Published in final edited form as:

Crit Rev Eukaryot Gene Expr. 2013 ; 23(1): 77–91.

Chemokines and Chemokine Receptors as Promoters of Prostate Cancer Growth and Progression

Nicole Salazar^{1,2}, Miguel Castellán³, Samir S. Shirodkar³, and Bal L. Lokeshwar^{1,2,3,4,5,*}

¹Sheila and David Fuente Graduate Program in Cancer Biology, University of Miami School of Medicine, Miami, Florida

²Sylvester Comprehensive Cancer Center, University of Miami School of Medicine, Miami, Florida

³Department of Urology, University of Miami School of Medicine, Miami, Florida

⁴Department of Radiation Oncology, University of Miami School of Medicine, Miami, Florida

⁵Research Service, Bruce W. Carter VA Medical Center, Miami, Florida

Abstract

Prostate cancer (CaP) is estimated to be first in incidence among cancers, with more than 240,000 new cases in 2012 in the United States. Chemokines and their receptors provide survival, proliferation, and invasion characteristics to CaP cells in both primary sites of cancer and metastatic locations. The emerging data demonstrate that many chemokines and their receptors are involved in the multistep process of CaP, leading to metastasis, and, further, that these factors act cooperatively to enhance other mechanisms of tumor cell survival, growth, and metastasis. Changes of chemokine receptor cohorts may be necessary to activate tumor-promoting signals. Chemokine receptors can activate downstream effectors, such as mitogen-activated protein kinases, by complex mechanisms of ligand-dependent activation of cryptic growth factors; guanosine triphosphate-binding, protein-coupled activation of survival kinases; or transactivation of other receptors such as ErbB family members. We describe vanguard research in which more than the classic view of chemokine receptor biology was clarified. Control of chemokines and inhibition of their receptor activation may add critical tools to reduce tumor growth, especially in chemo-hormonal refractory CaP that is both currently incurable and the most aggressive form of the disease, accounting for most of the more than 28,000 annual deaths.

Keywords

chemokines; chemokine receptors; prostate cancer; apoptosis; survival signaling; gene regulation; growth factor receptors; invasion; motility; metastasis

I. INTRODUCTION

The American Cancer Society's Cancer Facts and Figures for 2012 estimated prostate cancer to be first in incidence (241,740 cases) and second in estimated deaths (28,170) among cancers in the United States.¹ This trend has been the same for the 10 years, except in 2006, when colon and rectal cancers overtook mortality of CaP by 1%. If detected early by a simple blood test to determine the levels of prostate-specific antigen (PSA), CaP can be

treated effectively, with a median survival of >15 years. However, a significant fraction of the cancers, even if detected early, are aggressive and become lethal rapidly, in <5 years. The most significant danger of CaP is osteoblastic metastasis to bone, mostly to the lumbar vertebrae, even though soft tissue metastases are also common. The effective nonsurgical methods of treatment of CaP involve focused radiation to the pelvic area, total androgen blockade with a variety of drugs that bring the androgen level in the body to near zero, or androgen receptor blockers, which affect a much broader population of tumor cells and often are combined with both radiation and antiandrogen therapy. Nonsurgical treatments, especially those involving chemical castration, are temporary, and the disease recurs within 2–5 years.^{2–4} The recurrent disease is then treated by oncologists with a set of chemotherapeutic agents, notably docetaxel, and now with custom-made tumor vaccines.⁵ However, treatment failure is almost certain within 3–4 months; a rise of PSA in the blood is the surrogate marker for CaP recurrence and the median survival of patients with castration-resistant CaP (CRPC) treated with docetaxel, mitoxantrone, or other combination chemotherapy is about 19 months at present.⁶ There are many explanations for the failure of each therapeutic modality in CaP, a discussion of which is not the purpose of this review.

Nothing is needed more urgently than the development of better treatment for bone metastasis of CaP because it contributes to significant morbidity, including pain, impaired mobility, and increased fatality. Little is known about the mechanism of bone metastasis in human CaP. Lack of a testable animal model that recapitulates human bone metastasis patterns is another limitation. However, bone metastasis can be observed in a xenograft model by direct injection of susceptible human tumor CaP cells into the bone, by injecting into the ventricle and thus bypassing tumor cell circulation through the lungs, or by examining rare occurrences of microscopic tumor lesions in the bone as in the LNCaP C4-2B models. As a survival mechanism, CaP cells adapt to express factors that allow enhanced affinity to the bone marrow, leading to bone metastasis with osteoblastic changes in a high percentage of patients with advanced CaP. This adaptation by tumor cells includes morphological and physiological changes in response to existing therapy. Evidence is presented below to focus efforts to understand a class of secreted proteins and their cell surface receptors that participate in the process of metastasis and help tumor cells challenge their elimination by treatments.

