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Targeting chemokine (C-C motif) ligand 2 (CCL2) as an example of translation of cancer molecular biology to the clinic

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Introduction

Chemokines are a family of small and secreted proteins that play pleiotropic roles in inflammation-related pathological diseases including cancer. Among the identified 50 human chemokines, chemokine (C-C motif) ligand 2 (CCL2) is of particular importance in cancer development since it serves as one of the key mediators of interactions between tumor and host cells. CCL2 is produced by cancer cells and multiple different host cells within the tumor microenvironment. CCL2 mediates tumorigenesis in many different cancer types. For example, CCL2 has been reported to promote prostate cancer cell proliferation, migration, invasion, and survival, via binding to its functional receptor CCR2. Furthermore, CCL2 induces the recruitment of macrophages and induces angiogenesis and matrix remodeling. Targeting CCL2 has been demonstrated as an effective therapeutic approach in preclinical prostate cancer models and currently, neutralizing monoclonal antibody against CCL2 has entered into clinical trials in prostate cancer. In this chapter, targeting CCL2 in prostate cancer will be used an example to show translation of bench findings from cancer molecular biology to the clinic.

1. Biology of CCL2

1.1. CCL2 basics

Chemokines, a family of chemoattractant cytokines, are classified into four subfamilies as CXC, CC, CX3C and C based on the number and location of the cysteine residues at the N-terminus of the protein. Chemokine (C-C motif) ligand 2 (CCL2), also known as monocyte chemotactic protein-1 (MCP-1), is a small, secreted protein that belongs to the CC chemokine family. CCL2 was purified and cloned in 1989 from human gliomas and myelomonocytic cells by two independent research groups based on its ability to chemoattract monocytes (1, 2). Subsequent to its cloning, it was confirmed that this protein was also identical to the product of the human *JE* gene. The *JE* gene, originally identified in mouse fibroblasts, is a platelet derived growth factor (PDGF) inducible gene. Since then, CCL2 has been shown to display chemoattractic activity for not only monocytes, but also memory T cells, natural killer (NK) cells, and perhaps dendritic cells resulting in recruiting of these cells to sites of tissue injury and inflammatory responses (3, 4). The human *CCL2* cDNA encodes a 99 amino acid residue precursor protein with a hydrophobic signal peptide of 23 amino acids and a mature peptide of 76 amino acids (5, 6). The *CCL2* gene is located on the chromosome 17 where many of the genes of CC chemokine family are located. The mouse or rat CCL2 gene has about 75% homology to human. CCL2 functions through

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binding to a functional chemokine receptor CCR2, although it also binds to CCR4 (7). The roles of CCL2 have been implicated in the pathogenesis of various diseases that associate with monocyte infiltration, for example rheumatoid arthritis, atherosclerosis, and multiple types of cancer [see review in (8)].

1.2. CCL2 expression

CCL2 is expressed in a wide array of tissues. It can be produced by multiple cell types including fibroblasts, macrophages, lymphocytes, astrocytes, mast cells, endothelial cells, and osteoblasts (1, 9–14). In addition, CCL2 can also be produced by a variety of human and murine malignant cells [see review in (15–17)]. In prostate cancer, determined by immunohistochemical staining on a human tissue microarray, CCL2 positive staining was located mostly in epithelial and fibromuscular stromal cells (18). However, CCL2 positive staining was also observed in the extracellular areas surrounding neoplastic glands and epithelial cells (18) suggesting both autocrine and paracrine production of CCL2 in the tumor microenvironment. In at least one report, CCL2 expression levels positively correlated with Gleason score (a measure of tumor aggressiveness) and pathologic stages (18–20). CCL2 production has been determined by enzyme-linked immunosorbent assay (ELISA) in conditioned media collected from prostate cancer cell lines and compared to primary prostate epithelial cells (18). Prostate cancer cells produce higher amounts of CCL2 as compared to non-malignant prostate epithelial cells (18). Higher production of CCL2 at the sites of bone metastases was demonstrated by a clinically-related report in which Loberg et al collected tumor bone metastatic and normal bone specimens from vertebral lesions in three patients with prostate cancer (21). Total protein lysates were isolated and analyzed by cytokine array. Elevated CCL2 production was identified in the tumor-bone microenvironment compared to normal bone microenvironment suggesting that CCL2 plays a critical role in prostate tumorigenesis in bone metastases (21). *In vitro*, it has been further reported that human osteoblasts and bone marrow endothelial cells produced higher amount of CCL2 compared to prostate epithelial cells (13). CCL2 can also be produced by osteoclasts (22–25). Its production can be induced by receptor activator of NF-κB ligand (RANKL) and tumor necrosis factor alpha (TNFα) (22–25). To date, it remains unclear which cell type(s) play the major role in the production of CCL2 in the tumor microenvironment.

1.3. CCL2 functions

CCL2 functions as a chemoattractant through binding to its receptor on monocytes, macrophages, and lymphocytes [see review in (26)]. The existanceof CCL2/CCR2 axis has been validated using CCR2 knockout animals (27). In acute inflammatory response, CCL2 has been shown to actively recruit monocytes to the site of inflammation (28, 29). CCL2 also plays important roles in T-cell immunity and CCL2 expression is associated with Th2 response (30–32). For example, CCL2 is over produced in an animal model of Th2 immune-mediated asthma (33). CCL2 is potent factor in the polarization of Th0 cells towards a Th2 phenotype (34). It has been demonstrated that CCL2 induced interleukin-4 (IL-4) production through direct activation of IL-4 promoter in T cells (35). CCL2 knockout mice demonstrated impaired Th2 immunity but intact Th1 immune response (36). In chronic inflammatory diseases, the roles of CCL2 have also been reported. For example, there are a large body of evidence showing the crucial roles of CCL2/CCR2 axis in various chronic inflammatory conditions that are associated with macrophage infiltration such as atherosclerosis (37, 38), multiple sclerosis (39), rheumatoid arthritis (40), glomerulonephritis (41), pulmonary hypertension (42), and pulmonary fibrosis (43). It has been reported that CCL2 is expressed at the site of tooth eruption and bone resorption (44). It has been shown that CCL2 can induce osteoclast maturation and formation as represented by the formation of TRAP-positive, multinuclear cells in the absence of RANKL, but the

produced osteoclasts lack the ability to cause bone resorption (25, 45). In addition, CCL2 has been reported to cause the degranulation of basophiles and migration of mast cells (46, 47). This effect can be enhanced by pre-treatment with IL-3 and other cytokines (48). In tumor development, stimulation of infiltrating macrophages has been shown to either augment anti-tumor activity or promote tumor development, depending on cancer type [see review in (49)].

1.4. CCR2, the functional receptor for CCL2

CCR2, a G protein-coupled receptor, is the key functional receptor for CCL2. The activation of the ligand-receptor binding leads to the activation of intracellular signaling cascades that mediate chemotactic response. CCR2 has both pro-inflammatory (mediated by APC and T cells) and anti-inflammatory (mediated by regulatory T cells) effects [see review in (8)]. CCR2-deficient mice have been shown to have altered inflammatory responses in an allergic asthma model (50).

CCR2 can be expressed by both hematopoitic cells such as macrophages and non-hematopoietic cells such as endothelial cells (51), fibroblasts (52), and mesenchymal stem cells (53). In prostate cancer, it has been shown that CCR2 expression correlates with prostate cancer progression and metastasis as determined by in situ immunohistochemical staining (54, 55). Specifically, CCR2 expression correlated with Gleason score and pathological stages. However, these published reports were not able to distinguish which cell type(s) may produce CCR2 transcripts in the metastatic sites. In prostate cancer cell lines, differential expression of CCR2 has been reported (21, 54). In particular, more aggressive cancer cells express greater levels of CCR2 compared with the less aggressive cancer cells or non-neoplastic cells (54). These findings suggest that the CCL2/CCR2 axis may be a target for prostate cancer treatment. It has been recognized that CCR2 antagonists are potential therapeutic agents in preventing, treating, or ameliorating a CCR2-mediated inflammatory syndrome or disease such as psoriasis, uveitis, rheumatoid arthritis, multiple sclerosis, asthma, obesity, and chronic obstructive pulmonary disease [see review in (56)]. In a prostate cancer study, a CCR2 antagonist has been shown to diminish the prostate cancer cell proliferation and invasion *in vitro* (18).

Recently, CCR2 was reported as a key factor in balance the bone remodeling process (57). It was shown that CCR2 knockout mice had high bone mass and stability (biomechanical properties by compression) due to a decrease in number, size, and function of osteoclasts (57). RANK expression is diminished in CCR2 knockout mice, and CCL2 enhances RANK expression via NFkB and ERK1/2 pathways (57). Therefore, CCR2 could become a therapeutic target in postmenopausal bone loss.

