REVIEW

Prostate Cancer

The Role of Inflammation and Chemokines

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Prostate cancer (PC) is a leading cause of death in men. Inflammation is one of the initiating processes whereby cells are trafficked into the tumor microenvironment by specific cytokines termed chemokines. This recruitment is complex and involves diverse leukocyte subsets with procancer and anticancer functions. Chemokines promote/abrogate proliferation of cancerous cells, block/aid apoptosis, and are instrumental/detrimental in cancer cell migration required for metastasis. Chemokines guide the release/transport of immune cells that serve as chaperones at sites of inflammation, and after subsequent activation, they lead to an immune response. The variety of immune cells recruited at the site of tumor initiation possess unique functions, and the plethora of chemokines released by each cell derived from a progenitor cell activated under a defined set of conditions dictates its specific role in cancer progression/regression. Geographic consequences that govern the climate and endemic diseases, along with the associated evolutionary effects that at times protect populations from one disease, could lead to genetic variations that determine a role for ethnicity and race in PC risk and susceptibility. Dysregulated expression or an imbalance in the homeostatic mechanisms associated with chemokines is implicated in PC. This review discusses the role of inflammation and chemokines in PC. (*Am J Pathol* 2019, 189: 2119–2137; <https://doi.org/10.1016/j.ajpath.2019.07.007>)

Prostate cancer (PC) is the most common cause of cancer in men. Although a disease of elderly people, it strikes almost one in eight men over their lifetime (Prostate Cancer UK, <https://prostatecanceruk.org/prostate-information/about-prostate-cancer>, last accessed August 2, 2019).^{1,2} Unlike normal cells, which maintain their homeostasis by regulating expression and response to their immediate environment, cancer cells hijack the same homeostatic cells to generate an environmental milieu that supports its rabid multiplication, migration, and metastasis. Evidence from epidemiologic studies has revealed that chronic inflammation, due to hormones, chemicals, radiation, or viral infections, is one of the major risks for developing cancer and the same applies to PC (Figure 1). The key feature of cancer-related inflammation is the recruitment of leukocytes, production of cytokines and chemokines, and subsequent progression, angiogenesis, epithelial-mesenchymal transition (EMT), migration, and metastasis.³

Inflammation is one of the leading causes of cancer, and it had been postulated as a cause of cancer >150 years ago by Rudolph Virchow.⁴ Inflammation-associated cancer presents with a microenvironment consisting of chemokines produced by cancer cells and leukocyte subtypes. Chemokines (chemotactic cytokines) are small protein ligands that act through their receptors and play a fundamental role in development, inflammation, immune surveillance, and the progression of cancer.^{3,5}

Basically, the movement of cells is regulated spatiotemporally by chemokines. Chemokines act to recruit the first line of defense, the effector innate immune cells. These cells then cooperate and integrate the effects of other mediators (lipids, minerals, peptides, complement pathway proteins, and other chemoattractants) at the site of inflammation.

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Figure 1 Schematic representation of the factors influencing progression/remission of prostate cancer. The etiology of cancers and, thus, of prostate cancer is multifactorial, with inflammation being a focal point and the recruitment of immune cells along with the associated chemokines and soluble mediators being crucial in the determination of prostate cancer progression/regression.

These effects then play a critical role in the EMT, motility, invasion, and metastasis of cancer cells.⁶ With a complex chemokine ligand-receptor family and cascade of events involved in PC, their role in the tumor microenvironment (TME) becomes crucial; and this review discusses research performed so far in this field along with highlighting their use in therapeutics.

Chemokines

Chemokines are chemotactic cytokines that promote their effects by binding to their respective receptors. They are a group of chemoattractant regulatory proteins (approximately 8 to 12 kDa) that traffic leukocytes and position them, thus playing a critical role in orchestrating immune responses and inflammation.³ The chemokine landscape is diverse and involves interactions with and between different chemokines and their receptors that function in a combinatorial cascade of events with several other adhesion molecules. Altogether, the effect of chemokines could be either deleterious or advantageous, homeostatic or inflammatory, depending on the composition of/at the stages of cancer. Their main function is to bring about the coordinated movement of immune cells to the site of inflammation.

Chemokines and their corresponding receptors are divided into four groups based on the cysteine residue: CXC (α), CC (β), CX3C (γ) and XC (δ), where cysteine is denoted by C and the noncysteine residue by X. The CXC chemokines are divided into two subtypes, depending on the presence/absence

of a Glu-Leu-Arg motif at the N terminus. The chemokines transduce their signals via seven transmembrane G-protein coupled receptors. There are approximately 50 typical chemokines that bind to their respective typical receptors, of which there are 20. At least two chemokines have an alternative non-allelic form in humans: for chemokine (C-C motif) ligand CCL3 is termed CCL3-L1; and for CCL4, it is termed CCL4-L1.³ Although the *CCL3* and *CCL3L1* genes encode two biologically distinct proteins, the proteins encoded by *CCL4* and *CCL4-L1* are similar in activity.^{3,5}

Apart from the typical chemokine receptors, there are also atypical chemokine receptors. The atypical chemokine receptors are predominantly expressed on nonleukocytic cells and bind chemokines with a high affinity. They also regulate the expression of other typical chemokine receptors.⁷ The atypical chemokine receptors (ACKRs) do not transduce their signals via G proteins and present with a lack of chemotactic activity. This group of receptors is mainly composed of the five receptors: ACKR1 [Duffy antigen receptor for chemokines (DARC)], ACKR2 [chemokine binding protein 2 (CCBP2) and D6], ACKR3 (CXCR7), ACKR4 (CCRL1 and CCXCR), and ACKR5 (CCRL2).⁷

On the one hand, there is perfect binding of chemokines to their respective typical/atypical chemokine receptors, but on the other hand there are various studies that have demonstrated the binding of unknown/nonchemokines to chemokine receptors and chemokines binding to unknown/nonchemokine receptors, described later in the section on chemokine complexes.

Inflammation

Acute, followed by chronic, inflammation is said to be a determinant that predisposes individuals to various cancers and has been surmised by the laboratory of Mantovani et al⁸ as one of the hallmarks of cancer. Epidemiologic studies suggest that inflammatory diseases predispose individuals to an increased risk of cancer by generating a microenvironment that contributes to cancer cell multiplication, migration, and metastasis. It was Virchow,⁹ who in 1863 defined the role of inflammatory leukocytes at sites of cancer/tumor and thus made his observation that lymphoreticular infiltrate is reflective of chronic inflammation. Basically, early-stage PC develops into advanced and metastatic PC through a combination of factors associated within the TME, the mediators (noncellular), and immune cells (cellular) at the site of the tumor. The presence of inflammatory cells and mediators, such as chemokines and cytokines at the site of the cancer, is a poor prognostic factor because of the favorable conditions for metastasis. Some studies also have reported a negative correlation between inflammation and PC, suggesting a protective role of immune cell infiltrates.¹⁰ Recently, a cluster of molecules, defined as inflammasomes, have emerged as regulators of inflammation.

Inflammasomes are oligomeric complexes that typically consist of sensors [nucleotide binding domain—like receptors (NLRs): NLRP1, NLRP3, NLRP4, NLRP6, and NLRP12; absent in melanoma 2—like receptors; proteins that contain a tripartite motif, and pyrin, an adaptor molecule (apoptosis-associated speck-like protein containing a caspase-activation and recruitment domain; alias PYCARD)], and the cysteine protease procaspase-1.¹¹ On activation of the inflammasome complex, caspase-1 is catalyzed into its subunits p10 and p20, which then promotes processing and release of IL-1 β and IL-18.¹¹ Although it is understood that inflammasomes promote cancer through hypoxic conditions, an elevation in the serum levels of IL-1 β and IL-18 correlates with an increased risk and prognosis of PC; and elevated levels of serum IL-18 are a feature in patients exhibiting locally advanced PC.¹¹ With cytokines, inflammation, inflammasomes, and the immune cells playing an important role in cancer progression, targeting, and studying the TME with its various immune cells will facilitate an understanding of their individual role in PC.