One aspect that has not been exploited comprehensively until now is the contribution of autocrine and paracrine factors that affect CaP growth, survival in a hostile environment, avoidance of immune surveillance, and adoption of alternate survival mechanisms upon chemotherapy stress. CaP cells have been found to express survival, differentiation, chemotactic, and growth factors for their growth in ectopic organs, such as bone marrow. Although chemokines and their receptors are relatively new to this list of tumor-promoting paracrine and autocrine factors, they are involved in many critical normal functions as well as pathological processes.⁷ The purpose of this article is to highlight the contribution of chemokine receptors, which are integral to CaP development, survival, and acquisition of resistance to chemo-hormonal therapy.

II. CHEMOKINES

Chemokines, also known as chemoattractant cytokines, are a super family of small secreted proteins initially characterized by their ability to induce leukocyte migration. They are small proteins (8–12 kDa) known as cytokines that have chemoattractant properties and thus are called chemokines. One of their most critical functions is governed by the specific amino acid sequence (or motif) of Glu-Leu-Arg (or ELR) immediately preceding the first cysteine of their CXC motif. ELR⁺ CXC chemokines are considered angiogenic, whereas ELRCXC chemokines are considered angiostatic.⁸

The first chemokine, discovered in 1987, was interleukin (IL)-8 (or CXCL8).^{9,10} More than 50 chemokines and 20 chemokine receptors have been identified since. Chemokines and their receptors have a highly conserved sequence throughout the genetic tree.¹¹ They are grouped into 4 categories (C, CC, CXC, and CX3C) based on the number and position of conserved cysteine residues near the NH₂ terminus of the molecule.¹² Most chemokines are able to bind with high affinity to multiple receptors while their receptors usually are restricted to a single subclass. Chemokines generally act via 7-span transmembrane (TM) guanine nucleotide-binding protein (G protein)-coupled receptors (GPCRs) and induce an intensive signaling cascade of activated second messengers that lead to cell motility and multiple other functional effects in the target cells. Cellular trafficking and processes are influenced significantly by chemokines via their receptors.

III. CHEMOKINE RECEPTORS

Most chemokine receptors bind both specifically and promiscuously to their chemokine ligands. Chemokine receptors exist in many epithelial and hematopoietic cells. These receptors are members of the 7-TM GPCR super family. Like chemokines, chemokine receptors also share structurally conserved/homologous regions, which allow their subgrouping classification. In general, upon binding to their ligands, chemokine receptors undergo conformational changes that allow the binding of G proteins to intracellular loop epitopes and the carboxy terminal tail of the receptors.¹³ The second intracellular loop of the receptors contains a Asp-Arg-Tyr-Leu-Ala-Ile-Val (DRYLAIV) motif, which is missing in the nonsignaling receptors and does not permit G-protein coupling.¹² The chemokine receptors that do not bind G proteins may act in synchrony with other proteins that step in to support their effects. Examples of these alternative cohorts include β -arrestin, integrins, and growth factor receptors.^{14–19} A generalized signaling mechanism of chemokine-chemokine receptors in cell proliferation and motility is illustrated in Fig. 1.

IV. CHEMOKINE RECEPTORS EXPRESSED ON PROSTATE CANCER CELLS

Chemokines are vital for leukocyte migration and activation, and physiologically they have been implicated in various immune/inflammatory/activation responses such as allergic disease, atherosclerosis, and wound healing. Furthermore, chemokines play a key role in the neoplastic processes of many organs and in determining the location of the metastatic spread of cancer cells away from the primary tumor. Multiple chemokines and their receptors (including CCR5, CCR7, CCR9, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR7) are involved in the multistep process of CaP metastasis.^{15,18,20–28} A single chemokine receptor may recognize more than one chemokine, and a single chemokine may bind more than one receptor. The complex interactions between chemokines and their receptors regulate the activity and the response of their target cells, acting directly on tumor or host cells and giving rise to a diversity of effects that shape the malignant phenotype. Table 1 lists the currently known chemokines and their receptors that are expressed in CaP tissues or by established CaP cell lines. Here we highlight the significance and functions associated with some of the chemokines and their receptors. Since there are fewer receptors for CXCL chemokines—only 7 to date—than for chemokines (at least 17), we discuss the role of the chemokine receptors via binding or independent of ligands in CaP growth and metastatic progression.