1.5. Regulation of CCL2 and CCR2

1.5.1. CCL2 regulation—CCL2 production is elevated in various diseases that are associated with chronic inflammation and macrophage infiltration. Similar to other inflammation-associated soluble factors, CCL2 production can be induced by oxidative stress, cytokines, and growth factors. In prostate cancer, it has been reported that serum CCL2 levels are elevated in patients with skeletal metastases compared to localized prostate cancer (58). One of the key regulators has been suggested to be parathyroid hormone–related protein (PTHrP), a 141-amino acid protein that has limited homology to PTH, but binds the same receptor as PTH with similar biological activity (59, 60). It was also reported that PTHrP is highly expressed in metastatic bone lesions, compared to a moderate expression on localized prostate cancer tissues and cell lines (61–63). PTHrP has been shown to enhance bone metastases in animal models of both prostate cancer (64) and breast cancer (65–68). It has been demonstrated that PTHrP treatment of osteoblastic cells up-

regulates CCL2 (13, 69) and this induction can be blocked by a PTHrP antagonist (70, 71), suggesting that prostate cancer cell-derived PTHrP plays an important role in elevation of osteoblast-derived CCL2. It was further demonstrated that PTHrP induces CCL2 production in human bone marrow endothelial (HBME) cells (13). Investigation of the mechanisms through which CCL2 is upregulated in osteoblasts and HBME cells is needed to provide a better understanding of the roles of tumor microenvironment in skeletal metastasis.

The *CCL2* gene is regulated in a tissue-specific and stimulus-specific manner (13). In its promoter region, there are a pair of C/EBP binding sites (−2591 to −2579; −3118 to −3107) that are important for the response to insulin activation of PI3K (72), a pair of NFkB binding sites (−2639 to −2630; −2612 to −2603) that are important for interleukin-1 (IL-1) and TNFα stimulation, and a GC box (−64 to −59) that binds Sp1 and is important for CCL2 basal expression (73). Using CCL2 reporters that were transfected into human fetal osteoblast (hFOB) cells, PTHrP induced CCL2 promoter activity in hFOB cells through NFkB and C/EBP activation (13). However, it remains unknown (a) whether PTHrP can also upregulate the CCL2 promoter-luciferase reporter set in the HBME cells as well as in prostate cancer cell lines, (b) if PTHrP does so via NFkB and C/EBP activations as with hFOB, and (c) whether other stimuli such as IL-6 or TNFα or RANKL also can upregulate this promoter in these cells and, (d) if so, via which transcription factor, NFkB and/or C/EBP. It is worth to note that TNFa has been reported to induce CCL2 expression in sensory neurons (74–76), and RANKL induces CCL2 expression in osteoclasts, at transcriptional levels (25, 57).

Recently, a gene expression profile in individual human prostate cancer specimens before and after exposure to chemotherapy (docetaxel treatment) was determined (77). In that study, several genes including CCL2 were upregulated post the chemotherapy. In addition, docetaxel was shown to induce CCL2 expression in prostate cancer cell lines *in vitro*. CCL2-specific siRNA inhibited prostate cancer cell proliferation and enhanced the growth inhibitory effect of low-dose docetaxel. This protective effect of CCL2 was associated with activation of the ERK/MAP kinase and PI3K/AKT (77). These findings suggest a mechanism of chemotherapy resistance mediated by cellular stress responses involving the induction of CCL2 expression and indicate that inhibiting CCL2 activity could enhance therapeutic responses to taxane-based therapy.

1.5.2. CCR2 regulation—Little is known about the regulation of the *CCR2* gene in normal or cancerous tissues. It is down-regulated as monocytes move down the macrophage differentiation pathway while other related chemokine receptors are not (78). IFNγ+ M-CSF or PMA + ionomycin down-regulate CCR2 expression in monocytes and this can be replicated with a −1220/+115 hCCR2 promoter-pGL3 luciferase reporter (78). Peroxisome proliferator-activated receptor-gamma (PPAR-γ) ligands (i.e. Rosiglitazone) also down-regulate CCR2 in circulating monocytes while cholesterol slightly up-regulated CCR2 (79). While pro-inflammatory cytokines rapidly reduce CCR2 expression in monocytes, they up-regulate CCR2 expression in the brain (80). Constitutive tissue-specific expression of CCR2 in THP-1 monocyte cells has been shown to be dependent upon a 31-bp region (−89 to −59) adjacent to the TATA box that contains an Oct-1 binding site and a pair of tandem C/EBP binding sites located in the 5′UTR (+50 to +77 bp) (81). Besides the Oct-1 and C/EBP binding sites that function in monocyte CCR2 expression, the hCCR2 5′ flank and UTR contains an array of possible binding sites for PPAR/RXR, SREBP, GATA, STAT, NFAT, and AP-1, as well as additional C/EBP and Oct sites. It remains unknown if these sequences are sufficient for positive regulation in prostate cancer cells, but the monocyte expression of CCR2 suggests that they should function in osteoclast precursor cells, for example RAW264.7 cells.

2. CCL2 in prostate cancer

2.1. Proliferation and survival

CCL2 has been shown to promote prostate cancer cell proliferation and invasion *in vitro* via the phophatidylinositol 3-kinase (PI3K)/AKT signaling pathway (18, 21). CCL2 induces Akt phosphorylation in prostate cancer cells. In addition, CCL2 stimulates p70-S6 kinase phosphorylation which is a downstream target of Akt, resulting in actin rearrangement, which is a critical step in the formation of the migratory phenotype of the tumor cells (21). Activation of p70-S6 kinase alters the actin cytoskeleton microstructure (82) and the binding of CCL2 and CCR2 has been linked to the actin skeleton through interactions with FOUNT, a novel activator of C-C chemokines (83–85). Constitutive activation of this PI3K/AKT pathway has been implicated in prostate cancer progression (86–89) and activation of AKT pathway further induces survival benefits for the tumor cells (90). The later protective roles of CCL2 have shown to upregulate survivin gene expression, and therefore CCL2 plays an important role for the tumor cell survival, possibly through reduction of autophagosome formation (90, 91). Survivin has been demonstrated to serve as a key molecule that protects the tumor cells from autophagic death (90, 92).

2.2. Angiogenesis

Chemokines play an important role in the maintenance of hematopoietic homeostasis, regulation of cell proliferation, tissue morphogenesis, and angiogenesis (93). In human breast cancer, it was reported that CCL2 levels in the excised breast cancer tissue were correlated significantly with the level of vascular endothelial growth factor (VEGF), thymidine phosphorylase, TNFa and IL-8, which are potent angiogenic factors (94). Transfection of colon cancer cells with the CCL2 gene induces angiogenesis in a murine model (95). It has been demonstrated that both CCL2 and VEGF expression positively correlates with TAM infiltration and angiogenesis in breast cancer (96). In prostate cancer, it has been reported that CCL2 induces tumor cells to produce the pro-angiogenic factor VEGF-A that indirectly induce sprout formation in human bone marrow endothelial cells (71). *In vivo*, it has been shown that administration of neutralizing antibody against CCL2 significantly reduce tumor blood vessel density and decrease the prostate cancer tumor burden (Figure 1) (71, 97). Therefore, CCL2 is a key mediator of tumor angiogenesis.

2.3. Migration, invasion, and metastasis

Metastasis is a multi-step process that begins with a cell that has a phenotype that allows higher motility, invasion through tissue layers in the primary tumor, survival in the circulation, and establishment, expansion and growth in a “hostile” microenvironment at the distant target organ. In this process the interaction between the tumor cells and the tumor microenvironment has become the focus of therapeutic opportunities (98–104). There is growing evidence to suggest that CCL2 may act directly on the cancer epithelial cells and regulate the migration and invasion, thus enhancing matastatic potential. In particular, CCL2 has been shown to be a potent chemotactic factor, in both autocrine and paracrine manners, for prostate cancer cells *in vitro* (18, 21). It has been also reported that CCL2 acts as a chemoattractant for myeloma cell migration (24, 105, 106). In addition, CCL2 has been suggested as a predictor of colorectal cancer hepatic metastasis and poor survival (107).

2.4. Macrophage infiltration

Chronic inflammation has been recognized as a risk factor in a variety of cancer types, including prostate cancer (108, 109). In the tumor microenvironment, inflammatory components present as a large number of infiltrating macrophages (110). These macrophages are most likely derived from circulating monocytic lineage (111) and have

been termed tumor-associated macrophages (TAMs). It is well accepted that TAMs provide a direct link between inflammation and malignancy (49, 112–119). TAMs are increasingly recognized as important regulators to cancer progression and metastasis, both in positive or negative ways (49, 116–119). TAMs can be stimulated to inhibit tumor growth (49, 117–119), but on the other hand, they can produce soluble mediators, such as CCL2, to directly or indirectly promote cancer epithelial cell proliferation in the tumor microenvironment (110). CCL2 has been suggested to be one of the crucial determinants of human tumor macrophage content (120, 121) and a large number of TAMs has been identified in prostate cancer tissues compared to non-neoplastic tissues (122). In human breast cancer, CCL2 concentration from the excised tumor was associated with TAM accumulation (94, 123). TAM infiltration has been demonstrated in preclinical animal models in prostate (97, 122, 124), breast (123, 125), cervix (126), and pancreatic carcinoma (127, 128). Recently, CCL2-over expressing breast cancer cells were shown to promote macrophage chemotaxis *in vivo* in a mouse model (125). In colon cancer, it has been shown that blocking TNFa/TNF receptor axis reduces colorectal carcinogenesis, intracolonic macrophage infiltration, and CCL2 mRNA expression (129).