The TME is composed of tumor epithelial cells, extracellular matrix (ECM), myeloid-derived suppressor cells (MDSCs), mesenchymal stem cells (MSCs), cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), tumor-associated dendritic cells (DCs), tumor-infiltrating T cells, and several other immune-inflammatory cells.² Basically, almost all cells of the immune system, including monocytes, basophils, macrophages, B cells, CD4⁺ T cells, CD8⁺ T cells, type 17 helper T (Th17) cells, $\gamma\delta$ T cells, DCs, neutrophils, eosinophils, natural killer (NK) cells, NK T cells, and regulatory T (Treg) cells, are found associated with various tumors.² Chemokines mediate the activation of the adaptive arm of the immune system via stimulation of the innate immune cells through

pattern recognition receptors. Innate immune cells, like macrophages, dendritic cells, and mast cells, release soluble mediators (chemokines) that attract effector innate cells, like neutrophils and monocytes, to sites of inflammation.² The inflammatory TME in PC is a suitable niche containing several other mediators, like tumor necrosis factor (TNF), IL-6, IL-7, IL-2, IL-15, and IL-17.^{2,6} In addition, PC cells themselves secrete a set of soluble mediators, including colony stimulating factor 1 (CSF-1), granulocyte macrophage-colony stimulating factor (GM-CSF), granulocyte macrophage-colony stimulating factor receptor (GM-CSFR), and CCL2, which promote recruitment of immune cells and subsequent differentiation into their activated counterparts.¹² One of the chemokine receptors, CXCR3 (CD183), which binds to interferon (IFN)- γ -inducible ligands CXCL9, CXCL10, CXCL11, and CXCL4, is expressed on Th1 cells, CD8⁺ cells, NK T cells, NK cells, monocytes, DCs, and cancer cells.¹³ A list of the chemokines/receptors and associated immune cells is detailed in Table 1.^{14–31}

Effector innate immune cells additionally release their own set of soluble mediators (chemokines), thereby orchestrating the movement of innate immune cells and activating the adaptive immune response. Thus, chemokines and their corresponding receptors play a critical role in integrating the innate and adaptive arms of the immune system against cancer-related inflammation. The predominant subtypes of immune cells within the TME are listed below.

T Lymphocytes

T lymphocytes infiltrate the tumor at the sites of origin and at its margins. Infiltration of T cells had been studied to be associated with higher survival rates by Vesalainen et al¹⁴ in 1994, and further studies by several other groups have demonstrated their association with chemokines and chemokine receptors (Table 1). Since then, various studies have reported contrasting results, with studies defining a protumorigenic and antitumorigenic role for T lymphocytes, composed of CD4⁺, CD8⁺, Th17, Th9, Th22, T-follicular helper, and Treg cells.³² Each of the T-cell subsets produces proinflammatory and anti-inflammatory soluble mediators: the CD8⁺ T cells secrete IFN- γ , TNF- α , IL-2, granzyme, and perforin; Th1 cells secrete IFN- γ , TNF- α , and IL-2; Th2 cells secrete IL-4, IL-5, IL-13, IL-25, and IL-10; and Tregs secrete IL-10, transforming growth factor (TGF)- β , IL-35, CD39, CD73, and indoleamine 2,3-dioxygenase (IDO).³³ The Th2 cells express CCR3, CCR4, CCR5, CCR8, CXCR3, and CXCR4 on their surface; Th17 cells express CCR7, CXCR5, CCR4, CCR5, and CXCR6; T-follicular helper cells express CXCR5; Th9 cells express CCR3, CCR6, and CXCR3; Th22 cells express CCR4, CCR6, and CCR10; and Tregs express CXCR3, CCR6, and CCR4.⁵

The CD8⁺ T cells, or cytotoxic T lymphocytes, are generally considered to be associated with a favorable prognosis and a population of CD8⁺ T cells, CD8⁺CD44⁺, has been recognized to reduce tumor burden.³²

Table 1 List of Chemokines and Their Receptors along with the Immune Cell Expression and Known Therapeutic Agents¹⁴

Name	Immune cell expression	Ligand	Therapeutic agents	Reference
CC Family				
<i>CCR1</i>	Monocyte, macrophage, neutrophil, Th1, basophil, DC	CCL3, CCL4, CCL5, CCL7, CCL14, CCL15, CCL16, CCL23		6,15
<i>CCR2</i>	TAMs, MDSCs, monocytes, platelets Monocyte, macrophage, Th1, DC, basophil, NK	CCL2, CCL7, CCL8, CCL12, CCL13	CNTO-888 (anti-CCL2 Ab) C1142 (anti-CCL2 Ab)	12,15–20
<i>CCR3</i>	Platelets, eosinophil, basophil, mast cell	CCL5, CCL7, CCL11, CCL13, CCL15, CCL24, CCL26, CCR28		5
<i>CCR4</i>	Tregs, monocytes, platelets, Th2, Th17, CD8 ⁺ , B cell, DC	CCL2, CCL3, CCL5, CCL17, CCL22		5,19–21
<i>CCR5</i>	TAMs, monocyte, macrophage, Th1, NK, Treg, CD8 ⁺ , DC, neutrophil	CCL3, CCL4, CCL5, CCL8	Anti-CCR5 (TAK-779 maraviroc)	16,17,22,23
<i>CCR6</i>	Th17, DC, $\gamma\delta$ T, NKT, NK, Treg, TFH	CCL20		5,16
<i>CCR7</i>	T cells, Th22, Tregs, DC, B cells	CCL19, CCL21		16,24,25
<i>CCR8</i>	Th2, Treg, $\gamma\delta$ T, monocyte, macrophage	CCL1, CCL4, CCL17, CCL18		26
<i>CCR9</i>	B cell, DC	CCL25		16,27
<i>CCR10</i>	T cells, Tregs	CCL27, CCL28		5
CXC Family				
<i>CXCR1</i>	Neutrophils, MDSCs, NK, mast cell, basophil, CD8 ⁺	CXCL6, CXCL8		16,28
<i>CXCR2</i>	Neutrophils, MDSCs, platelets, NK, mast cell, basophil, CD8 ⁺ T	CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8	Anti-CXCL1 Ab	16,17,28
<i>CXCR3</i>	T cells, NKT cells, platelets, Th1, CD8 ⁺ and T _{EM} , NK, NKT, pDC, B cell, Treg, TFH	CXCL9, CXCL10, CXCL11, CXCL12		5,16,22
<i>CXCR4</i>	MDSCs, most (if not all) leukocytes	CXCL12	Anti-CXCR4s (plerixafor AMD3100 CTCE9908)	16,17,22
<i>CXCR5</i>	B cell, TFH, TFR, CD8 ⁺	CXCL13		5,29
<i>CXCR6</i>	Th1, Th17, $\gamma\delta$ T, ILC, NKT, NK, plasma cell	CXCL16	Anti-CXCL16 Ab	5,17,30
<i>CXCR7 (ACKR3)</i>	Endothelial cells	CXCL11, CXCL12		16,31
CX3C Family				
<i>CX3CR1</i>	TAMs, monocyte, macrophage, Th1, CD8 ⁺ , NK, $\gamma\delta$ T cell, DC	CX3CL1		5,16
XC Family				
<i>XCR1</i>	T cells, NK, CD8 ⁺ , DC, thymic DC	XCL1		5
<i>ACKR5 (CCRL2)</i>		CCL2, CCL5, CCL7		7

Adapted from *Janeway's Immunobiology*,⁵ Griffith et al,¹⁵ Marcuzzi et al,¹⁶ and Coniglio.¹⁷

Ab, antibody; CCL, chemokine (C-C motif) ligand; DC, dendritic cell; ILC, innate lymphoid cell; MDSC, myeloid-derived suppressor cell; NK, natural killer; pDC, plasmacytoid dendritic cell; TAM, tumor-associated macrophage; TEM, T-cell effector memory; TFH, T-follicular helper; TFR, T follicular regulatory cell; Th, helper T cell; Treg, regulatory T.

In addition to the presence of CD8⁺ T cells at the site of tumor margins, there are CD4⁺ Th1 cells that are endowed with the presence of chemokine receptors (CCR5, CXCR3, and CXCR4) on their cell surface in addition to producing cytokines and soluble mediators, like IL-2 and IFN- γ .²² An increase in the inflammatory cell infiltrate has been associated with a higher risk of tumor recurrence; and enhanced presence of CD4⁺ T cells due to chemokines, like CXCL9, in the TME is associated with poor survival in PC patients.³⁴ The infiltrating cells promote invasion by PC cells through modulation of the fibroblast growth factor 11

(FGF11):miRNA541: androgen receptor (AR):matrix metalloproteinase 9 (MMP9) signaling pathway.³⁴

CCL5 and its receptor CCR5 promote antitumor activity by recruiting immune cells with antitumor activity. In mice, CCR5 deficiency leads to adenocarcinoma and lymphoma, although it has been demonstrated that expression of CCR5 on CD4⁺ and CD8⁺ T cells leads to immunity against cancers and maximal antitumor response.²³ Another study, examining the same chemokine axis in PC, suggests that the CCL5:CCR5 axis promotes PC progression, with both CCL5 and CCR5 being expressed in PC cell lines and tissues.