A. CXCR1 and CXCR2

These GPCRs are expressed on the surface of CaP cells, tumor tissues, and metastatic lesions. Increased expression of IL-8 (CXCL8) has been correlated with increased

angiogenesis, tumor progression, and incidence of lymph node metastasis arising from androgen-independent CaP cells implanted in athymic nude mice.^{22,29,30} Elevated IL-8 and IL-8 receptor expression (CXCR1, CXCR2) has been shown in cancer cells from prostate biopsy tissue, and the intensity of IL-8, CXCR1, and CXCR2 staining increased with stage of disease.^{22,31} IL-8 levels also correlated with more aggressive CaP with a higher Gleason score and higher lymph node metastasis.³² Most interesting is that the IL-8/receptor axis has been shown to be a method of resistance in androgen-independent CaP cells by means of increased nuclear factor (NF)- κ B activity. Wilson et al.³³ demonstrated how expression of CXCR1 and CXCR2 increased in CaP cells after treatment with oxaliplatin, a drug whose activity is reportedly sensitive to NF- κ B activity. Surprisingly, this is the only chemokine/receptor in the literature directly associated with resistance; evidence for any of the others still is lacking.

CXCR1 is a coreceptor for the inflammatory chemokine IL-8 and it heterodimerizes with CXCR2 upon binding to IL-8/CXCL8. In addition, it binds weakly to Gro- α and CXCL6, although little is known about the physiological consequences of binding to these ligands. Unlike CXCR1, CXCR2 is more promiscuous: it interacts with other protein ligands besides as Gro- α , such as Gro- β , Gro- γ , neutrophil activating protein-2, and granulocyte chemotactic protein-2.^{34–36} We and others have shown that CXCR1 activation by CXCL8 triggers mitogenic response in tumor cells, including that of CaP cells, and there is increased accumulation of CXCR1 intracellularly.^{22,37–40} Silencing the expression of CXCR1 in CaP leads to growth arrest, increased apoptosis by an intrinsic (mitochondria-mediated) mechanism, and reduced tumor growth in human CaP xenografts in athymic mice. Furthermore, CXCR1 knock out also decreases functions associated with CXCR2, such as decreased angiogenesis and secretion of vascular endothelial growth factor (VEGF)-1, but not motility and invasive potential.⁴⁰ The role of CXCR1 as a mitogenic activator via CXCL8 binding is more pronounced in CRCP cell lines, and possibly in humans as well, because the circulating levels of CXCL8 are higher in patients with CRCP.⁴¹ Studies also have shown that increased levels of CXCL8, and therefore potentially more active CXCR1 in primary tumors, may predict biochemical (PSA) progression of CaP.⁴² These observations suggest that CXCL8 may be one of the several chemokines that support tumor cell survival and proliferation in the absence of androgens (i.e., the emergence of CRPC).^{23,43}

B. CXCR4

The chemokine receptor CXCR4 is expressed predominantly on lymphocytes, where it activates chemotaxis. Although it is not expressed in normal human epithelial cells, it is the most common chemokine receptor overexpressed in human cancers. CXCR4 and its ligand CXCL12/SDF-1 have been shown to participate in the proliferation, differentiation, and meta-stasis of many cancers, including CaP; CXCR4 levels increased in metastatic CaP compared with localized CaP.⁴⁴ Mochizuke et al.⁴⁵ evaluated the expression level of SDF-1 and CXCR4 in CaP cell lines (LNCaP, PC3, and DU145) and normal prostate epithelial cell line. The PC3, DU145, and LNCaP cell lines originally were isolated from vertebral, brain, and lymph node metastases of patients with CaP. CXCR4 expression was detected in all 3 CaP cell lines, but not in prostate epithelial cells. In bone marrow, CXCL12 is expressed by osteoblasts, fibroblasts, and endothelial cells, and high levels of CXCL12 are present in lymph nodes. During normal cellular development, the CXCL12/CXCR4 axis plays a crucial role in the migration and patterning of many embryonic cell lineages, the homeostatic transport of hematopoietic stem cells, and the transport of lymphocytes. In addition, the complex may be important in the proangiogenic development of tumors because CXCL12 is an efficacious chemoattractant for endothelial cells that express the CXCR4 receptor.⁴⁶ Darash-Yahana et al.⁴⁷ examined the role of high expression of receptor