Macrophages are classified as M1-type, the “classically activated macrophages” and M2-type, the “alternatively activated macrophages” that contribute to the tumor progression (110, 114). Furthermore, CCL2, in concert with IL-6, has been shown to promote survival of human CD11b positive peripheral blood mononuclear cells and induce M2-type macrophage polarization (122). The mechanistic studies have shown that both cytokines inhibit the apoptotic cleavage of caspase-8 and promote enhanced autophagic activity to protect the monocyte recruited to the tumor (Figure 2) (122). CCL2’s anti-tumor activity has been demonstrated *in vitro* by its ability to augment cytostatic activity against tumor cells upon addition to macrophages in tissue culture and by its ability to induce FAS ligand expression in cultured endometrial stromal cells, thus driving the cells to apoptosis (130–133). These findings suggest that targeting both CCL2 and IL-6 could become an optional therapeutic approach in prostate cancer treatment.

2.5. Osteoclast recruitment and activation

Several cancer types including prostate, breast, and lung cancer preferentially metastasize to the skeleton. Unlike breast cancer that usually cause bone resorptive osteolysis, prostate cancer bone metastatic lesions usually represent a mixture of predominant osteoblastic response (woven bone formation) and osteolytic (bone resorptive) activity. It is well-documented that the tumor-induced osteoclast activity may be a pre-requisite for prostate cancer establishment as micrometastases in the bone microenvironment [see review in (134)]. CCL2 participates in the recruitment of osteoclast precursor cells, osteoclast activation, and maturation (25, 45, 135).

Bone is a dynamic tissue, being continuously remodeled by the coordinated actions of osteoclasts and osteoblasts. Osteoblasts, the bone-forming cells, are derived originally from pluripotent mesenchymal stem cells. Osteoblasts express protease-activated receptor-1 and VEGF (136). Osteoclasts arise from monocytic precursor cells. Cytokines and hormones regulate osteoclast formation and activity. Most osteotropic factors, such as PTH, 1,25-hydroxy vitamin D3, TNFα, and prostaglandins promote osteoclast formation mediated by induction of RANKL on marrow stromal cells and osteoblasts. Osteoprotegerin (OPG), a decoy receptor for RANKL, inhibits osteoclast formation and activity. In prostate cancer bone metastasis, the number of osteoclasts is increased because of cytokines and chemokines produced or induced by tumor cells increase the ratio of RANKL to OPG, and thereby increase osteoclast formation (137). Tumors metastatic to bone increase osteoclast numbers, which in turn increase bone destruction and create space for the tumor growth.

In prostate cancer animal models, CCL2 has been shown to induce osteoclast differentiation and maturation using human bone marrow monocytes (138) and peripheral blood mononuclear cells (139). It has been shown that *in vivo*, CCL2 mediates prostate cancer cell-induced osteoclast activity (71, 140). It has been reported that CCL2 knockdown in prostate cancer cells by shRNA methodology significantly reduced the conditioned media (collected from the CCL2 knockdown cells compared to the scramble control knockdown cells)-induced osteoclast maturation *in vitro* and diminish partially prostate cancer growth in bone in an intratibial-injection mouse model (58). Similar inhibition of tumor growth in bone was demonstrated in other cancer types for example in lung cancer (141) and breast cancer (125). Another chemokine RANTES can also stimulate the differentiation of pre-osteoclasts into mature osteoclasts (45).

3. CCL2 development as a therapeutic target

3.1. Preclinical animal models

Due to the bench findings that CCL2 directly stimulate the tumor cell growth, survival, invasion, and migration, and indirectly promotes macrophage infiltration and osteoclast maturation and activity [reviewed in (15, 16, 142)], anti-CCL2 has been tested as a therapeutic option in preclinical animal models in prostate cancer (55, 71) and breast cancer (125). In one study, using neutralizing antibodies against human CCL2 (CNTO888) and/or the mouse CCL2 (C1142), it was shown that treatment with C1142 attenuated prostate cancer PC3 cell-mediated overall tumor burden in an intracardiac injection model by 96% at weeks post the tumor cells injection, although targeting the human CCL2 derived from the human tumor cells modestly inhibited the tumor growth (97). This suggest that host-derived CCl2 plays a prominent role in tumor progression and metastasis (21, 97). In addition, it was shown that the combination of chemotherapy drug docetaxel with the neutralizing antibodies against CCL2 further reduced the tumor growth compared to using either treatment alone (97). Future work is needed to delineate the role of host-derived and the tumor-derived CCL 2 in prostate caner tumorigenesis and metastasis.

The effects of CCR2 deficiency from host cells have been initially tested in a preclinical colon cancer model (143). In that study, murine colon adenocarcinoma colon 26 cells were intraportally injected into wile type and CCR2 knockout mice (143). After 10 days, the number and size of tumor foci were significantly reduced in CCR2-deficient mice, with a concomitant reduction in the macrophage accumulation in the tumor, compared to wild-type mice, although tumor formation occurred at similar rates in wild-type and CCR2-deficient mice up to 10 days after tumor cell injection. Further evaluation is still needed to determine the effects of CCL2 in this model.

TAMs are pivotal member of stromal cells in the tumor microenvironment, releasing a variety of growth factors, proteolytic emzymes, cytokines, and inflammatory mediators. Therefore, TAMs have been implicated as therapeutic targets. It was demonstrated, for example, that extratumoral macrophages promote tumor and vascular growth in an orthotopic rat prostate tumor model (144). This study was performed using Dunning R-3327 AT-1 rat prostate tumor cells that have been demonstrated to produce CCL2 *in vivo*. Recently, clodronate- or other bisphosphonate liposome-mediated macrophage depletion regimens has been tested in preclinical models (144–147). It has been shown that combined with antibodies against VEGF, depletion of TAMs was accompanied by significant inhibition of tumor growth in tumor models (146). In a human melanoma xenograft model, it has been shown that targeting TAMs by clodronate liposomes reduced the tumor growth associated with less angiogenesis and macrophage infiltration (145).

In a preclinical lung cancer model, it was very recently shown that the combination of neutralizing antibody against CCL2 with a tumor vaccine effectively augmented efficacy with enhanced reduction in tumor volume and cures of approximately 50% of the tumors (148). The combined therapy generated more total intratumoral CD8+ T cells that were more activated and more antitumor antigen-specific, as measured by tetramer evaluation. A potential mechanism is suggested by the reduction in intratumoral T regulatory cells in this model. These findings suggest that CCL2 is indeed a key chemokine that mediates immune suppression in the tumors.

3.2. Clinical studies

Neutralizing monoclonal antibody against CCL2 (CNTO888) has entered into Phase I trials for safety and Phase II clinical tests in prostate cancer to test efficacy. In the near future, a combination of therapeutic approaches such as neutralizing antibody against CCL2/CCR2 axis or small molecule CCR2 antagonist with other therapeutic approaches such as chemotherapy or other immune modulators should provide new therapeutic approaches for prostate and other cancers.

4. Conflict reports on the roles of CCL2 in cancer

There are a few conflicting reports on the role of CCL2 in tumor progression and metastasis. Specifically, it was reported that in 4T1E breast cancer parental cells, CCL2 is highly expressed but shows low bone metastasis based on incidence of metastasis and histology from a group of 10 to 11 animals (149). The 4T1E-derived 4T1E/M3 cells have extremely low levels of CCL2 expression but have a high incidence of metastasis (149). Transfection of the *CCL2* gene into a highly metastatic murine colon carcinoma CT-26 cells reduced tumorigenicity and suppressed metastatic potential (150). The same group presented a similar report in the renal adenocarcinoma cell line RENCA (151). TAM-associated modulation of tumor growth *in vivo* in Panc-1, a human pancreatic carcinoma cell line, and WM115, a human melanoma cell line, was reported using antibodies (152).. Addition of CCL2 did not have effects on cancer cell proliferation and apoptosis (153). Monocyte recruitment was blocked using a rat monoclonal antibody against murine CD11b and CCL2 was blocked using a mouse monoclonal antibody against human CCL2. In another study, the CCL2 gene was introduced into Chinese hamster ovary (CHO) cells and the ability of tranfected cells to form tumors in vivo was evaluated (154). Clones transfected with human CCL2 or murine CCL2, via mammalian expression vector did not show significant differences in growth rate *in vivo* compared with clones transfected with vectors. Finally, it was demonstrated that when non-tumorigenic melanoma cells were transfected with CCL2 expression vector and injected *in vivo*, high level of CCL2 production resulted in extensive monocyte invasion and elimination of the tumor growth, and low level CCL2 production resulted a low level of monocyte recruitment and promotion of tumor angiogenesis (155). Based on the above reports, CCL2 needs further investigation in different tumor types. Like CCL2, CCR2 has low sequence homology between human and lower species which could raise the question of whether blocking the CCL2/CCR2 axis could generate sufficient clinical efficacy in certain diseases, as predicted by many preclinical animal modes (56).

5. Conclusions

It has been demonstrated that CCL2 promotes prostate cancer tumorigenesis and metastasis via 1) direct promotional effects on tumor cell growth and survival, and 2) indirect modulatory effects on macrophage infiltration and osteoclast activation (16) (Figure 3). The laboratory investigations of CCL2 have been successfully translated to the clinic: (1) studies of CCL2 were initiated by discovery of highly production of CCL2 in bone metastasis compared to primary prostate cancer; (2), the functional roles of CCL2 in the tumor

development in vitro were investigated; (3), the roles of CCL2 in vivo in preclinical animal models were confirmed; (4) neutralizing antibodies against CCL2 are currently being evaluated in clinical trials. CCL2 can serve as an example of other chemokines and cytokines for therapeutic development in cancer.