The Th17 cells secrete IL-17A, IL-17F, IL-21, and IL-22; and a loss of function in the Th17 lineage leads to a prevention in the development of microinvasive PC in mouse models.¹³ IL-17A is secreted by a plethora of other T-cell subsets, including NK T cells, $\gamma\delta$ T cells, and CD8⁺ T cells; and its binding to respective receptors promotes the release of a separate set of soluble mediators, like IL-6, IL-1 β , TNF- α , β -defensins, and CXC chemokines, like CXCL8, CXCL9, CXCL10, and CXCL11, which may each again have protumorigenic and antitumorigenic effects, depending on their individual effects.¹³ In a pilot study, the detection of lower NK cell activity in whole blood from PC patients resulted in a better outcome at prostate biopsy.³⁵ The same was confirmed by another study in androgen-deprived PC patients, in which elevated levels of CD56⁺ NK cells correlated negatively with PC progression.³⁶ The same study also demonstrated increased levels of CD3⁺ and CD8⁺ T cells, which correlated positively with PC progression.³⁶

Another subset of cells involved in PC is the $\gamma\delta$ T cells, which are classified on the basis of the T-cell receptor δ chains into V δ 1, V δ 2, V δ 3, and V δ 5 types. Each of the types of δ variants are further divided into subsets based on the γ chains. Proliferation of PC tumor cells was suppressed in severely immunodeficient mice, and an initial study using the $\gamma\delta$ T-cell agonist zoledronate in combination with IL-2 presented with promising results in patients with metastatic PC.³⁷

Immune responses against the tumors are dependent on the subset of T cell recruited to the site of the tumor, which is dependent on the chemokine and cytokine gradient defining the specific type of inflamed cells. The Tregs, through their immunosuppressive effects, favor tumor-promoting characteristics; and the same has been demonstrated in PC patients, in whom elevated levels of Tregs conferred poor survival rates and a greater possibility of death.³⁸ Various immune populations are trafficked to the tumor site, and Treg trafficking is mainly mediated through the production of CCL17 and CCL22 by tumor-infiltrating T cells and TAMs through the CCL17/CCL22:CCR4 axis.²¹

B Lymphocytes

Tumor-infiltrating B-lymphocyte cells are provided the right niche by Th2, T-follicular helper, and other infiltrating cells for the generation of memory B-cell and associated responses. Tumor-infiltrating B-lymphocyte cells promote an antitumor effect through the production of tumor-specific antibodies for recognition by macrophages. Tumor-infiltrating B-lymphocyte cells secrete IL-2, IL-4, TNF- α , IL-22, IL-6, IL-15, and GM-CSF, whereas regulatory B cells produce protumorigenic and antitumorigenic soluble factors, like IL-10, IL-12, TNF- α , FasL, CXCL8, lymphotoxin, TGF- β , granzyme B, and PDL1.³³

Demonstrating a pivotal role of B cells, a study of anti-androgen therapy in mouse models showed apoptosis of cells at the PC site, which subsequently led to the recruitment of leukocytes, including B cells, and PC recurrence.

Recruitment of B cells to the site of tumor is dependent on CXCL13; and the recruited B cells secrete lymphotoxin, which activates inhibitory- κ B kinase α (IKK α) and STAT3, thereby promoting survival and proliferation of androgen-deprived PC cells and progression to castration-resistant PC (CRPC).³⁹ Thus, PC cells progress to CRPC on activation of the CXCL13/IKK α /STAT3/E2F1/BMI1 (RNF51) pathway, which causes ubiquitination of histones within the PC cell nuclei.³⁹ Inflammation due to advanced PC, which may contribute to castration-resistant PC, is due to leukocytes and B-cell infiltration that produce cytokines/chemokines, which eventually activate the IKK α and STAT3 pathways.⁴⁰

IL-21-induced regulatory B cells, producing granzyme B, have a role in PC, where these cells modulate adaptive immune responses and allow cancer progression through the recruitment of Tregs.³⁹ Genotoxic stress due to chemotherapeutic drugs leads to changes in B-cell 1 (a subset of B cells with CD27⁺CD43⁺), promoting the secretion of proteins like the Wnt family member wingless-type mouse mammary tumor virus integration site family member 16B, which is regulated by NF- κ B.⁴¹ Expression of wingless-type mouse mammary tumor virus integration site family member 16B in the TME leads to the progression of PC.⁴¹

Thus, B cells play a protumorigenic or antitumorigenic role, depending on the soluble mediators released and the regulation of associated immune cells.

Tumor-Associated Macrophages

TAMs are a prominent component of the infiltrating immune cells in the TME of PC. Recruitment of leukocyte populations to the primary cancer site is a hallmark of cancer; and studies have predominantly reported the presence of macrophages, which is made possible by cytokines and chemokines, like vascular endothelial growth factor (VEGF) and CCL2. TAMs originate and differentiate from the bone marrow-derived monocytes on activation through soluble mediators and contact with different types of epithelial cells. Essentially, macrophages are heterogeneous and, with plasticity being an important characteristic, are classified into M1, M2, and a spectrum of macrophage phenotypes at various stages of differentiation between the M1/M2 subtypes.¹² Although M1 is predominantly proinflammatory and tumoricidal, the M2 subtype is proangiogenic and anti-inflammatory and plays a role in immune tolerance and, thus, a protective role, thereby promoting cancer progression.¹² The recruitment of specific subsets of macrophages is dependent on the soluble mediators released by the tumor cells and immune cells present within the TME. Macrophages and monocytes are attracted toward inflammatory sites due to CCL2, and this chemokine also acts as a Th2-polarizing factor along with modulating the secretion of IL-4 by T cells. Thus, CCL2 appears to be a macrophage polarizing factor favoring macrophages gaining an anti-inflammatory phenotype.⁴²

TAMs are recruited to cancer sites under the influence of chemokines, like CCL2, CCL3, CCL4, CCL5, CCL22,

CXCL1, CXCL2, CXCL8, CCL25, CCL27, CXCL9, and CXCL10.⁶ TAM infiltration of PCs leads to either a poor prognosis or a good prognosis, depending on the secretion of soluble mediators (IL-6, IL-10, IL-12, IL-23, TNF- α , CXCL5, CXCL9, CXCL10, IL-1 β , inducible nitric oxide synthase, TGF- β , VEGF, arginase, and MMPs) and their individual effects, with distinct mechanisms defining their role on distinct PC clones and the associated function affected: proliferation, angiogenesis, and/or metastasis. The density of TAMs at the cancer site correlated positively with poor prognosis and had a shorter median cancer-specific survival time.⁴³ During the same period (2000), another study demonstrated the opposite effect of TAM infiltration, with an inverse association observed with PC clinical stage.⁴³ In a separate study, CD68⁺ macrophages, which comprise both M1 and M2 macrophages, favored the progression of PC to CRPC in androgen deprivation therapy (ADT)-treated PC patients.³⁶ The CD163 marker (alias, the scavenger receptor that is more specific to the M2 macrophage) is indicative of poor prognosis in PC.³⁸

A growing body of evidence suggests that the chemokines secreted in the TME of PC by TAMs mediate activation of the CCL2:CCR2, CCL17:CCR4, and CCL22:CCR4 pathways, thereby promoting PC progression.¹⁹ In the laboratory, Izumi et al²⁰ studied the role of TAMs in PC metastasis and demonstrated a role for the CCL2/CCR2-mediated STAT3 and the CCL17/CCL22:CCR4 axis in PC progression. CCL2 has been identified as an inflammation-promoting chemokine through its response against tumors mediated by the migration of monocytes and macrophages. The CCL2-dependent STAT3 activation induces EMT, and inhibition of the CCL2 axis leads to an enhanced suppression of the migratory and invasive properties when compared with AR targeting alone. This was verified in AR siRNA-treated PC cells, where metastasis was observed and the mechanism elucidated a role for an enhanced recruitment of TAMs, which further activated the CCL2/STAT3 axis, thereby promoting metastasis bypassing the AR signaling axis.⁴⁴ A separate study identified a fine interplay between chemokines and their receptors, where the CCL2:CCR2 axis further activated the CCL17/CCL22:CCR4 axis to promote PC progression by their immunosuppressive properties perpetuated through TAMs. TAMs at the PC site secrete CCL22, which is abundantly expressed in PC; this then promotes the recruitment of Tregs to the PC site, and Tregs inhibit anti-tumor immunity.²¹ It has been demonstrated that CCR4 antagonists, a class of Treg recruitment inhibitors, block signaling through the CCL17/CCL22:CCR4 axis, prevent Treg/Th2 recruitment, and are associated with better prognosis and survival in a mouse model.²¹