CXCR4 on prostate tumor growth *in vivo* and *in vitro*. Mice injected with tumor cells expressing high levels of CXCR4 developed increased tumor burden versus control tumors; thus high levels of CXCR4 seem to confer a more aggressive behavior to CaP cells. Sun et al.^{48,49} also evaluated the role of CXCL12/CXCR4 expression and its association with increased malignant potential by examining human CaP cells for expression of CXCR4 and CXCL12 using high-density tissue microarrays constructed from samples obtained from patients who had undergone radical retropubic prostatectomy. They observed that the protein expression of CXCR4 was elevated significantly in both localized and metastatic CaP, and levels of CXCL12 were higher in metastatic lesions than in primary tumors, suggesting that CXCL12 might serve as a growth factor for metastatic disease once the tumor had traveled to, and lodged in, a metastatic site. They also observed that after adding a neutralizing antibody to CXCL12, there was a significant decrease in the proliferation of bone-homing LNCaP and PC3 metastatic tumor cells, suggesting that CXCL12 derived from CaP cells acts in an autocrine fashion. CXCR4 expression has been hypothesized to be a useful prognostic factor for patients with metastatic CaP. Akashi et al.^{50,51} evaluated CXCR4 expression in 52 patients with metastatic CaP who received hormonal therapy. Before any treatment, specimens were obtained using transperineal needle biopsy and were stained with antihuman CXCR4 antibody. A statistically significant association was noted between CXCR4 expression and cancer-specific survival, and CXCR4 was detected in 94.2% of patients with metastatic CaP. Patients with an elevated expression of CXCR4 in tumors had lower cancer-specific survival than those with low expression.

Not all research shows CaP cells express CXCR4. In one study, Darash-Yahana et al.⁴⁷ detected no or low CXCR4 surface expression in all 3 CaP cell lines (PC3, LNCaP, DU-145). In their study, transfection of CXCR4 into CaP cells did not up-regulate the chemotactic potential of the tumor cells. Similarly, Heresi et al.²¹ reported a patient with metastatic CaP presenting with generalized lymphadenopathy who failed to express messenger RNA (mRNA) for CXCR4 but did express mRNA for CCR1, CCR4, and CCR5. Engl et al.¹⁵ published an inverse relationship between CXCR4 expression and the malignant behavior of prostate tumor cells but a positive relationship between intracellular CXCR4 content and tumor malignancy. In their studies, flow cytometry showed that the number of CXCR4-positive results was higher in cells with less malignant potential (LNCaP > DU-145 > PC-3). They also observed that treatment of LNCaP cells with the antitumor and differentiation-inducing compound valproic acid caused CXCR4 down-regulation within the cytoplasm and a concomitant accumulation of CXCR4 on the plasma membrane. They hypothesized that the absolute amount of receptors on the cell surface might not determine the grade of malignancy but rather the CXCR4 shifting into the cytoplasm. Mochizuki et al.⁴⁵ performed Boyden chamber migration (*in vitro*) assays to investigate the chemotactic effect and growth-promoting effect of SDF-1 on DU145 and PC3 cells. SDF-1 significantly enhanced the migration of PC3 and DU145 cells in a dose-dependent manner, and anti-CXCR4 antibody inhibited this chemotactic effect. They suggest that the interaction between the SDF-1 and CXCR4 ligand-receptor system is involved in the process of CaP metastasis by the activation of cancer cell migration. They also performed immunohistochemical analysis of CXCR4 expression in tissues from 35 cases of human CaP. Of 35 clinical CaP samples, 20 (57.1%) were found to be positive for the CXCR4 protein, and the positive expression was an independent predictor factor of bone metastasis. CXCR4 expression is associated with increased tumor aggressiveness, and it was an independent prognostic factor in CaP with bone metastasis.

Engl et al.⁵² studied the importance of the CXCL12/CXCR4 axis as a mediator for the adherence of prostate tumor cells to the endothelium and for interactions with extracellular matrix proteins such as laminin, fibronectin, and collagen. Their study showed that CXCR4 expressed on the surface of LNCaP and DU-145 cells enables migration toward a CXCL12

gradient. Anti-CXCR4 antibodies or CXCR4 knockout cells significantly impaired CXCL12-related tumor cell binding. The CXCL12/CXCR4 complex triggers tumor cell adhesion to endothelial cells as well as to extracellular matrix proteins, playing a key role in controlling binding events before invasion.

The role of the CXCL12/CXCR4 axis in the directional migration of metastatic CaP cells to specific organs has been studied by Arya et al.⁵³ They studied the role of the CXCL12/CXCR4 chemokine ligand receptor complex in determining the organ-specific metastasis of CaP in 3 metastatic prostate cell lines, a primary CaP cell line, and in normal prostate epithelial cell lines. Migration studies revealed that chemotaxis of the metastatic cell lines was enhanced by the CXCL12 ligand and inhibited by the antibody to CXCR4. They concluded that in human prostate cell lines derived from metastases the CXCL12/CXCR4 ligand receptor interaction enhances their migratory capabilities and supports directional migration of metastatic CaP cells to specific organs.