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References

1. Yoshimura T, Robinson EA, Tanaka S, Appella E, Kuratsu J, Leonard EJ. Purification and amino acid analysis of two human glioma-derived monocyte chemoattractants. J Exp Med. 1989; 169:1449–59. [PubMed: 2926329]
2. Matsushima K, Larsen CG, DuBois GC, Oppenheim JJ. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. J Exp Med. 1989; 169:1485–90. [PubMed: 2926331]
3. Balkwill F. Cancer and the chemokine network. Nat Rev Cancer. 2004; 4:540–50. [PubMed: 15229479]
4. Charo IF, Taubman MB. Chemokines in the pathogenesis of vascular disease. Circ Res. 2004; 95:858–66. [PubMed: 15514167]
5. Cochran BH, Reffel AC, Stiles CD. Molecular cloning of gene sequences regulated by platelet-derived growth factor. Cell. 1983; 33:939–47. [PubMed: 6872001]
6. Van Coillie E, Van Damme J, Opdenakker G. The MCP/eotaxin subfamily of CC chemokines. Cytokine Growth Factor Rev. 1999; 10:61–86. [PubMed: 10379912]
7. Graves DT, Jiang Y, Valente AJ. Regulated expression of MCP-1 by osteoblastic cells in vitro and in vivo. Histol Histopathol. 1999; 14:1347–54. [PubMed: 10506949]
8. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res. 2009; 29:313–26. [PubMed: 19441883]
9. Barna BP, Pettay J, Barnett GH, Zhou P, Iwasaki K, Estes ML. Regulation of monocyte chemoattractant protein-1 expression in adult human non-neoplastic astrocytes is sensitive to tumor necrosis factor (TNF) or antibody to the 55-kDa TNF receptor. J Neuroimmunol. 1994; 50:101–7. [PubMed: 8300851]
10. Brown Z, Strieter RM, Neild GH, Thompson RC, Kunkel SL, Westwick J. IL-1 receptor antagonist inhibits monocyte chemotactic peptide 1 generation by human mesangial cells. Kidney Int. 1992; 42:95–101. [PubMed: 1386129]
11. Cushing SD, Berliner JA, Valente AJ, Territo MC, Navab M, Parhami F, Gerrity R, Schwartz CJ, Fogelman AM. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. Proc Natl Acad Sci U S A. 1990; 87:5134–8. [PubMed: 1695010]
12. Standiford TJ, Kunkel SL, Phan SH, Rollins BJ, Strieter RM. Alveolar macrophage-derived cytokines induce monocyte chemoattractant protein-1 expression from human pulmonary type II-like epithelial cells. J Biol Chem. 1991; 266:9912–8. [PubMed: 2033076]
13. Lu Y, Xiao G, Galson DL, Nishio Y, Mizokami A, Keller ET, Yao Z, Zhang J. PTHrP-induced MCP-1 production by human bone marrow endothelial cells and osteoblasts promotes osteoclast differentiation and prostate cancer cell proliferation and invasion in vitro. Int J Cancer. 2007; 121:724–33. [PubMed: 17390372]
14. Yoshimura T, Yuhki N, Moore SK, Appella E, Lerman MI, Leonard EJ. Human monocyte chemoattractant protein-1 (MCP-1). Full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. FEBS Lett. 1989; 244:487–93. [PubMed: 2465924]
15. Zhang J, Patel L, Pienta KJ. CC chemokine ligand 2 (CCL2) promotes prostate cancer tumorigenesis and metastasis. Cytokine Growth Factor Rev. 2010; 21:41–8. [PubMed: 20005149]

16. Zhang J, Lu Y, Pienta KJ. Multiple roles of chemokine (C-C motif) ligand 2 in promoting prostate cancer growth. J Natl Cancer Inst. 2010; 102:522–8. [PubMed: 20233997]
17. Craig MJ, Loberg RD. CCL2 (Monocyte Chemoattractant Protein-1) in cancer bone metastases. Cancer Metastasis Rev. 2006; 25:611–9. [PubMed: 17160712]
18. Lu Y, Cai Z, Galson DL, Xiao G, Liu Y, George DE, Melhem MF, Yao Z, Zhang J. Monocyte chemotactic protein-1 (MCP-1) acts as a paracrine and autocrine factor for prostate cancer growth and invasion. Prostate. 2006; 66:1311–8. [PubMed: 16705739]
19. Mazzucchelli L, Loetscher P, Kappeler A, Uguccioni M, Baggiolini M, Laissue JA, Mueller C. Monocyte chemoattractant protein-1 gene expression in prostatic hyperplasia and prostate adenocarcinoma. Am J Pathol. 1996; 149:501–9. [PubMed: 8701989]
20. Chetcuti A, Margan S, Mann S, Russell P, Handelsman D, Rogers J, Dong Q. Identification of differentially expressed genes in organ-confined prostate cancer by gene expression array. Prostate. 2001; 47:132–40. [PubMed: 11340636]
21. Loberg RD, Day LL, Harwood J, Ying C, St John LN, Giles R, Neeley CK, Pienta KJ. CCL2 is a potent regulator of prostate cancer cell migration and proliferation. Neoplasia. 2006; 8:578–86. [PubMed: 16867220]
22. Stifter S. The role of nuclear factor kappaB on angiogenesis regulation through monocyte chemotactic protein-1 in myeloma. Med Hypotheses. 2006; 66:384–6. [PubMed: 16253428]
23. Hurwitz AA, Lyman WD, Berman JW. Tumor necrosis factor alpha and transforming growth factor beta upregulate astrocyte expression of monocyte chemoattractant protein-1. J Neuroimmunol. 1995; 57:193–8. [PubMed: 7706436]
24. Johrer K, Janke K, Krugmann J, Fiegl M, Greil R. Transendothelial migration of myeloma cells is increased by tumor necrosis factor (TNF)-alpha via TNF receptor 2 and autocrine up-regulation of MCP-1. Clin Cancer Res. 2004; 10:1901–10. [PubMed: 15041705]
25. Kim MS, Day CJ, Morrison NA. MCP-1 is induced by receptor activator of nuclear factor-{kappa}B ligand, promotes human osteoclast fusion, and rescues granulocyte macrophage colony-stimulating factor suppression of osteoclast formation. J Biol Chem. 2005; 280:16163–9. [PubMed: 15722361]
26. Balkwill F. Chemokine biology in cancer. Semin Immunol. 2003; 15:49–55. [PubMed: 12495640]
27. Kurihara T, Warr G, Loy J, Bravo R. Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. J Exp Med. 1997; 186:1757–62. [PubMed: 9362535]
28. Ajuebor MN, Flower RJ, Hannon R, Christie M, Bowers K, Verity A, Perretti M. Endogenous monocyte chemoattractant protein-1 recruits monocytes in the zymosan peritonitis model. J Leukoc Biol. 1998; 63:108–16. [PubMed: 9469480]
29. Kurth I, Willimann K, Schaerli P, Hunziker T, Clark-Lewis I, Moser B. Monocyte selectivity and tissue localization suggests a role for breast and kidney-expressed chemokine (BRAK) in macrophage development. J Exp Med. 2001; 194:855–61. [PubMed: 11561000]
30. Chensue SW, Warmington KS, Lukacs NW, Lincoln PM, Burdick MD, Strieter RM, Kunkel SL. Monocyte chemotactic protein expression during schistosome egg granuloma formation. Sequence of production, localization, contribution, and regulation. Am J Pathol. 1995; 146:130–8. [PubMed: 7856722]
31. Chensue SW, Warmington KS, Ruth JH, Sanghi PS, Lincoln P, Kunkel SL. Role of monocyte chemoattractant protein-1 (MCP-1) in Th1 (mycobacterial) and Th2 (schistosomal) antigen-induced granuloma formation: relationship to local inflammation, Th cell expression, and IL-12 production. J Immunol. 1996; 157:4602–8. [PubMed: 8906839]
32. Handel TM, Domaille PJ. Heteronuclear (1H, 13C, 15N) NMR assignments and solution structure of the monocyte chemoattractant protein-1 (MCP-1) dimer. Biochemistry. 1996; 35:6569–84. [PubMed: 8639605]
33. Gonzalo JA, Lloyd CM, Wen D, Albar JP, Wells TN, Proudfoot A, Martinez AC, Dorf M, Bjerke T, Coyle AJ, Gutierrez-Ramos JC. The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. J Exp Med. 1998; 188:157–67. [PubMed: 9653092]
34. Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. Nature. 2000; 404:407–11. [PubMed: 10746730]