Although CCR8 is expressed by Th2 cells, NK cells, monocytes, and endothelial cells, it is up-regulated in TAMs and tumor-infiltrating myeloid cells; and when complexed with CCL1, it is a major source of IL-6, CCL3, CCL4, VEGF, and CXCL8.²⁶ The CCL1:CCR8 axis presents itself

as an important pathway in cancer-associated inflammation.²⁶ In a co-culture experiment of monocyte lineage cells with PC cells, CCL2 was up-regulated and this promoted the up-regulation of CCR2 and CCR4 in the PC cells, thereby leading to PC cell proliferation and defining their role in PC progression.¹⁹

TAMs, on activation by the Th2 cytokine IL-4, express CCL18; and the CCL18/phosphatidylinositol transfer protein, membrane associated 3 (PITPNM3/NIR1/ACKR6) complex recruits naïve CD4 T cells to the tumor site.³ It has been demonstrated that patient-derived monocyte-derived macrophages lead to resistance to enzalutamide therapy in AR-positive PC cells. Patient-derived monocyte-derived macrophages, on stimulation by CSF1 produced by LNCaP cells, lead to a concomitant increase in the CSF1-regulated genes *IL10* and *CCL18*, which is associated with aggressive PC in patients.⁴⁵ Studies targeting the CSF1 pathway in combination with enzalutamide hold promise in combating aggressive disease.

A recent study reported that WNT5A led to an up-regulation of CCL2 in androgen-sensitive cells through the activation of extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK), AKT, and NF- κ B pathways, followed by the recruitment of TAMs.⁴⁶ The retinoic acid receptor-related orphan receptor 2 (ROR2) and c-Jun kinase/c-Jun proteins play an important role in WNT5A-led regulation of metalloproteinase in the acquisition of tumor aggressiveness.⁴⁶ Elevated levels of WNT5A correlated with an increase in the Gleason score in PC patients.⁴⁶

Mesenchymal Stem Cells

MSCs are multipotent cells that exhibit plasticity and differentiate into cells of different lineages, including osteoblasts, chondrocytes, adipocytes, fibroblasts, neurons, and smooth myocytes, among many others. MSCs are characterized by expression of *CD73*, *CD90*, and *CD105*; and they are cells that home into regions of inflammation and, thus, also at sites of tumors and cancer. At present, MSCs represent at least 1% of the total cells in PC that is dependent on the known markers and have been noted to promote metastasis through their effects on migration and invasiveness.⁴⁷

Homing of PC cells expressing CXCR4 into the metastatic niches with a cluster of MSCs, like in the bone marrow, is supported through chemoattractants, similar to the production of SDF-1 α in the bone marrow.⁴⁷ Basically, CXCR4 is a transmembrane receptor that belongs to the family of G-protein coupled receptors and modulates their functions through the stromal-derived-factor-1 (SDF-1; alias CXCL12):CXCR4 axis; and studies have also demonstrated extracellular ubiquitin as a CXCR4 ligand. Ubiquitin is understood to possess anti-inflammatory/immune-suppressive properties; and both the CXCR4 ligands, SDF-1 (CXCL12) and ubiquitin, promote the release of the anti-inflammatory cytokine IL-10, while suppressing the release of the proinflammatory cytokine TNF- α .⁴⁸

Interestingly, SDF-1 and ubiquitin have demonstrated anti-apoptotic effects in endothelial progenitor cells and cardiac myocytes through the phosphatidylinositol 3-kinase (PI3K)/Akt/endothelial nitric oxide synthase and glycogen synthase kinase-3 β (GSK3 β)/c-jun kinase pathways, respectively.⁴⁸

MSC-dependent PC metastasis progresses through mechanisms dependent on the CXCL12, CCL5, CCL2, and TGF- β gradients.⁴⁷ MSCs, under hypoxic conditions, activate the PI3K/AKT/I κ B α and JAK2/STAT3 signaling pathways, along with supporting the migration of PC3 cells mediated via the VEGF-C receptor (VEGF receptor-3) along with CCR7.⁴⁴

Another study demonstrated an increase in PC stem cells and MSC recruitment to tumor sites during castration-induced inflammation.⁴⁹ This was attributed to a crucial role of MSCs in enhancing the stem cell properties of PC cells by CCL5.⁴⁹

Other Tumor-Infiltrating Cells

The cells infiltrating tumors are composed of various other subtypes, which include MDSCs, dendritic cells, tumor-associated neutrophils, CAFs, and ECM. MDSCs are a group of immature myeloid cells with immunosuppressive properties mediating their effects by suppressing the activity of T and NK cells. The IL-23 produced by MDSCs activates the AR pathway and promotes a shift of PC to CRPC under ADT.⁵⁰

The DCs, as is common knowledge, are well known for being antigen-presenting cells (APCs) that uptake tumorigenic antigens and present the antigens, thereby regulating T-cell responses. The maturation of DCs is induced on stimulation with tumor-derived soluble mediators, like heat shock protein and high mobility group box 1 protein, and those secreted by tumor-infiltrating immune cells.

Tumor-associated neutrophils that play a role in inflammation have been studied with respect to their ratio with lymphocytes as a prognostic factor in PC, termed as neutrophil/lymphocyte ratio. A high neutrophil/lymphocyte ratio is associated with poor response in CRPC patients treated with docetaxel, enzalutamide, or abiraterone.⁵¹ The MDSC concentrations are associated with increased neutrophil/lymphocyte ratio and are slightly higher in metastatic PC patients when compared with localized PC.⁵²

Another core population of stromal cells found in PCs is the CAFs, which are a heterogeneous population of cells derived from localized tissue fibroblasts, bone marrow-derived MSCs, hematopoietic stem cells, endothelial cells, and epithelial cells, among few other possible cell types.⁴¹ Because of their heterogeneous origins, they possess opposing functions, leading to apoptosis of cancer cells under certain conditions (antitumorigenic) and contributing to cancer progression (protumorigenic) under certain other conditions.⁴¹ Basically, CAFs promote progression of cancer through their invasive capability, gained by inducing apoptosis of cancer cells, thereby releasing apoptotic vesicles, which serve as hubs

for fibroblasts.⁴¹ Although named as CAFs, they differ from normal fibroblasts in their phenotypic and functional characteristics, with CAFs generally identified through presence of markers, including those of cytoskeletal and cytoplasmic origin, ECM-associated proteins, soluble mediators, like chemokines and cytokines, along with receptors and membrane-associated signaling proteins. These CAF markers range from α -smooth muscle actin, fibroblast-specific protein-1, vimentin, desmin, tenascin, fibroblast activation protein, platelet-derived growth factor receptor, tenascin-C, periostin, and CXCL12 (SDF-1), among several others.⁴¹ CAFs induce epithelial tumorigenesis in normal but initiated/immortal prostate epithelial cells when co-cultured with normal fibroblasts.⁵³ CAFs induce EMT, CXCR4, and IL-6 receptor expression through the monoamine oxidase A/mammalian target of rapamycin (mTOR)/hypoxia-inducible factor (HIF)-1 α in the PC3 PC cells.⁵³ Monoamine oxidase promotes EMT and PC metastasis by stabilizing the hypoxia-associated gene *HIF1 α* and inducing hypoxia through oxidative stress.^{53,54} The monoamine oxidase-dependent regulation of EMT is activated through the HIF-1 α /VEGF/forkhead box A1 (FOXA1)/TWIST1 pathway, and a knockdown of monoamine oxidase led to a reduction in the proliferation and metastasis in xenograft PC mouse models.^{41,53} Basically, CAFs at the site of cancer/tumor promote progression by secreting CXCL12, CXCL14, CCL2, CCL5, CCL7, and CCL8. CXCL8 (IL-8) regulates cell proliferation, invasion, migration, and metastasis in cancer cells. On stimulation of CAFs with testosterone, the AR binds at *CCL2* and *CXCL8* loci, thereby down-regulating the *CCL2* and *CXCL8* genes and regulating PC migration and invasion.⁵⁵

ECM is the noncellular matrix component of the tissue that provides structural and metabolic support to the cellular components. The ECM serves as a functionally active compartment composed of and supporting the production of collagen, elastin, laminin, and proteoglycans.^{27,41} Cancers, under hypoxic conditions, support an ECM that facilitates metastasis due to the hypoxic TME generated by rapidly proliferating cancer cells.