An interesting study by Chinni et al.¹⁶ showed that the CXCL12/CXCR4 complex interacts with the ErbB family in CaP cells. They explored the cell membrane activity of CXCR4 and found that CXCR4 and human epidermal growth receptor 2 (HER2) heterodimerize and localize to lipid rafts. They also showed that over-expression of CXCR4 leads to increased HER2 phosphorylation and migratory properties of CaP cells, suggesting the key role of lipid raft signaling in CaP metastasis to the bone microenvironment. Another exciting study compared 2-dimensional and 3-dimensional (3D) cultures and found that growing PC3 CaP cells in 3D culture actually up-regulates the expression of CXCR4, which does not occur in 2-dimensional culture. This result is more consistent with human prostate tissue biopsies, where a highly invasive phenotype is correlated with high expression of CXCR4.⁵⁴

Taken together, these data show that CaP cells express CXCR4 in specific areas and that high levels of CXCR4 are associated with increased malignant potential and likelihood of metastasis. These studies demonstrate that the CXCL12/CXCR4 axis plays a significant role in the migration of CaP cells to specific organs.

V. OTHER CHEMOKINES ON PROSTATE CANCER CELLS

CaP cells produce large amounts of proangiogenic CXC chemokines. Normal prostatic epithelial cells produce low levels of angiogenic ELR⁺ CXC chemokines and high levels of angiostatic ELR⁻ CXC chemokines, whereas CaP cells produce high levels of angiogenic ELR⁺ CXC chemokines and low levels of angiostatic ELR⁻ CXC chemokines.⁸ These altered productions of CXC chemokines promote neovascularization and growth of tumor cells.

A. CXCR2 and CXCR3

In vivo and *in vitro* studies have identified the angiogenic chemokine receptors CXCR2 and CXCR3 as the receptors mediating the angiostatic properties. In 2006 Shen et al.²⁴ reported that alteration in the balance of these receptors resulted in the growth of prostate tumors. In their study, mice with transgenic adenocarcinoma of the prostate (TRAMP) were bred with CXCR2 or CXCR3 knockout mice. Tumors in mice with TRAMP/CXCR3^{-/-} were palpable much earlier than those in mice with TRAMP/CXCR3^{+/+} and had greatly increased angiogenesis. These data showed that the malignant transformation of prostatic epithelial cells results in an increased production of pro-angiogenic chemokines and a decrease in angiostatic chemokines that through interaction with their receptors, CXCR2 and CXCR3, contribute to an angiogenic balance that ultimately may regulate prostate tumor growth. These observations were corroborated by Engl et al.¹⁵ when they reported a comparative analysis of 3 different prostate tumor cell lines (PC-3, DU-145, and LNCaP) and showed

evidence that alterations in tumor cell growth and adhesion are paralleled by alterations in CXCR expression. CXCR3 was expressed on the plasma membranes of the 3 cell lines, and the CXCR3/CXCL10 axis was considered the element responsible for regulation of prostate tumor growth, with an inverse relationship between CXCR3 surface expression and growth capacity. Another study found that for CXCR3, 2 mRNA isoforms were expressed differentially in CaP. In a CaP specimen, the CXCR3A mRNA level was up-regulated while CXCR3B mRNA was down-regulated. They also found that normal prostate epithelial cells (RWPE-1 cell line) expressed half the CXCR3A level that the invasive and metastatic DU-145 and PC-3 cells did.²⁵

B. CXCR5

CXCR5 is a specific chemokine receptor for CXCL13. CXCL13 is expressed by osteoblasts, osteoclasts, and vascular endothelial cells after stimulation with inflammatory factors. It is believed that CXCL13 mediates enhanced invasive capacity and is related to modulation of matrix metalloproteinases (MMPs).²⁸ In 2009 Singh et al.²⁸ reported that CXCR5 mRNA and protein expression are significantly elevated in CaP cells lines compared with matched normal tissues. CaP cells with an elevated Gleason score showed higher expression of nuclear CXCR5. In another study, Singh et al.⁵⁵ showed that the CXCL13-mediated adhesion of CaP cells to human bone marrow endothelial cells works through a CXCR5- α v β 3-integrin association and found that the chemokine CXCL13 was a better predictor of CaP than PSA. This type of critical information may improve the false-positive risks associated with the current standard of screening mechanisms (the PSA test). The same group also showed that activation of the Akt, extracellular signal-regulated kinases 1 and 2, and JNK pathways in CaP specifically employs dedicator of cytokinesis 2 as a means of mediating growth and metastatic potential in hormone-refractory, CXCR5-positive CaP cells.⁵⁶ Taken together, these data demonstrate the clinical and biological relevance of the CXCR5/CXCL13 axis in CaP progression and metastasis.