35. Karpus WJ, Lukacs NW, Kennedy KJ, Smith WS, Hurst SD, Barrett TA. Differential CC chemokine-induced enhancement of T helper cell cytokine production. J Immunol. 1997; 158:4129–36. [PubMed: 9126972]
36. Dewald O, Zymek P, Winkelmann K, Koerting A, Ren G, Abou-Khamis T, Michael LH, Rollins BJ, Entman ML, Frangogiannis NG. CCL2/Monocyte Chemoattractant Protein-1 regulates inflammatory responses critical to healing myocardial infarcts. Circ Res. 2005; 96:881–9. [PubMed: 15774854]
37. Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2−/− mice reveals a role for chemokines in the initiation of atherosclerosis. Nature. 1998; 394:894–7. [PubMed: 9732872]
38. Namiki M, Kawashima S, Yamashita T, Ozaki M, Hirase T, Ishida T, Inoue N, Hirata K, Matsukawa A, Morishita R, Kaneda Y, Yokoyama M. Local overexpression of monocyte chemoattractant protein-1 at vessel wall induces infiltration of macrophages and formation of atherosclerotic lesion: synergism with hypercholesterolemia. Arterioscler Thromb Vasc Biol. 2002; 22:115–20. [PubMed: 11788470]
39. Izikson L, Klein RS, Charo IF, Weiner HL, Luster AD. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. J Exp Med. 2000; 192:1075–80. [PubMed: 11015448]
40. Shahrara S, Proudfoot AE, Park CC, Volin MV, Haines GK, Woods JM, Aikens CH, Handel TM, Pope RM. Inhibition of monocyte chemoattractant protein-1 ameliorates rat adjuvant-induced arthritis. J Immunol. 2008; 180:3447–56. [PubMed: 18292571]
41. Wada T, Yokoyama H, Furuichi K, Kobayashi KI, Harada K, Naruto M, Su SB, Akiyama M, Mukaida N, Matsushima K. Intervention of crescentic glomerulonephritis by antibodies to monocyte chemotactic and activating factor (MCAF/MCP-1). FASEB J. 1996; 10:1418–25. [PubMed: 8903512]
42. Kasahara Y, Kimura H, Kurosu K, Sugito K, Mukaida N, Matsushima K, Kuriyama T. MCAF/MCP-1 protein expression in a rat model for pulmonary hypertension induced by monocrotaline. Chest. 1998; 114:67S. [PubMed: 9676637]
43. Moore BB, Paine R 3rd, Christensen PJ, Moore TA, Sitterding S, Ngan R, Wilke CA, Kuziel WA, Toews GB. Protection from pulmonary fibrosis in the absence of CCR2 signaling. J Immunol. 2001; 167:4368–77. [PubMed: 11591761]
44. Volejnikova S, Laskari M, Marks SC Jr, Graves DT. Monocyte recruitment and expression of monocyte chemoattractant protein-1 are developmentally regulated in remodeling bone in the mouse. Am J Pathol. 1997; 150:1711–21. [PubMed: 9137095]
45. Kim MS, Day CJ, Selinger CI, Magno CL, Stephens SR, Morrison NA. MCP-1-induced human osteoclast-like cells are tartrate-resistant acid phosphatase, NFATc1, and calcitonin receptor-positive but require receptor activator of NFkappaB ligand for bone resorption. J Biol Chem. 2006; 281:1274–85. [PubMed: 16280328]
46. Alam R, Kumar D, Anderson-Walters D, Forsythe PA. Macrophage inflammatory protein-1 alpha and monocyte chemoattractant peptide-1 elicit immediate and late cutaneous reactions and activate murine mast cells in vivo. J Immunol. 1994; 152:1298–303. [PubMed: 8301133]
47. Taub D, Dastych J, Inamura N, Upton J, Kelvin D, Metcalfe D, Oppenheim J. Bone marrow-derived murine mast cells migrate, but do not degranulate, in response to chemokines. J Immunol. 1995; 154:2393–402. [PubMed: 7532669]
48. Valent P. Cytokines involved in growth and differentiation of human basophils and mast cells. Exp Dermatol. 1995; 4:255–9. [PubMed: 8528598]
49. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004; 4:71–8. [PubMed: 14708027]
50. Kim Y, Sung S, Kuziel WA, Feldman S, Fu SM, Rose CE Jr. Enhanced airway Th2 response after allergen challenge in mice deficient in CC chemokine receptor-2 (CCR2). J Immunol. 2001; 166:5183–92. [PubMed: 11290802]
51. Salcedo R, Ponce ML, Young HA, Wasserman K, Ward JM, Kleinman HK, Oppenheim JJ, Murphy WJ. Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. Blood. 2000; 96:34–40. [PubMed: 10891427]

52. Carulli MT, Ong VH, Ponticos M, Shiwen X, Abraham DJ, Black CM, Denton CP. Chemokine receptor CCR2 expression by systemic sclerosis fibroblasts: evidence for autocrine regulation of myofibroblast differentiation. Arthritis Rheum. 2005; 52:3772–82. [PubMed: 16320328]
53. Klopp AH, Spaeth EL, Dembinski JL, Woodward WA, Munshi A, Meyn RE, Cox JD, Andreeff M, Marini FC. Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment. Cancer Res. 2007; 67:11687–95. [PubMed: 18089798]
54. Lu Y, Cai Z, Xiao G, Liu Y, Keller ET, Yao Z, Zhang J. CCR2 expression correlates with prostate cancer progression. J Cell Biochem. 2007; 101:676–85. [PubMed: 17216598]
55. Loberg RD, Ying C, Craig M, Day LL, Sargent E, Neeley C, Wojno K, Snyder LA, Yan L, Pienta KJ. Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor regression in vivo. Cancer Res. 2007; 67:9417–24. [PubMed: 17909051]
56. Xia M, Sui Z. Recent developments in CCR2 antagonists. Expert Opin Ther Pat. 2009; 19:295–303. [PubMed: 19441905]
57. Binder NB, Niederreiter B, Hoffmann O, Stange R, Pap T, Stulnig TM, Mack M, Erben RG, Smolen JS, Redlich K. Estrogen-dependent and C-C chemokine receptor-2-dependent pathways determine osteoclast behavior in osteoporosis. Nat Med. 2009; 15:417–24. [PubMed: 19330010]
58. Lu Y, Chen Q, Corey E, Xie W, Fan J, Mizokami A, Zhang J. Activation of MCP-1/CCR2 axis promotes prostate cancer growth in bone. Clin Exp Metastasis. 2009; 26:161–9. [PubMed: 19002595]
59. Juppner H, Abou-Samra AB, Freeman M, Kong XF, Schipani E, Richards J, Kolakowski LF Jr, Hock J, Potts JT Jr, Kronenberg HM, et al. A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. Science. 1991; 254:1024–6. [PubMed: 1658941]
60. Burtis WJ. Parathyroid hormone-related protein: structure, function, and measurement. Clin Chem. 1992; 38:2171–83. [PubMed: 1424107]
61. Dougherty KM, Blomme EA, Koh AJ, Henderson JE, Pienta KJ, Rosol TJ, McCauley LK. Parathyroid hormone-related protein as a growth regulator of prostate carcinoma. Cancer Res. 1999; 59:6015–22. [PubMed: 10606251]
62. Iwamura M, di Sant'Agnese PA, Wu G, Benning CM, Cockett AT, Deftos LJ, Abrahamsson PA. Immunohistochemical localization of parathyroid hormone-related protein in human prostate cancer. Cancer Res. 1993; 53:1724–6. [PubMed: 8467485]
63. Iwamura M, Gershagen S, Lapets O, Moynes R, Abrahamsson PA, Cockett AT, Deftos LJ, di Sant'Agnese PA. Immunohistochemical localization of parathyroid hormone-related protein in prostatic intraepithelial neoplasia. Hum Pathol. 1995; 26:797–801. [PubMed: 7628854]
64. Deftos LJ, Barken I, Burton DW, Hoffman RM, Geller J. Direct evidence that PTHrP expression promotes prostate cancer progression in bone. Biochem Biophys Res Commun. 2005; 327:468–72. [PubMed: 15629138]
65. Guise TA, Yin JJ, Thomas RJ, Dallas M, Cui Y, Gillespie MT. Parathyroid hormone-related protein (PTHrP)-(1–139) isoform is efficiently secreted in vitro and enhances breast cancer metastasis to bone in vivo. Bone. 2002; 30:670–6. [PubMed: 11996903]
66. Yin JJ, Selander K, Chirgwin JM, Dallas M, Grubbs BG, Wieser R, Massague J, Mundy GR, Guise TA. TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. J Clin Invest. 1999; 103:197–206. [PubMed: 9916131]
67. Dunbar ME, Wysolmerski JJ. Parathyroid hormone-related protein: a developmental regulatory molecule necessary for mammary gland development. J Mammary Gland Biol Neoplasia. 1999; 4:21–34. [PubMed: 10219904]
68. Bouizar Z, Spyratos F, De vernejoul MC. The parathyroid hormone-related protein (PTHrP) gene: use of downstream TATA promotor and PTHrP 1–139 coding pathways in primary breast cancers vary with the occurrence of bone metastasis. J Bone Miner Res. 1999; 14:406–14. [PubMed: 10027905]
69. Li X, Qin L, Bergenstock M, Bevelock LM, Novack DV, Partridge NC. Parathyroid hormone stimulates osteoblastic expression of MCP-1 to recruit and increase the fusion of pre/osteoclasts. J Biol Chem. 2007; 282:33098–106. [PubMed: 17690108]