Chemokines and their receptors are pivotal during an inflammatory response and in defining the homing of cancer cells at the metastatic sites. The prominent chemokines and the receptors involved in PC are CCR5, CCR7, CCR9, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, and CXCR7 (ACKR3). CXCL8 binds to the heterodimer CXCR1/CXCR2, and CXCL13 binds to its receptor CXCR5 to modulate its inflammatory effects.²⁷ CXCL5 promotes inflammation, malignancy, and metastasis in PC cells, both *in vivo* and *in vitro*. The chemokines, CXCL1 and CXCL2, on binding to their receptor, the CXCR2 expressed on immune cells, promote inflammation and progression of tumor growth.² The laboratory of Podgorski and colleagues⁵⁶ showed that an increase in adiposity led to an increase in proinflammatory factors, CCL2 and cyclooxygenase (COX)-2, which is implicated in tumorigenesis of the bone. They also demonstrated recently that the levels

of CXCL1 and CXCL2 in prostate bone tumors are enhanced under experimental conditions of high marrow adipocytes.⁵⁶ The high mobility group box 1 protein forms a complex with CXCL12 and binds the CXCR4 ligand to mediate its effects, which include recruitment of neutrophils to the TME and acquisition of an anticancer response elicited by treatment with cabozantinib, an receptor tyrosine kinase (RTK) inhibitor.^{3,57}

Another mediator, the serum levels of macrophage inhibitory chemokine (MIC-1), has been associated with PC metastases. It has been demonstrated that PC cells expressing MIC-1 are prone to sclerotic bone metastasis when compared with PC lacking MIC-1, supporting the hypothesis for a pivotal role of MIC-1.⁵⁸ Another study revealed a contradictory role for MIC-1 in which the serum levels were reduced in PC.⁵⁹ The antitumorigenic effects promoted by MIC-1 are mediated through the p53 and ERK1/2 pathways. MIC-1 protein expression was also verified to be enhanced in PC when compared with the homing of cancer cells (BPH) patients and controls.⁵⁹ The TAMs play a crucial role in PC progression through MIC-1, which is secreted in response to TGF- β and through its autocrine effects negatively regulates the production of TNF- α .⁵⁹

Some mediators are protective toward PC and can halt its progression. One such cytokine involved during inflammation is IL-15, and it leads to a TME that nullifies tumor progression by halting the invasive and migratory capability of PC cells.⁶⁰ IL-15 increases the neutrophil infiltration at

the site of tumor and essentially promotes an inflammatory TME that mediates a decrease in PC cell proliferation. In addition, IL-15, along with CCL4 and CX3CL1, is a predictive biomarker of PC recurrence after radical prostatectomy.⁶¹

Binding of a chemokine to its respective receptor brings about a cascade of events that leads to a conformational change, thereby leading to prostate cancer proliferation, migration, EMT, angiogenesis, and metastasis.

Prostate Cancer

Prostate cancer is a heterogeneous disease, is the most common male cancer in the UK, accounts for approximately 26% of all new cancer cases, and is the second most common cause of death in men in the UK (Cancer Research UK, <https://www.cancerresearchuk.org/health-professional/cancer-statistics/mortality/common-cancers-compared#heading-One>, last accessed February 8, 2019). Localized PC can be treated surgically or through radiation therapy. Studies have reported that the progression of PC is supported through signaling mediated by the androgen/AR pathway. ADT constitutes a major aspect in PC therapy, and a large proportion of patients who are ADT responsive eventually develop castration-resistant metastatic disease (CRPC). The emergence of CRPC leads to alternative treatment options, including secondary hormonal manipulations and eventually chemotherapy. There are few therapeutic drugs to manage

Figure 2 Immune cells and the chemokines influencing prostate cancer (PC) progression/remission: The combined efforts from each of these factors indicates prostate cancer progression/remission. The main chemokines that have been studied to date and their effects on each of these processes have been illustrated. Although most of the chemokines produced in the tumor microenvironment favor poor prognosis in PC, the natural killer (NK) cells promote favorable responses by combating PC progression. The combined effects from these known chemokines and much more unknown decide the fate of prostate cancer progression and metastasis/regression. CCL, chemokine (C-C motif) ligand; DC, dendritic cell; MSC, mesenchymal stem cell; TAM, tumor-associated macrophage; TFH, T-follicular helper; Treg, regulatory T.

and treat this stage of CRPC. Docetaxel and prednisone are the standard chemotherapeutic agents used in CRPC patients, although there are several other options; nevertheless, it is an incurable disease at this point.^{62,63}

Progression of PC is determined by several factors, including chemokines, and it becomes imperative to study the role of each and every cell/matrix in the tumor and TME (Figure 2).

Cancer Proliferation

Proliferation of the cancer cells is crucial to the progression of prostate cancer. This is achieved through the control of the mitotic processes involved in cell cycle regulation. Several proteins and transcription factors mediate the homeostasis or rapid expansion of cells, aberrant expression of which leads to changes in the exponential proliferation of cells. The control of cyclin–cyclin-dependent kinase (CDK) proteins, along with driver mutations, determines malignant transformations. On binding of androgen to the AR, the mTOR/D-cyclins/p21^{Cip1}/CDK4/6 pathway is activated, which leads to phosphorylation of the retinoblastoma pathway that controls the G₁-S phase transition and, thus, CDK4/6 is a therapeutic target in PC.⁶⁴ Clinical trials using CDK4/6 inhibitors are ongoing; and one such is in a phase 2 study of palbociclib, a CDK4/6 inhibitor in patients with metastatic CRPC (ClinicalTrials.gov Identifier: NCT02905318).

The proliferation of cancer cells is regulated by a plethora of signal transduction pathways, including the WNT/β-catenin, NOTCH, insulin-like growth factor (IGF), PI3K/mTOR, NF-κB, and hedgehog pathways, among many others. The WNT/β-catenin signaling pathway represents a pivotal mechanism promoting cell proliferation and localization at specific sites. Studies have determined a pivotal role for the WNT/β-catenin/AR pathway in PC and resistance to enzalutamide. The AR and β-catenin proteins bind to and regulate several common genes, including the prostate-specific antigen gene (*KLK3*). Wingless-type mouse mammary tumor virus integration site family member 16B is expressed by fibroblasts around the PC TME and is regulated by NF-κB, which promotes EMT and, thus, drug resistance.⁶⁴ PC stem cells play an important role in proliferation; and *LGR5*, a WNT target gene, promotes CRPC.

NOTCH is a family of proteins that consist of four receptors (Notch 1 to 4) and five ligands [jagged1,2 (JAG1/2) delta-like 1, 3, 4 (DLL1,3,4)] and mediates its actions by regulating its target genes, like *cyclin D*, *p21^{CIP1}*, *NF-κB*, and *c-MYC*. Up-regulated NOTCH signaling leads to the aggressiveness of PC cells and docetaxel resistance.⁶⁵ The NOTCH signaling pathway plays both procancerous and anticancerous roles in PC, depending on the downstream proteins activated. *HES1*, a downstream target of NOTCH, is up-regulated in PC3 and PC3M cancer cell lines.⁶⁵ Hes-related family BHLH transcription factor with YRPW motif 1 (HEY1), HEY2, HEY3 (HEYL) are a part of the HEY family of transcriptional repressors, with *HEY1* being amplified in a subset of patients

with androgen-independent PC.⁶⁵ Prostate tumor overexpressed 1 (PTOV1) is up-regulated in PC and down-regulates the NOTCH target genes, *HES1* and *HEY1*, which leads to proliferation and metastasis *in vivo* along with enhancing PC3 cell proliferation.⁶⁵ There is a loss of the tumor suppressor, phosphatase and tensin homolog (PTEN), in PC; and NOTCH mediates a suppression of the PTEN through *HES1* gene.⁶⁵ The NOTCH/HES1 signaling pathway, although an important hub in the control of PC progression, has not been exploited as a therapeutic target in PC.

IGF-IR is the receptor that binds IGF and insulin to regulate the proliferation of PC cells.⁶⁶ This occurs through several mechanisms, one of which is the activation of the PI3K/AKT pathway and a subsequent loss of PTEN, in the absence of androgen to promote progression to CRPC. An up-regulation of both the IGF-I and IGF-IR correlates positively with progression of PC to CRPC.⁶⁶ Studies have also demonstrated that IGF-I mimics androgens, thereby activating the AR pathway and promoting progression to CRPC in androgen-deprived models. Interestingly, there are redundant proteins/transcription factors used by both pathways, the androgen/AR and IGF-I/AR pathways, in the induction of common genes. The IGF-IR pathway, being crucial in PC progression, has been studied by using antibodies targeting the IGF-IR axis. A phase 2 study (ClinicalTrials.gov Identifier: NCT00313781) studied the human anti IGF-IR monoclonal antibody, figitumumab (CP-751,871), in combination with docetaxel/prednisone in chemotherapy-naïve (arm A) and docetaxel/prednisone refractory (arm B) patients with hormone-insensitive PC, and it was observed that prostate-specific antigen levels were reduced in arm B of the study.⁶⁶ A phase 1/2 trial was undertaken to study the effects and efficacy of the human anti IGF-IR, antibody, cixutumumab (IMC-A12/LY3012217) and temsirolimus, an mTOR inhibitor in metastatic PC patients (ClinicalTrials.gov Identifier: NCT01026623).