C. CXCR6

CXCR6 is a chemokine receptor present in prostate tissue and bone marrow. CXCL16 has been identified as a ligand for receptor CXCR6, and the interaction between CXCL16 and CXCR6 has been shown to intervene in multiple biological activities. Using immunohistochemistry, Hu et al.⁵⁷ investigated the protein expression of CXCL16/CXCR6 in human CaP and benign prostatic hyperplasia and CXCL16 expression in human osseous tissue samples. Moderate to strong CXCR6 protein expression was demonstrated in primary CaP cells and was more elevated than expression in the cells from hyperplastic epithelia. CXCL16 was expressed positively by osteocytes and endothelial cells in human bone sections. *In vitro* studies showed that PC3 and LNCaP cells expressed CXCR6 at the mRNA and protein levels, and exogenous CXCL16 had the potential to stimulate the invasion of these cells *in vitro*. These data suggest that, besides CXCL12/CXCR4, the CXCL16/CXCR6 axis has a potential role mediating CaP selective metastasis to bone. Wang et al.⁵⁸ examined the protein expression of CXCR6 using high-density tissue microarrays and immunohistochemistry. *In vivo* and *in vitro* studies showed that alterations in CXCR6 were associated with invasive activities and tumor growth of CaP cells. CXCR6 expression was able to regulate the expression of the proangiogenic factors IL-8 and VEGF. CXCL16 signaling induced the activation of the Akt/mammalian target of rapamycin pathways, which have been shown to play a central role in the development of CaP by regulating the progression of cell growth and cell cycle. By using rapamycin, Wang et al. significantly reduced CXCL16-induced CaP cell invasion and growth, which may be beneficial in the development of a therapeutic strategy for CaP.

D. CXCR7

CXCR7 is the newest member of the chemokine receptor family. CXCR7 functions as a chemokine receptor for SDF-1/CXCL12, which is historically known to be a potent chemoattractant for mature and immature hematopoietic cells and a regulator of a spectrum of normal and pathological processes. In 2007, Begley et al.⁵⁹ reported that PC3 cells express CXCR7. CXCR7 affects a spectrum of important biological and pathological processes in CaP, including cell growth, survival, and adhesion. Van Rechem et al.⁶⁰ linked the inactivation of HIC-1 with overexpression of CXCR7. Wang et al.²⁷ studied the role of CXCR7 in CaP progression and metastasis. In their work, staining of high-density CaP tissue microarrays showed that more aggressive tumors correlated with higher expression of CXCR7. They provided evidence of a survival advantage in cells that overexpress CXCR7 *in vivo* and *in vitro* and functional evidence showing how CXCR7 expression affects blood vessel formation in CaP cells by altering the response to IL-8 and VEGF. They also showed that CXCR4 regulates CXCR7 levels and that signaling by CXCR7 activates the Akt pathway. In another facet of the same receptor, our laboratory has demonstrated that CXCR7 interacts with the epidermal growth factor receptor (EGFR) to promote CaP growth.¹⁸ We showed that in CaP there is a physical association of CXCR7 with EGFR, and down-regulation of CXCR7 affects downstream activation of the growth factor receptor, leading to suppressed growth of prostate tumors through cell cycle arrest. The implication of this work for future studies is significant because it sets a foundation in understanding the dynamics that can illustrate the interaction between the chemokine receptor super family and the epithelial growth factor family heterodimerization.

E. CCR9 and CCR2

CCR9 is another chemokine of the CC subfamily that is overexpressed in CaP cell lines versus prostatic epithelial cells.²⁰ The expression of functional CCR9 may facilitate tumor cell migration and invasion. When induced by CCL25, CCR9 expression correlates with enhanced migration and invasion of CaP cells. Inhibition of CCL25-CCR9 interactions reduced the migration and invasive competence of LNCaP and PC3 cells. LNCaP cells express higher levels of CCR9 compared with PC3 cells, and this lymph node-derived cell line has higher migration and invasion potential when stimulated by CCL25. CCL25 differentially modulates MMP expression of LNCaP and PC3 cell lines. Lu et al.^{61,62} reported that the ligand for CCR2, CCL2 or MCP-1, acts as a paracrine and autocrine factor for CaP growth and invasion. They also reported that expression of the receptor is increased in more aggressive CaP cells compared with less aggressive and benign prostate cells, suggesting a potential role of CCR2 in CaP progression.

Chemokines play a critical role in determining the fate of the developing tumor by regulating the migration of different leukocyte subtypes to tumors. Although the process of metastasis formation is complex, and even today is still not entirely understood, many studies, like the ones highlighted here, indicate that chemokines and their receptors play a major role in the survival of prostatic cancer cells. Multiple chemokines and their corresponding receptors are involved in the multistep process of CaP cell development, progression, and metastasis. A single chemokine and receptor might not regulate tumor growth and metastasis because these are multistage processes. Therefore, the expression of multiple and varied chemokine receptors working together on the surface of tumor cells may be what ultimately leads to adhesion and survival advantages. In view of the importance of several chemokine axes, manipulation of these signaling pathways may be of therapeutic benefit against CaP. Identification and elucidation of the roles of different receptors in tumor genesis and progression may give us new tools for potential therapeutic interventions in CaP. However, the clinical application of such an approach should be done with great care. Targeting the chemokine receptor pathway alone may be dangerous, a double-edged sword,

because of the seminal role of chemokine receptors in the immune system and normal processes (such as bone and stem cell homeostasis), which is why an approach that is either double-targeted or multitargeted (such as chemokine and growth factor receptor interaction) may be more beneficial: it may relieve the tension applied to just one point (the chemokine receptors) and be more efficiently distributed across the diseased cell. In Fig. 2, we illustrate the expression pattern of chemokine receptors in various locations and various stages of CaP.