70. Liao J, Li X, Koh AJ, Berry JE, Thudi N, Rosol TJ, Pienta KJ, McCauley LK. Tumor expressed PTHrP facilitates prostate cancer-induced osteoblastic lesions. Int J Cancer. 2008; 123:2267–78. [PubMed: 18729185]
71. Li X, Loberg R, Liao J, Ying C, Snyder LA, Pienta KJ, McCauley LK. A destructive cascade mediated by CCL2 facilitates prostate cancer growth in bone. Cancer Res. 2009; 69:1685–92. [PubMed: 19176388]
72. Sekine O, Nishio Y, Egawa K, Nakamura T, Maegawa H, Kashiwagi A. Inslin activates CCAAT/enhancer binding proteins and proinflammatory gene expression through the phosphatidylinositol 3-kinase pathway in vascular smooth muscle cells. J Biol Chem. 2002; 277:36631–9. [PubMed: 12145301]
73. Ueda A, Okuda K, Ohno S, Shirai A, Igarashi T, Matsunaga K, Fukushima J, Kawamoto S, Ishigatsubo Y, Okubo T. NF-kappa B and Sp1 regulate transcription of the human monocyte chemoattractant protein-1 gene. J Immunol. 1994; 153:2052–63. [PubMed: 8051410]
74. Milligan ED, Twining C, Chacur M, Biedenkapp J, O'Connor K, Poole S, Tracey K, Martin D, Maier SF, Watkins LR. Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats. J Neurosci. 2003; 23:1026–40. [PubMed: 12574433]
75. Twining CM, Sloane EM, Milligan ED, Chacur M, Martin D, Poole S, Marsh H, Maier SF, Watkins LR. Peri-sciatic proinflammatory cytokines, reactive oxygen species, and complement induce mirror-image neuropathic pain in rats. Pain. 2004; 110:299–309. [PubMed: 15275780]
76. Jung H, Miller RJ. Activation of the nuclear factor of activated T-cells (NFAT) mediates upregulation of CCR2 chemokine receptors in dorsal root ganglion (DRG) neurons: a possible mechanism for activity-dependent transcription in DRG neurons in association with neuropathic pain. Mol Cell Neurosci. 2008; 37:170–7. [PubMed: 17949992]
77. Qian DZ, Rademacher BL, Pittsenbarger J, Huang CY, Myrthue A, Higano CS, Garzotto M, Nelson PS, Beer TM. CCL2 is induced by chemotherapy and protects prostate cancer cells from docetaxel-induced cytotoxicity. Prostate. 70:433–42. [PubMed: 19866475]
78. Phillips RJ, Lutz M, Premack B. Differential signaling mechanisms regulate expression of CC chemokine receptor-2 during monocyte maturation. J Inflamm (Lond). 2005; 2:14. [PubMed: 16259633]
79. Chen Y, Green SR, Ho J, Li A, Almazan F, Quehenberger O. The mouse CCR2 gene is regulated by two promoters that are responsive to plasma cholesterol and peroxisome proliferator-activated receptor gamma ligands. Biochem Biophys Res Commun. 2005; 332:188–93. [PubMed: 15896316]
80. Glabinski AR, Bielecki B, Kolodziejski P, Han Y, Selmaj K, Ransohoff RM. TNF-alpha microinjection upregulates chemokines and chemokine receptors in the central nervous system without inducing leukocyte infiltration. J Interferon Cytokine Res. 2003; 23:457–66. [PubMed: 13678434]
81. Yamamoto K, Takeshima H, Hamada K, Nakao M, Kino T, Nishi T, Kochi M, Kuratsu J, Yoshimura T, Ushio Y. Cloning and functional characterization of the 5′-flanking region of the human monocyte chemoattractant protein-1 receptor (CCR2) gene. Essential role of 5′-untranslated region in tissue-specific expression. J Biol Chem. 1999; 274:4646–54. [PubMed: 9988701]
82. Raymond CR, Redman SJ, Crouch MF. The phosphoinositide 3-kinase and p70 S6 kinase regulate long-term potentiation in hippocampal neurons. Neuroscience. 2002; 109:531–6. [PubMed: 11823064]
83. Gavrilin MA, Gulina IV, Kawano T, Dragan S, Chakravarti L, Kolattukudy PE. Site-directed mutagenesis of CCR2 identified amino acid residues in transmembrane helices 1, 2, and 7 important for MCP-1 binding and biological functions. Biochem Biophys Res Commun. 2005; 327:533–40. [PubMed: 15629146]
84. Terashima Y, Onai N, Murai M, Enomoto M, Poonpiriya V, Hamada T, Motomura K, Suwa M, Ezaki T, Haga T, Kanegasaki S, Matsushima K. Pivotal function for cytoplasmic protein FROUNT in CCR2-mediated monocyte chemotaxis. Nat Immunol. 2005; 6:827–35. [PubMed: 15995708]

85. Satoh M, Akatsu T, Ishkawa Y, Minami Y, Nakamura M. A novel activator of C-C chemokine, FROUNT, is expressed with C-C chemokine receptor 2 and its ligand in failing human heart. J Card Fail. 2007; 13:114–9. [PubMed: 17395051]

86. Majumder PK, Sellers WR. Akt-regulated pathways in prostate cancer. Oncogene. 2005; 24:7465–74. [PubMed: 16288293]

87. Zi X, Singh RP, Agarwal R. Impairment of erbB1 receptor and fluid-phase endocytosis and associated mitogenic signaling by inositol hexaphosphate in human prostate carcinoma DU145 cells. Carcinogenesis. 2000; 21:2225–35. [PubMed: 11133812]

88. Miyake H, Nelson C, Rennie PS, Gleave ME. Overexpression of insulin-like growth factor binding protein-5 helps accelerate progression to androgen-independence in the human prostate LNCaP tumor model through activation of phosphatidylinositol 3′-kinase pathway. Endocrinology. 2000; 141:2257–65. [PubMed: 10830316]

89. Murillo H, Huang H, Schmidt LJ, Smith DI, Tindall DJ. Role of PI3K signaling in survival and progression of LNCaP prostate cancer cells to the androgen refractory state. Endocrinology. 2001; 142:4795–805. [PubMed: 11606446]

90. Roca H, Varsos Z, Pienta KJ. CCL2 protects prostate cancer PC3 cells from autophagic death via phosphatidylinositol 3-kinase/AKT-dependent survivin up-regulation. J Biol Chem. 2008; 283:25057–73. [PubMed: 18611860]

91. Roca H, Varsos ZS, Mizutani K, Pienta KJ. CCL2, survivin and autophagy: new links with implications in human cancer. Autophagy. 2008; 4:969–71. [PubMed: 18758234]

92. Roca H, Varsos ZS, Pienta KJ. CCL2 is a negative regulator of AMP-activated protein kinase to sustain mTOR complex-1 activation, survivin expression, and cell survival in human prostate cancer PC3 cells. Neoplasia. 2009; 11:1309–17. [PubMed: 20019839]

93. Karnoub AE, Weinberg RA. Chemokine networks and breast cancer metastasis. Breast Dis. 2006; 26:75–85. [PubMed: 17473367]

94. Ueno T, Toi M, Saji H, Muta M, Bando H, Kuroi K, Koike M, Inadera H, Matsushima K. Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. Clin Cancer Res. 2000; 6:3282–9. [PubMed: 10955814]

95. Nakashima E, Mukaida N, Kubota Y, Kuno K, Yasumoto K, Ichimura F, Nakanishi I, Miyasaka M, Matsushima K. Human MCAF gene transfer enhances the metastatic capacity of a mouse cachectic adenocarcinoma cell line in vivo. Pharm Res. 1995; 12:1598–604. [PubMed: 8592656]

96. Valkovic T, Dobrila F, Melato M, Sasso F, Rizzardi C, Jonjic N. Correlation between vascular endothelial growth factor, angiogenesis, and tumor-associated macrophages in invasive ductal breast carcinoma. Virchows Arch. 2002; 440:583–8. [PubMed: 12070596]

97. Loberg RD, Ying C, Craig M, Yan L, Snyder LA, Pienta KJ. CCL2 as an important mediator of prostate cancer growth in vivo through the regulation of macrophage infiltration. Neoplasia. 2007; 9:556–62. [PubMed: 17710158]

98. Roodman GD. Mechanisms of bone metastasis. N Engl J Med. 2004; 350:1655–64. [PubMed: 15084698]

99. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer. 2002; 2:584–93. [PubMed: 12154351]

100. Taichman RS, Loberg RD, Mehra R, Pienta KJ. The evolving biology and treatment of prostate cancer. J Clin Invest. 2007; 117:2351–61. [PubMed: 17786228]

101. Keller ET, Zhang J, Cooper CR, Smith PC, McCauley LK, Pienta KJ, Taichman RS. Prostate carcinoma skeletal metastases: cross-talk between tumor and bone. Cancer Metastasis Rev. 2001; 20:333–49. [PubMed: 12085970]

102. Lu X, Kang Y. Organotropism of breast cancer metastasis. J Mammary Gland Biol Neoplasia. 2007; 12:153–62. [PubMed: 17566854]

103. Akhtari M, Mansuri J, Newman KA, Guise TM, Seth P. Biology of breast cancer bone metastasis. Cancer Biol Ther. 2008; 7:3–9. [PubMed: 18059174]

104. Morrissey C, Vessella RL. The role of tumor microenvironment in prostate cancer bone metastasis. J Cell Biochem. 2007; 101:873–86. [PubMed: 17387734]