Among the several chemokines, CXCL8 plays an important role in PC proliferation, promoting phosphorylation and signaling through Src-kinases, focal adhesion kinase (FAK), PI3K/AKT/mTOR/cyclin D1, and MAPK in cancer cells, thereby promoting CRPC progression and proliferation in PC3 cells.⁵⁵ CXCL8 also leads to an up-regulation of the AR. While the CXCL8: CXCR1 axis leads to proliferation, the CXCL8: CXCR2 axis leads to angiogenesis.²⁸ Apart from the CXCL8 axis, which plays a crucial role through expression of *VEGF*, EGF receptor (*EGFR*), and *MMP9* to modulate its actions, CCR6 and CXCR6 receptors play an equally important role in proliferation in PC cells.³⁰ In this study, the CXCL16: CXCR6 axis plays an important role in that both CXCL16 and CXCR6 colocalize on cancer cells and their expression correlates positively with the grade of PC. The study also demonstrated an up-regulation of CXCL16 and CXCR6 in the prostatic epithelium on induction with inflammatory cytokines in the inflammatory TME. T cells within the TME

express CXCL16 and CXCR6; and the CXCL16, through an autocrine loop, leads to the proliferation of T cells.³⁰

The CCL5:CCR5 axis also promotes antitumor immunity and PC progression, with both CCL5 and CCR5 being expressed in PC cell lines and tissues, details of which have been elucidated in the section on inflammation.²³ The proliferative and invasive capacity of PC is inhibited by a CCR5 inhibitor, which goes on to confirm the tumorigenic ability of the CCL5/CCR5 axis.⁶⁷ A separate study demonstrates an up-regulation of prostate-specific membrane antigen, which subsequently led to an increase in the concentration of CCL5 and IL-6. The researchers observed that IL-6 and CCL5 enhanced proliferation of LNCaP cells in a synergistic and dose-dependent manner. These observations implicated a role for the infiltrating CD8⁺ T cells, release of CCL5, and modulation of the CCL5/STAT5/cyclin D1 pathway, which controls cell proliferation.⁶⁸ STAT5 is constitutively activated in PC; almost 60% of PC cases with distant metastases present with STAT5 activation, and it is predominantly associated with CRPC. STAT5 up-regulates B-cell lymphoma-extra large (BCL-xL) and cyclin D1, and this subsequently leads to PC cell survival and proliferation, which is the basis of the finding that STAT5 promotes the metastatic potential of PC.⁶⁹

CXCR4 plays a pivotal role in the proliferation of cancerous cells, and inhibition of the CXCL12:CXCR4 axis leads to apoptosis of oncogenic cells. CXCR4 was constitutively up-regulated in metastatic breast cancer, liver, and lymph node.⁷⁰ In PC3 cells, the CXCL12 α induces CXCR4 expression, mitogen activated protein kinase kinase (MAPKK/MEK):ERK NF- κ B pathways pivotal in cancer cell proliferation. CXCR4 antibodies led to a suppression in the cancer proliferation and vascularization.⁷¹ Basically, the CXCL12:CXCR4 axis induces migration, angiogenesis, and survival of PC cells.²⁷

The CXCL9:CXCR3 axis plays a role in PC in that PC cells secrete CXCL9 and this leads to the recruitment of CD4⁺ T cells via modulation of the FGF11/miRNA541/AR/MMP9 pathway.³⁴ A loss or absence of CXCL9 affects leukocyte migration and tissue infiltration. CXCL9 is associated with occurrence of cancers/tumors and is regulated by IFN- γ /STAT1 axis, where myeloid transcription factor PU.1 may be recruited, as well as NF- κ B. Another study demonstrated that CXCL9 regulated the expression of *IL6* and *TGFB*, and they were inversely correlated. Thus, over-expression of CXCL9 led to a concomitant reduction in the levels of IL-6 and TGF- β , and this could also affect the transcription factors regulated by the two cytokines (IL-6 and TGF- β) and promoted the development of PC. The expression of CXCL9 is associated with clinical grading of PC.

The expression of CXCL5 is up-regulated in PC cells and tissues, and up-regulated CXCL5 further regulates the expression of tumor-associated genes, like *BAX*, *NDRG3*, *IL18*, *BCL2*, and *CASP3*.⁷²

In the past few years, several new therapies targeting the proliferation of PC cells have been designed; and new drugs

have been approved to control its progression to metastatic PC and CRPC.

Hypoxia and Angiogenesis

Hypoxic conditions in the TME lead to angiogenesis, and it serves as the mode through which tumors gain access to oxygen through vasculogenesis and promoting the proliferation and expansion of malignant cells. Tumor/cancer cells are confronted with the reality of overgrowing proliferating cells and, thus, hypoxic conditions are generated due to unavailability of blood vessels and oxygen within the most central cells of the tumor. For cancer cells to flourish and grow under these harsh hypoxic conditions, tumor cells remodel and reshape their environment to adjust proliferation of the cells under their new hypoxic inflammatory conditions. Hypoxia leads to tumors growing under conditions of diminished blood supply due to aberrant or unavailable blood vessels. The intratumoral pressure of oxygen generates a TME that not only supports cell proliferation, but also serves as an important indicator of tumor metastasis. Hypoxia correlates positively with aggressive advanced PC disease and treatment resistance.⁷³

Angiogenesis is the formation of a vascular network to newly formed cancers that require adequate oxygen and nutrients, along with eliminating metabolic waste. This is even more important in solid cancers, where the supply of nutrients to the inner mass of cells is required to keep the cancerous cells from undergoing apoptosis. Indeed, studies have implicated chemokines in cancer angiogenesis.

The transcription factor, HIF, is activated during hypoxia and plays a pivotal role in the inflammatory response by inducing chemokines and soluble mediators.⁷⁴ HIF is induced under hypoxia; and it regulates several proangiogenic genes and proteins, including VEGF and its receptors [FMS-like tyrosine kinase 3 (FLT-1) and fetal liver kinase 1 (FLK-1)], PDGF-B, ANG-1, angiopoietin 2 (ANG-2), and MMP-9, among a few others. The prominent angiogenic factors are VEGF, FGF, TGF- β , TNF- α , platelet-derived endothelial growth factor, GM-CSF, CXCL8, hepatocyte growth factor, and epidermal growth factor (EGF). Hypoxia regulates all the stages in cancer progression, including proliferation, angiogenesis, stemness, EMT, and metastasis, including progression to CRPC. These effects are mediated via several signaling pathways, including PI3K/AKT/mTOR, Wnt/ β -catenin, hedgehog, NF- κ B, and STAT3.⁷⁴ Hypoxia in a tumor is measured by HIF regulated genes or by using the pimonidazole dye, which, when injected intravenously and studied using robot-assisted laparoscopic radical prostatectomy, could characterize the stage of prostate cancer and report progression based on the Gleason score.⁷³ The recruitment, mobilization, and homing of MSCs are induced by the hypoxic preconditioning of tumor cells, which activates HIF-1 α and leads to an up-regulation of CXCR4 and, thus, an induction of the CXCL12:CXCR4 axis.⁶ The CXCL13:CXCR5 axis plays a regulative role in

PC; and under androgen-deprived conditions, it has been observed that hypoxia leads to an induction of CXCL13 by fibroblasts in an HIF/TGF- β /IGF1/connective tissue growth factor-dependent manner. In the PC3 cell line, which are hormone-independent PC cells promoting bone metastasis, an inhibition of CXCL13 led to delay in the progression of PC and subsequent bone metastasis.²⁹

Exosomes are particles that carry proteins, miRNAs, and lipids and relay information from the hypoxic cells into the TME to promote angiogenic effects. Inflammation and hypoxia play a key role in PC through the nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3 (NLRP3) and absent in melanoma 2-like receptor inflammasomes. Inflammation-associated enhancement in IL-1 β and IL-18 correlates with PC by promoting their immunosuppressive effects and promoting progression of PC to a metastatic state. An IL-1 receptor antagonist has shown efficacy as a therapeutic drug in PC.⁷⁵