VI. DISCUSSION AND FUTURE PERSPECTIVES

Many chemokines and their receptors affect the development and progression of CaP. We have included only the chemokines and chemokine receptors that are expressed endogenously by CaP cells and lead to cancer development. The most evidence is provided for CXCR1, 2, and 4. The more aggressive tumors and CaP cell lines express higher levels of CXCR1, CXCR2, and CXCR4 compared with less aggressive cells. However, CXCR2 and CXCR7 levels are high in prostate tumors, while in human prostate tumors, CXCR2 and CXCR7 levels are high compared to non cancerous tissue.^{22, 23, 63}

However, many other factors affect CaP development, including hormonal state and genetic and epigenetic contributions; the microenvironment also contributes heavily.^{64–67} Chemokine receptors in cancer have become a field of increasing interest and exploration. They are connected deeply with some of the critical hallmarks of cancer development, mainly growth, angiogenesis, and metastasis. We have described some examples of how the expression of these receptors compare in CaP. However, many questions remain for consideration. An important component missing throughout the literature is a standardization of the levels of each receptor type and their expression relative to each other within the cell lines and in tissue from human biopsy samples. Establishing normal levels may prove to be very informative for understanding their mechanistic functional importance at a basal level and how their overexpression (or loss) dysregulates downstream signaling events. Elucidation of cross-talk mechanisms within CaP cells, as seen with CXCR4-HER2 and CXCR7-EGFR, demonstrates the importance of chemokine receptor levels and their impact on other receptor families and the membrane environment.^{16,18}

Further work needs to explore the complexity of these interactions and the role of other notable factors such as VEGF, key metalloproteinases (i.e. ADAMs, MMPs), and ligand shedding, which may be critical for chemokine receptor transactivation of other receptor families. Receptor level and dynamics may vary considerably because of the autocrine feedback loops and other key factors of the cells and cellular microenvironment. It is critical to use improved models that simulate the real tumor microenvironment within CaP, such as improved osteoblastic CaP metastasis models *in vivo*. Studying CaP cells in 3D tissue and showing that the level of a chemokine in serum is an improved measure of CaP over PSA is innovative work that pushes the field forward because it simulates the human microenvironment more closely.^{54,55}

The relevance of chemokines and their receptors in CaP has been demonstrated by many recent studies, as described here. However, specific details about the cause of this expression are scarce in the literature. In CaP, there are several critical factors that influence progression. Among these, androgen receptor expression is the leader. The growth of CaP cells is primarily regulated by androgen, its activation of the androgen receptor, and eventually androgen-independent receptor activation. It was shown recently that the CXCL12/CXCR4 axis can directly affect the activation of androgen receptor in CRCP.⁶⁸ In a previous study, Begley et al.⁵⁹ also demonstrated how this chemokine axis mediates a complex transcriptional response in prostate epithelial cells, suggesting that CXCL12 has

significant weight in the etiology of benign proliferative disease. Epigenetic changes may also be responsible for dysregulation of chemokines and their receptors, as shown with the overexpression of CXCR7 upon inactivation of HIC-1. Only a few reports have demonstrated chemokine and control at the promoter level, but they make it clear that pathways controlled by NF- κ B and HIC-1 may be responsible for their control and dysregulation.^{33,60} These 2 instances (pathways controlled by NF- κ B and HIC-1) are arguably the only explanations of how these factors are triggered thus far.

Finally, another important factor that may affect chemokine and receptor dysregulation in CaP progression is the effect of inflammatory mechanisms that can influence tumorigenesis. CaP progression and metastasis is associated with the infiltration of lymphocytes/macrophages into advanced tumors and the up-regulation of tumor necrosis factor family members such as receptor activator of NF- κ B ligand and lymphotoxin.⁶⁹ Other factors released by the primary tumor that many trigger expression of chemokines and their receptors include VEGFA, transforming growth factor- β , and tumor necrosis factor- α .^{67,70} These changes in environment may explain why chemokine and chemokine receptor expression is triggered; however, how these factors are triggered and controlled is still not clearly understood.