105. Vanderkerken K, Vande Broek I, Eizirik DL, Van Valckenborgh E, Asosingh K, Van Riet I, Van Camp B. Monocyte chemoattractant protein-1 (MCP-1), secreted by bone marrow endothelial

cells, induces chemoattraction of 5T multiple myeloma cells. Clin Exp Metastasis. 2002; 19:87–90. [PubMed: 11918087]

106. Vande Broek I, Asosingh K, Vanderkerken K, Straetmans N, Van Camp B, Van Riet I. Chemokine receptor CCR2 is expressed by human multiple myeloma cells and mediates migration to bone marrow stromal cell-produced monocyte chemotactic proteins MCP-1, -2 and -3. Br J Cancer. 2003; 88:855–62. [PubMed: 12644822]
107. Hu H, Sun L, Guo C, Liu Q, Zhou Z, Peng L, Pan J, Yu L, Lou J, Yang Z, Zhao P, Ran Y. Tumor cell-microenvironment interaction models coupled with clinical validation reveal CCL2 and SNCG as two predictors of colorectal cancer hepatic metastasis. Clin Cancer Res. 2009; 15:5485–93. [PubMed: 19706805]
108. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002; 420:860–7. [PubMed: 12490959]
109. DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. Pathological and molecular aspects of prostate cancer. Lancet. 2003; 361:955–64. [PubMed: 12648986]
110. Sica A, Schioppa T, Mantovani A, Allavena P. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. Eur J Cancer. 2006; 42:717–27. [PubMed: 16520032]
111. Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L. The origin and function of tumor-associated macrophages. Immunol Today. 1992; 13:265–70. [PubMed: 1388654]
112. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell. 2005; 7:211–7. [PubMed: 15766659]
113. Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit Rev Oncol Hematol. 2008; 66:1–9. [PubMed: 17913510]
114. Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer. 2008; 8:618–31. [PubMed: 18633355]
115. Mantovani A. La mala educacion of tumor-associated macrophages: Diverse pathways and new players. Cancer Cell. 2010; 17:111–2. [PubMed: 20159603]
116. Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. J Leukoc Biol. 2009; 86:1065–73. [PubMed: 19741157]
117. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001; 357:539–45. [PubMed: 11229684]
118. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol. 2002; 196:254–65. [PubMed: 11857487]
119. Brigati C, Noonan DM, Albini A, Benelli R. Tumors and inflammatory infiltrates: friends or foes? Clin Exp Metastasis. 2002; 19:247–58. [PubMed: 12067205]
120. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002; 23:549–55. [PubMed: 12401408]
121. Mantovani A. Tumor-associated macrophages in neoplastic progression: a paradigm for the in vivo function of chemokines. Lab Invest. 1994; 71:5–16. [PubMed: 7518882]
122. Roca H, Varsos ZS, Sud S, Craig MJ, Ying C, Pienta KJ. CCL2 and interleukin-6 promote survival of human CD11b+ peripheral blood mononuclear cells and induce M2-type macrophage polarization. J Biol Chem. 2009; 284:34342–54. [PubMed: 19833726]
123. Lin EY, Gouon-Evans V, Nguyen AV, Pollard JW. The macrophage growth factor CSF-1 in mammary gland development and tumor progression. J Mammary Gland Biol Neoplasia. 2002; 7:147–62. [PubMed: 12465600]
124. Mizutani K, Roca H, Varsos Z, Pienta KJ. Possible mechanism of CCL2-induced Akt activation in prostate cancer cells. Anticancer Res. 2009; 29:3109–13. [PubMed: 19661323]
125. Lu X, Kang Y. Chemokine (C-C motif) ligand 2 engages CCR2+ stromal cells of monocytic origin to promote breast cancer metastasis to lung and bone. J Biol Chem. 2009; 284:29087–96. [PubMed: 19720836]

126. Pahler JC, Tazzyman S, Erez N, Chen YY, Murdoch C, Nozawa H, Lewis CE, Hanahan D. Plasticity in tumor-promoting inflammation: impairment of macrophage recruitment evokes a compensatory neutrophil response. Neoplasia. 2008; 10:329–40. [PubMed: 18392134]

127. Eisold S, Schmidt J, Ryschich E, Gock M, Klar E, von Knebel Doeberitz M, Linnebacher M. Induction of an antitumoral immune response by wild-type adeno-associated virus type 2 in an in vivo model of pancreatic carcinoma. Pancreas. 2007; 35:63–72. [PubMed: 17575547]

128. Gocheva V, Wang HW, Gadea BB, Shree T, Hunter KE, Garfall AL, Berman T, Joyce JA. IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. Genes Dev. 2010; 24:241–55. [PubMed: 20080943]

129. Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. J Clin Invest. 2008; 118:560–70. [PubMed: 18219394]

130. Palframan RT, Jung S, Cheng G, Weninger W, Luo Y, Dorf M, Littman DR, Rollins BJ, Zweerink H, Rot A, von Andrian UH. Inflammatory chemokine transport and presentation in HEV: a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. J Exp Med. 2001; 194:1361–73. [PubMed: 11696600]

131. Brown Z, Robson RL, Westwick J. Regulation and expression of chemokines: potential role in glomerulonephritis. J Leukoc Biol. 1996; 59:75–80. [PubMed: 8558071]

132. Haberstroh U, Stilo K, Pocock J, Wolf G, Helmchen U, Wenzel U, Zahner G, Stahl RA, Thaiss F. L-arginine suppresses lipopolysaccharide-induced expression of RANTES in glomeruli. J Am Soc Nephrol. 1998; 9:203–10. [PubMed: 9527396]

133. Zachariae CO, Anderson AO, Thompson HL, Appella E, Mantovani A, Oppenheim JJ, Matsushima K. Properties of monocyte chemotactic and activating factor (MCAF) purified from a human fibrosarcoma cell line. J Exp Med. 1990; 171:2177–82. [PubMed: 2161898]

134. Martin TJ, Mundy GR. Bone metastasis: can osteoclasts be excluded? Nature. 2007; 445:E19. discussion E-20. [PubMed: 17314931]

135. Kyriakides TR, Foster MJ, Keeney GE, Tsai A, Giachelli CM, Clark-Lewis I, Rollins BJ, Bornstein P. The CC chemokine ligand, CCL2/MCP1, participates in macrophage fusion and foreign body giant cell formation. Am J Pathol. 2004; 165:2157–66. [PubMed: 15579457]

136. Steinbrech DS, Mehrara BJ, Saadeh PB, Greenwald JA, Spector JA, Gittes GK, Longaker MT. VEGF expression in an osteoblast-like cell line is regulated by a hypoxia response mechanism. Am J Physiol Cell Physiol. 2000; 278:C853–60. [PubMed: 10751333]

137. Dovio A, Data V, Angeli A. Circulating osteoprotegerin and soluble RANKL: do they have a future in clinical practice? J Endocrinol Invest. 2005; 28:14–22. [PubMed: 16550717]

138. Lu Y, Cai Z, Xiao G, Keller ET, Mizokami A, Yao Z, Roodman GD, Zhang J. Monocyte chemotactic protein-1 mediates prostate cancer-induced bone resorption. Cancer Res. 2007; 67:3646–53. [PubMed: 17440076]

139. Mizutani K, Sud S, Pienta KJ. Prostate cancer promotes CD11b positive cells to differentiate into osteoclasts. J Cell Biochem. 2009; 106:563–9. [PubMed: 19170075]

140. Mizutani K, Sud S, McGregor NA, Martinovski G, Rice BT, Craig MJ, Varsos ZS, Roca H, Pienta KJ. The chemokine CCL2 increases prostate tumor growth and bone metastasis through macrophage and osteoclast recruitment. Neoplasia. 2009; 11:1235–42. [PubMed: 19881959]

141. Cai Z, Chen Q, Chen J, Lu Y, Xiao G, Wu Z, Zhou Q, Zhang J. Monocyte chemotactic protein 1 promotes lung cancer-induced bone resorptive lesions in vivo. Neoplasia. 2009; 11:228–36. [PubMed: 19242604]

142. Zhang J, Patel L, Pienta KJ. CC chemokine ligand 2 (CCL2) promotes prostate cancer tumorigenesis and metastasis. Cytokine Growth Factor Rev. 2009

143. Yang X, Lu P, Ishida Y, Kuziel WA, Fujii C, Mukaida N. Attenuated liver tumor formation in the absence of CCR2 with a concomitant reduction in the accumulation of hepatic stellate cells, macrophages and neovascularization. Int J Cancer. 2006; 118:335–45. [PubMed: 16052523]

144. Halin S, Rudolfsson SH, Van Rooijen N, Bergh A. Extratumoral macrophages promote tumor and vascular growth in an orthotopic rat prostate tumor model. Neoplasia. 2009; 11:177–86. [PubMed: 19177202]

145. Gazzaniga S, Bravo AI, Guglielmotti A, van Rooijen N, Maschi F, Vecchi A, Mantovani A, Mordoh J, Wainstok R. Targeting tumor-associated macrophages and inhibition of MCP-1 reduce angiogenesis and tumor growth in a human melanoma xenograft. J Invest Dermatol. 2007; 127:2031–41. [PubMed: 17460736]