Among the CXC chemokines, the Glu-Leu-Arg-positive chemokines are powerful regulators of angiogenesis, whereas the Glu-Leu-Arg-negative chemokines do not have a role in angiogenesis and are considered to be angiostatic (prevent angiogenesis). The formation of blood vessels is essential for cancer growth and proliferation, which is promoted by the CXC chemokines, CXCLs 1, 2, 3, 5, 6, 7, and 8, which occurs through their interactions with CXCR2. Hypoxic conditions up-regulate the CXCL12/CXCL8 axis, which subsequently up-regulates the expression of a well-studied angiogenic factor, *VEGF*, which acts through a cascade of events to promote angiogenesis in cancers.²⁷ Interestingly, few chemokines, like CXCL6 and CXCL8, the ligands for CXCR1, regulate angiogenesis by promoting the recruitment of neutrophils that are responsible for matrix degradation.²⁸ However, chemokines can also play opposing roles; and the angiostatic chemokines CXCL4, CXCL9, CXCL10, CXCL11, and CXCL14 are known to suppress angiogenesis.⁷⁶ CXCL14 is up-regulated in PC and correlated with increased Gleason scores, and overexpression of *CXCL14* in LAPC4 cells led to a 43% reduction in tumor growth, thus confirming its angiostatic function.⁶ In an ADT model of prostate cancer, a gene signature of *CXCL3*, *CXCL5*, *CXCL6*, and *CXCL8* was verified as the chemokines associated with angiogenesis.⁷⁷ CXCL12 activates CXCR4 to induce migration, angiogenesis, and survival of PC cells.²⁷

Cancer Cell Migration and Invasion

EMT is one of the processes that provides a cancer cell with enhanced migratory ability and invasiveness. EMT is a process that promotes an epithelial cell to undergo multiple biochemical changes, thereby enabling it to assume a mesenchymal-like cell phenotype. EMT is completed with the formation of a mesenchymal cell that disseminates itself from its origin to invade and metastasize in another suitable organ/tissue. EMT involves the CXCL8:CXCR1 axis and also *CXCR4*, the knockdown of which leads to a loss in the

migration ability. Another study demonstrated the role of CXCR7 (ACKR3) in EMT, migration, and invasion, where TGF- β 1 led to an up-regulation of CXCR7 (ACKR3) and a knockdown of CXCR7 (ACKR3) led to a reduction in the invasive and migratory capacity of cancer cells, which was unaffected on knocking down CXCR4.³¹ The CXCL12:CXCR4 axis leads to activation of several intracellular signaling pathways, leading to cellular migration and invasion; and the expression of *CXCR4* is regulated by transmembrane protease serine 2 V-ets erythroblastosis virus E26 oncogene like (TMPRSS2-ERG) in PC.⁷⁰ A stable isotope labeling with amino acids in cell culture analysis on PC cells identified PI4KIII α and SacI as CXCR4 interacting proteins that induced PC cell invasion.⁷⁰ The suppression of PI4KIII α in CXCR4 expressing PC3 cells led to phosphatidylinositol-4-phosphate (PI4P) production and a reduction in the invasiveness.

A high expression of testicular nuclear receptor 4, which plays protective roles against oxidative stress, was observed in PC. Mechanistic dissection of its precise role identified the CCL2:CCR2 axis as being driven by testicular nuclear receptor 4 to mediate the migratory, invasive, and metastatic potential of PC cells.⁷⁸ The testicular nuclear receptor 4/CCL2:CCR2 axis regulates several PC metastasis and EMT-associated genes, like *COX2*, *TGFA*, and a few others. CCL2 activates the PI3K/AKT pathway and regulates the survivin gene, thereby protecting the PC cells from autophagic death.⁷⁸ CCL2 plays a pivotal role in CAF-based migration and invasion of PC cells, as studied using a genomewide assay.⁵⁵ Binding of the AR to androgen response element sites on the *CCL2* gene on stimulation with testosterone led to a down-regulation of *CCL2* expression, and blocking CCL2 with a specific CCL2 antibody led to an annulment of migratory and invasive properties of the PC cells.⁵⁵

The CCL5:CCR5 axis plays an important role in PC, and both the components are expressed in PC. CCL5 promotes invasion and is inhibited by the CCR5 antagonist TAK-779, which blocks signaling through the CCL5/CCR5 axis. In addition, both CCR5 and CCR1 interact with each other to promote invasion of taxane-resistant PC3 cells by enhancing the secretion of MMP-2 and MMP-9 and regulating ERK and Rac signaling.²³ The CCL2:CCR5 axis also plays a role in tumor invasion in the bone. The CCL25:CCR9 axis is associated with enhanced migration and invasion of PC cells, and stimulation with CCL25 increased secretion of MMP-2 and MMP-9, thereby facilitating migration and invasion.²⁷ The MMPs, MMP-2, MMP-9, and MMP-13, modulate their action by cleaving CXCL12, thereby preventing the actions of CXCL12/CXCR4 on migration.^{6,27}

The CCR7 receptor is involved in invasion and metastasis of PC. This could be attributed to the role of TNF- α in the regulation of target genes through the NF- κ B and AP-1 signaling pathways, which lead to regulation of angiogenic VEGF factors.⁷⁹ A recent study demonstrated that TNF- α induced PC cell migration to lymph node metastatic sites

was facilitated through the TNF α -ERK-CCR7/CCL21/p38 signaling axis.²⁴ The miRNA let-7a is associated with EMT in PC, along with invasion and migration, via the CCR7/p38/ERK signaling axis.²⁵ Basically, down-regulation of the miRNA let7a leads to an up-regulation of *CCR7*; *CCR7* is a direct target of let7a.²⁵ The CCL21:CCR7 axis is involved in PC, and TNF- α phosphorylates ERK to induce *CCR7* expression in PC cells. The CCL21:CCR7 axis pathway phosphorylates p38 and promotes migration of PC cells.⁸⁰

CXCL8 plays a pivotal role in CAF-based migration and invasion of PC cells, as studied using a genomewide assay.⁵⁵ Binding of the AR to androgen response elements on the CXCL8 genome on stimulation with testosterone led to a down-regulation of *CCL2* and *CXCL2* expression, and blocking CXCL8 with a specific CXCL8 antibody led to an annulment of migratory and invasive properties of the PC cells.⁵⁵ The CCL18/PITPNM3 complex enhances EMT and invasion through enhanced NF- κ B activity and regulating the PI3K/AKT/GSK3 β -SNAIL pathway.¹

The CXCL13: CXCR5 axis plays an important role in PC progression through the control of the migratory and invasive potential of PC cells. An enhanced secretion of IL-6 in the PC TME promotes secretion of CXCL13 by endothelial cells and myofibroblasts. CXCL13 is expressed both under androgen-deprived conditions and on stimulation with androgens in AR expressing PC cells. Under AR responsive conditions, the androgen/AR target gene *CXCL13* promoted migration and invasion of PC cells through the regulation of AR-regulated genes (*ETS1*, *SNAIL*, and *cyclin B1*).²⁹ A mechanistic dissection indicated a role of the ERK1/2 pathway mediated through the PI3Kp110 isoforms, steroid receptor coactivator (SRC), and FAK.²⁹ An inhibition of the SRC, focal adhesion kinase (FAK), and CXCR5 pathways using inhibitors and antibodies led to impaired PC cell migration and invasion in response to CXCL13.

The CCL18:PITPNM3 complex enhances EMT and invasion through enhanced NF κ B activity and regulating the PI3K/AKT/GSK3 β -SNAIL pathway.

Eventually, the progression of cancer cells and subsequently metastasis depends on the mechanisms supporting and promoting the migration and invasion.

Cancer Metastasis

PC metastasis is regulated by a complex network of chemokines that regulate leukocyte activation, trafficking, and recruitment. Metastasis is defined as the mechanism that directs cancer cells from their initiation site to generate beachheads in other organ destinations. Chemokines serve as crucial mediators of cellular migration and interaction, and the chemotactic signals involved determine the hospitable organ for metastasis. The most common organs of metastasis are the lymph nodes, liver, lungs, brain, and bone marrow.

CCR2 is expressed under varied immune conditions in T cells, B cells, NK cells, basophils, and dendritic cells.