VII. CONCLUSION

These experiments demonstrate a proof of principle for the potential combination therapy approach to treat CaP progression and metastasis. So far, studies have focused on finding that CaP expresses chemokine receptors and have tried to understand their function. This has led to a vastly improved understanding that undoubtedly will lead to improved targeted therapy applicable beyond the field of CaP. However, it will be critical to understand what factors trigger the dysregulated expression of chemokine receptors in CaP. This type of insight could lead to what is better than improved therapeutic intervention: prevention. CaP is mainly a disease of the elderly. Because the life expectancy of the world's population is increasing and the percentage of adults older than 60 years in industrialized countries is the largest quartile of the population because of industrialization, technology, and medical advancements, it is urgent that we find alternatives to treat the morbidity and lethality of cancer. The synergy between current treatments and chemokine receptors may be an answer to provide improved therapy and even complete ablation of this disease.

Acknowledgments

The authors thank Ms. Maite Lopez for the illustrations (Fig. 2). This work is supported by funding from U.S. Public Health Service (National Institutes of Health [NIH]) grant nos. 5R01CA 63108-14, 1R01 AT 003544, and VA MERIT Review Program (5312.1 and 5312.2) (to BLL, Department of Urology and Sylvester Cancer Center). NS was supported in part by NIH Initiative for Maximizing Student Development Grant 1R25GM076419.

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FIGURE 1.

Chemokines receptor interactions in prostate cancer. These receptors are members of the 7-transmembrane guanine nucleotide-binding protein (G protein)-coupled receptor super family. In general, upon binding to their ligands, chemokine receptors undergo conformational changes that allow the binding of G proteins to intracellular loop epitopes and the carboxyl terminal tail of the receptors. The chemokine receptors that do not bind G proteins may act in synchrony with other proteins such as b-arrestin, integrins, and growth factor receptors to support their downstream effects.

FIGURE 2.

Chemokines and their receptors in the promotion of prostate cancer (CaP) growth and metastasis. Multiple chemokines and their corresponding receptors are involved in the multistep process of CaP cell development, progression, and metastasis. As CaP progresses, survival factors are activated that trigger the more aggressive CaP cells to express higher levels of chemokine receptors, leading to survival advantages. ECM, extracellular membrane.

TABLE 1

Chemokine Receptors Currently Linked to Prostate Cancer

Chemokine Receptor	Chemokine Ligands*	Description	Human Prostate Tissue Expression	CaP Cell Lines	References
CCR2	CCL2 , 8, 16	Metastasis	Yes	C4-2B, DU145, and PC3	Lu et al., 2007 ⁶²
CCR5	CCL5 , 3,4, CCL3L1, CCL4L1	Metastasis	Yes	PC-3, DU-145, and LNCaP	Vaday et al., 2006 ²⁶
CCR7	CCL19, 21	Metastasis	Yes	Unknown	Heresi et al., 2005 ²¹
CCR9	CCL25	Migration and invasion	Unknown	LNCaP and PC3	Vaday et al., 2006 ²⁶ Singh et al., 2004 ²⁰
CXCR1	CXCL8 , 6	Proliferation and metastasis	Yes	LNCaP, PC3, and DU-145	Murphy et al., 2005 ²² Heresi et al., 2005 ²¹ Inoue et al., 2000 ²⁹
CXCR2	CXCL 1, 2, 3, 5, 6, 7, 8	Metastasis	Yes	LNCaP and DU-145	Murphy et al., 2005 ²² Heresi et al., 2005 ²¹ Shen et al., 2002 ²⁶
CXCR3	CXCL9, 10 , 11, 14	Growth	Yes	PC-3, DU-145, and LNCaP	Shen et al., 2002 ²⁶ Engl et al., 2006 ¹⁵
CXCR4	CXCL12	Adhesion, proliferation, and metastasis	Yes	PC-3, DU-145, and LNCaP	Akashi et al., 2006 ⁵⁰ Mochizuki et al., 2006 ⁴⁵ Engl et al., 2006 ⁵² Chinni et al., 2008 ¹⁶ Wang et al., 2005 ¹⁴ Sun et al., 2003 ⁴⁸
CXCR5	CXCL13	Invasion and MMP modulation	Yes	LNCaP and PC-3	Singh et al., 2009 ²⁸
CXCR6	CXCL16	Adhesion and chronic inflammation	Yes	LNCaP and PC-3	Hu et al., 2008 ⁵⁷ Wang et al., 2008 ⁵⁸
CXCR7	CXCL11 , 12	Growth and proliferation	Yes	LNCaP	Wang et al., 2008 ²⁷ Singh and Lokeshwar, 2011 ¹⁸ Begley et al., 2007 ⁵⁹ Van Rechem et al., 2009 ⁶⁰

* Ligands in bold are those reported to bind the receptors. All others are those implicated in progression of prostate cancer.