave the way for personalized treatments in OA.
   - Diagnostic tools investigating genetic and molecular signatures associated with CCL2/CCR2 dysregulation could help stratify patients and guide treatment decisions.

3. Dual Benefit of Targeting CCL2/CCR2 Axis:
   - Targeting the CCL2/CCR2 axis not only alleviates symptoms and improves joint function but may also modify the disease's course by suppressing the inflammatory cascade within the joint, potentially altering OA's natural history.

4. Combination Therapies:
   - Combining CCL2/CCR2-targeted therapies with other anti-inflammatory agents like TNF-α or IL-1β inhibitors may offer synergistic benefits, enhancing treatment efficacy while minimizing adverse effects.

5. Nanoparticle-Based Therapies:
   - Nanoparticle-based delivery systems can enhance the therapeutic potential of CCL2/CCR2-targeted therapies by improving bioavailability, prolonging circulation, and enhancing accumulation in arthritic joints, potentially optimizing treatment outcomes for OA.

findings highlight the importance of local sustained delivery in the optimization of CCR2-targeted therapies for PTOA.

By overcoming the challenges associated with conventional systemic drug delivery, nanoparticle-based drug delivery approaches could revolutionize the treatment landscape for OA.

6 Future directions and concluding notes

It is expected that targeted therapies for OA will continue to grow and innovate in the future (Box 1). There is potential for further advancements in research focusing on refining treatment strategies, understanding patient-specific responses, and finding novel therapeutic targets within the inflammatory cascade. Despite the promising progress in developing targeted therapies targeting the CCL2/CCR2 axis, translating preclinical successes into clinical success remains challenging. Understanding individualized treatment approaches is complex due to patient heterogeneity, disease phenotypes, and the intricate interplay among multiple signaling pathways. Moreover, in the context of chronic diseases such as arthritis, it is important to assess the long-term safety and tolerability of these interventions carefully. Future research efforts should focus on improving patient stratification strategies, understanding molecular mechanisms underlying treatment responses, and exploring combinatorial approaches to OA's multifaceted pathology. To fully resolve the symphony of OA management, academia, industry, and regulatory agencies must collaborate to accelerate the development and approval of targeted therapies for OA.

Targeting the CCL2/CCR2 axis in OA has significant clinical implications and therapeutic opportunities. In addition to personalized medicine and disease modification, these emerging strategies offer hope to millions suffering from OA through innovative drug delivery and combination therapies. As the symphony of OA management evolves, CCL2/CCR2-targeted therapies are becoming increasingly significant in alleviating the burden associated with this disease. However, the variability in outcomes underscores the complexity of OA pathology and the requirement for personalized approaches in targeting the CCL2/CCR2 axis.

Author contributions

MA: Writing – original draft, Writing – review & editing. HK: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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