146. Zeisberger SM, Odermatt B, Marty C, Zehnder-Fjallman AH, Ballmer-Hofer K, Schwendener RA. Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new and highly effective antiangiogenic therapy approach. Br J Cancer. 2006; 95:272–81. [PubMed: 16832418]

147. Kimura YN, Watari K, Fotovati A, Hosoi F, Yasumoto K, Izumi H, Kohno K, Umezawa K, Iguchi H, Shirouzu K, Takamori S, Kuwano M, Ono M. Inflammatory stimuli from macrophages and cancer cells synergistically promote tumor growth and angiogenesis. Cancer Sci. 2007; 98:2009–1. [PubMed: 17924976]

148. Fridlender ZG, Buchlis G, Kapoor V, Cheng G, Sun J, Singhal S, Crisanti C, Wang LC, Heitjan D, Snyder LA, Albelda SM. CCL2 blockade augments cancer immunotherapy. Cancer Res. 70:109–18. [PubMed: 20028856]

149. Takahashi M, Miyazaki H, Furihata M, Sakai H, Konakahara T, Watanabe M, Okada T. Chemokine CCL2/MCP-1 negatively regulates metastasis in a highly bone marrow-metastatic mouse breast cancer model. Clin Exp Metastasis. 2009; 26:817–28. [PubMed: 19629725]

150. Huang S, Singh RK, Xie K, Gutman M, Berry KK, Bucana CD, Fidler IJ, Bar-Eli M. Expression of the JE/MCP-1 gene suppresses metastatic potential in murine colon carcinoma cells. Cancer Immunol Immunother. 1994; 39:231–8. [PubMed: 7954525]

151. Huang S, Xie K, Singh RK, Gutman M, Bar-Eli M. Suppression of tumor growth and metastasis of murine renal adenocarcinoma by syngeneic fibroblasts genetically engineered to secrete the JE/MCP-1 cytokine. J Interferon Cytokine Res. 1995; 15:655–65. [PubMed: 7553238]

152. Zhang L, Yoshimura T, Graves DT. Antibody to Mac-1 or monocyte chemoattractant protein-1 inhibits monocyte recruitment and promotes tumor growth. J Immunol. 1997; 158:4855–61. [PubMed: 9144501]

153. Monti P, Leone BE, Marchesi F, Balzano G, Zerbi A, Scaltrini F, Pasquali C, Calori G, Pessi F, Sperti C, Di Carlo V, Allavena P, Piemonti L. The CC chemokine MCP-1/CCL2 in pancreatic cancer progression: regulation of expression and potential mechanisms of antimalignant activity. Cancer Res. 2003; 63:7451–61. [PubMed: 14612545]

154. Hirose K, Hakozaki M, Nyunoya Y, Kobayashi Y, Matsushita K, Takenouchi T, Mikata A, Mukaida N, Matsushima K. Chemokine gene transfection into tumour cells reduced tumorigenicity in nude mice in association with neutrophilic infiltration. Br J Cancer. 1995; 72:708–14. [PubMed: 7669585]

155. Nesbit M, Schaider H, Miller TH, Herlyn M. Low-level monocyte chemoattractant protein-1 stimulation of monocytes leads to tumor formation in nontumorigenic melanoma cells. J Immunol. 2001; 166:6483–90. [PubMed: 11359798]

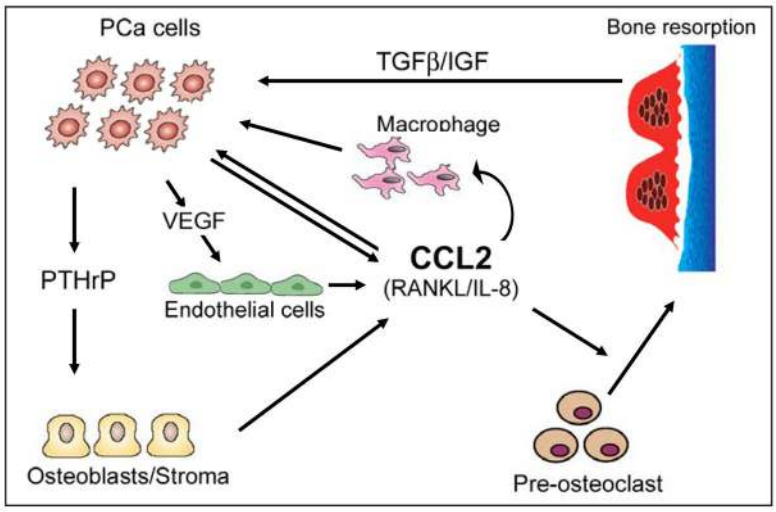

Figure 1.

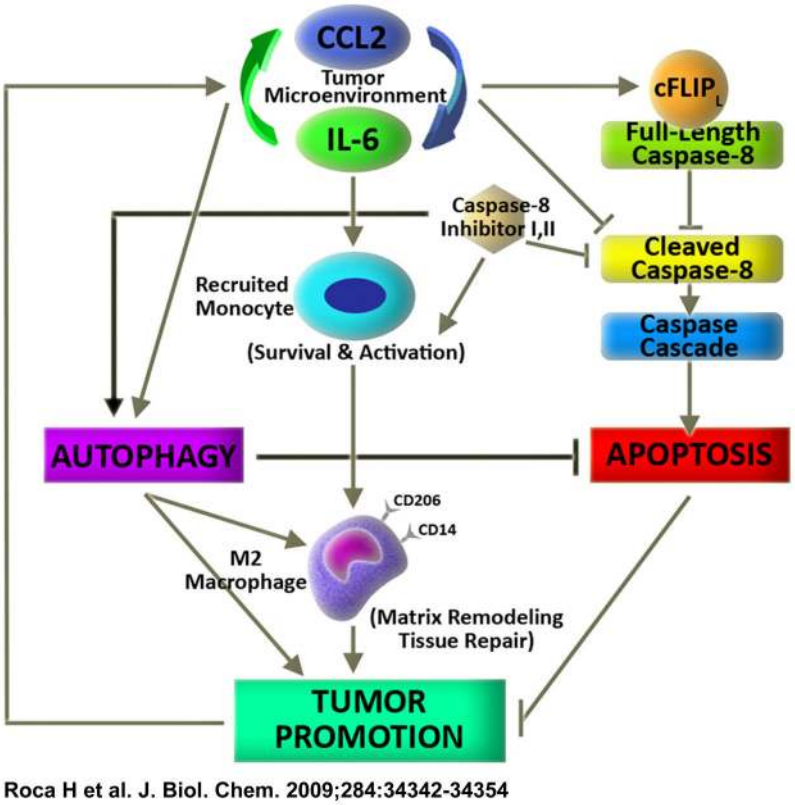

Figure 2.

Proposed mechanism by which CCL2 and IL-6 potentiate tumor progression by protecting tumor infiltrating monocytes and inducing their differentiation toward M2-type macrophages.

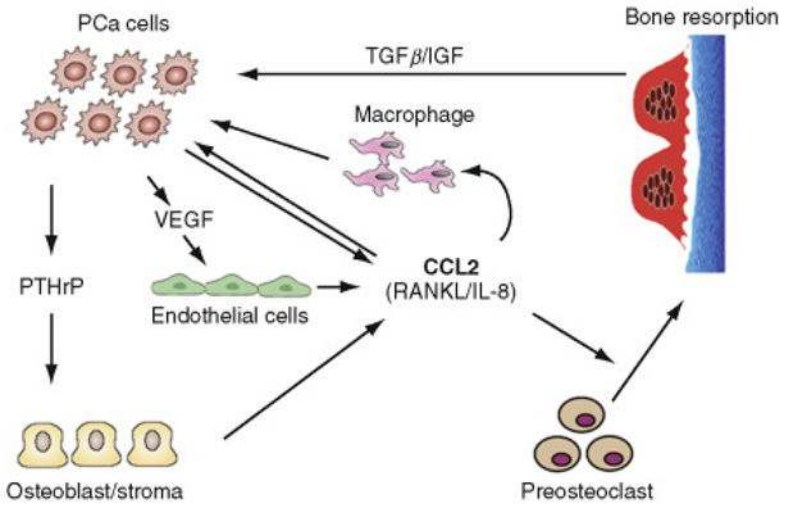

Figure 3.

Roles of chemokine (C-C motif) ligand 2 (CCL2) in prostate cancer cells and the bone microenvironment. The CCL2/CCR2 axis has been identified as an important contributor to prostate tumorigenesis. CCL2, by binding to its receptor CCR2, directly stimulates prostate cancer cell proliferation, survival, and migration. In addition, CCL2 contributes to the development of metastases in the bone microenvironment by stimulating macrophage recruitment and education, angiogenesis, and activation of osteoclastogenesis. Prostate cancer cells produce parathyroid hormone-related peptide (PTHrP), which stimulates CCL2 expression from osteoblasts. CCL2 appears to mediate the interactions between tumor-derived factors, such as PTHrP, and host-derived chemokines and cytokines, which act together to promote metastatic tumor growth in bone. IGF = insulin-like growth factor; IL-8 = interleukin 8; PCa = prostate cancer; RANKL = receptor activator of NF-κB ligand; TGF-β = transforming growth factor β; VEGF = vascular endothelial growth factor.[16]