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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 as 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 chemoattractant 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 chemoattractive 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

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- κ B ligand (RANKL) and tumor necrosis factor alpha (TNF α) (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 existence of 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 hematopoietic 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 NF κ B 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 NFκB 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 NFκB 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 NFκB 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, NFκB and/or C/EBP. It is worth to note that TNFα 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 phosphatidylinositol 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, TNF α 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 metastatic 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 TNF α /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, TNF α , 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 (CNT0888) 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 CCL2 in prostate cancer 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 wild 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 enzymes, 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 transfected 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 models (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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Figure 1.

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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