Furthermore, CCR2 is expressed in cancer tissues, and an up-regulation correlates with advanced cancer/tumor, metastasis, and relapse. *CCL2* is up-regulated in PC and metastatic PC cells have higher protein and mRNA expression.¹⁸ The common chemotherapeutic drug docetaxel led to an increase in tumor cell survival, and the mechanistic rationale was that docetaxel led to an up-regulation of *CCL2* through the c-jun kinase pathway, which led to an autocrine stimulation of the ERK/MAPK and PI3K/AKT signaling. *CCL2* signaling leads to CRPC through an autocrine mechanism, and it was demonstrated that *CCL2* also leads to the cabazitaxel resistance in PC.⁸¹ Moreover, carlumab (CNTO 888), a human monoclonal antibody with specificity for *CCL2*, was used as part of a clinical trial in CRPC patients; and although anti-*CCL2* blockers may hold promise, carlumab did not show any significant antitumor activity.¹²

A recent study reported that WNT5A led to an up-regulation of *CCL2* in androgen-sensitive cells and that overexpression of WNT5A leads to induction of CRPC through the effects of *CCL2* on infiltrating macrophages.⁴⁶ Also, higher levels of WNT5A correlated with high-grade PC, and a mechanistic dissection detailed its role in regulating metalloproteinase through ROR2.⁴⁶ WNT5A promotes chemokine production by cells in the TME through the MAPKs, PI3K/AKT, and NF- κ B.

CXCL7 is associated with high-grade metastatic lesions and aggressive prostate cancer.²⁷ CXCL7 forms a complex with the EGFR, thereby activating the EGF-mediated EGFR pathway and subsequent effects on tumor cell proliferation and survival.²⁸ CXCL7 also mediates other signaling cascades on inhibition of the AR by ADT and facilitates its actions by up-regulating CXCR7 (ACKR3) during ADT.³¹

CXCL8 expression at the mRNA level is positively correlated with the grade of prostate cancer, and serum levels of CXCL8 are higher in metastatic PC patients. Immunohistochemistry could predict recurrence of PC based on an analysis of CXCL8; and stimulation of PC cells with CXCL8 leads to regulation of cell cycle genes, like *cyclin D1*, thereby promoting PC progression.²⁸ Activation of the AR in the absence of its ligand is promoted by CXCL8, and this leads to progression of PC and metastasis.

CXCL12, and its receptor CXCR4, is one of the most prominent axis implicated in metastasis of various cancers: prostate, lung, breast, and glioblastoma. CXCL12 and its receptor CXCR4 act in concert to promote bone metastasis of PC cells.⁷⁰ Up-regulation of CXCR4 in PC leads to an enhancement in the invasive capacity of cancer cells, and its inhibition diminishes their metastatic ability.⁷⁰ Recent studies have gone on to suggest that CXCR4 could not only serve as a therapeutic target, but also a prognostic marker. The CXCL12: CXCR4 axis signals to promote colonization of cancer cells at metastatic sites in PC. Up-regulation of CXCR4 leads to enhanced metastatic activity and is associated with poor survival in PC.⁷⁰ A meta-analysis from two studies on prostate cancer patients involving 109 patients,

with 54 patients in the CXCR4-positive arm and 55 patients in the CXCR4-negative arm, predicted an unfavorable overall survival in the CXCR4-positive arm of the study (hazard ratio, 2.67; 95% CI, 1.61–4.42).⁷¹

The CCL25/CCR9, CCL27/CCL28:CCR10, CXCL13/CXCR5, CXCL9/CXCL10/CXCL11: CXCR3 axes have all been implicated in cancer metastasis because of their role in migration and invasion.^{13,82} The impact of CXCL13/CXCR5 on various types of cancer, including prostate cancer, has been discussed in the section on hypoxia and angiogenesis, migration, and PTEN. Dysregulated activation of the SRC and FAK pathways on stimulation with chemokines correlates with enhanced proliferation and metastasis in cancer cells. The CXCL13: CXCR5 axis regulates SRC, FAK, and G-protein β/γ -dependent pathways in promoting the metastatic potential of PC3 cells.²⁹

The atypical chemokine receptor *CCRL2* (*ACKR5*) is up-regulated in the metastatic PC cell line PC-3 when compared with the PWR-1E cells, which are non-tumorigenic. It was also demonstrated that *ACKR5* is significantly expressed in the epithelial cells of cancerous acini when compared with those of the benign acini.⁷

Chemokines and their receptors promote the proliferation and metastasis of cancer cells by varied mechanisms, some of which are associated with genetic alterations, chemokine complexing, and regulating signaling pathways and molecules.

Genetic Alterations

Clustering of genetic alterations leads to transformation of normal cells into malignant cells. The TME dictates, to a large extent, the possibility of genetic and epigenetic alterations due to the recruitment of inflammatory cells directed through chemokine–chemokine receptor complexes. For example, CAFs have been demonstrated to remodel the extracellular matrix and are a major component of the TME. Although CAFs promote proliferation, invasion, and metastasis of PC, any genetic/epigenetic change within these cells could configure the TME accordingly.

The various types of genetic alterations regulating cellular homeostasis are the mutations, single-nucleotide polymorphisms, copy number variation, loss of heterozygosity, neutral heterozygosity, allelic imbalance, microsatellite instability, gene fusions, and epigenetic changes.⁸³ Whole genome sequencing, whole exome sequencing, and several other targeting approaches have identified a plethora of genomic alterations with potential implication in the progression and metastasis of cancer cells, including those of PC cells. The Cancer Genome Atlas (TCGA) integrated the results from various platforms, including whole exome sequencing, array-based methods for profiling, mRNA, and miRNA sequencing, along with reverse-phase protein array low-pass/high-pass whole-genome sequencing and a comprehensive analysis of primary prostate carcinomas. These platforms revealed seven molecular classes that

defined the oncogenic drivers for this set: drivers involving *ERG*, *ETV1/4*, and *FLI1*, the most common being *TMPRSS2/ERG*, *TMPRSS2/ETV1*, *SLC45A3/ETV4* and *SLC45A3/FLI1*, along with those involving the gene *NDRG1*, and mutations involving the genes *SPOP*, *FOXA1*, and *IDH1*. The hypermethylation pattern of fusion genes versus nonfusion genes indicates a distinctive pattern demonstrating clinical associations with PC and could be studied in further detail because of the array of molecular, cellular, and biological diversity.⁸³ One of the most significant studies is that of the androgen/AR regulated *TMPRSS2* gene fusion with *ERG*, which is present in approximately 50% of PC and 20% of high-grade prostatic intraepithelial neoplasia. The *TMPRSS2/ERG* fusion results in an over-expression of *ERG* commonly observed in early PC and CRPC cases.⁷⁰ Gene fusions involving *TMPRSS2*, *KRAS*, *RAF1*, and *BRAF* genes (namely, *TMPRSS2-ERG*, *UBE2L3-KRAS*, *ESRP1-RAF1*, and *SLC45A3-BRAF*) have been commonly observed in PC.³⁴ Contrastingly, somatic point mutations are less frequent, with the most common being mutations within the genes *SPOP*, *TP53*, *FOXA1*, and *PTEN*.⁸³ *PTEN* is a well-known tumor suppressor in PC; and the *PTEN* gene is commonly mutated (deletions/inactivation mutations) at the *PTEN* locus, which occurs in a large proportion of PCs, with inactivating mutations more common in the metastatic setting.⁸⁴ *TP53* is another tumor suppressor, and deletions/point mutations at the *TP53* locus occur in PC. Mutations in *BRCA1* and *BRCA2* genes have been associated with PC progression and metastasis. TCGA study also identified a subset of tumors with mutations within the *IDH1* gene at R132, which correlated with the diagnosis of PC at a younger age.⁸³ A proportion of the tumors studied in TCGA cohort had mutations within the *TP53*, *KDM6A*, and *KMT2D* genes with somatic copy number amplifications of the genes spanning *MYC* on chromosome 8 and *CCND1* on chromosome 11.⁸³ Apart from the inactivating mutation of *PTEN*, PC carcinomas presented with activating mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) at seven loci, of which most of them had mutations at the hotspots E545K, Q546K, N345I, and C420R, along with another containing a rare double mutation at E542A and N345I loci within the *PIK3CA* gene.⁸³ At least two tumors within TCGA cohort of PC samples presented with comutated *PIK3CB* and *PTEN* together.⁸³ Another study also identified the coexistence of *PIK3CA* mutations and a *PTEN* loss that facilitated the progression of PC to CRPC.⁸⁵

TCGA study also identified mutations within the *AKT1*, *MTOR*, *RAC1*, *KRAS*, and *BRAF* genes.⁸³ Almost a quarter of the tumors studied had mutations in the genes of the PI3K/AKT/MAPK/mTOR pathway. Another 20% of the tumors had mutations/deletions within the *BRCA2*, *BRCA1*, *CDK12*, *ATM*, *FANCD2*, or *RAD51C* gene.⁸³ Genetic amplifications and a *PIK3CA* gain of function result in a hyperactive pathway, which occurs in PC.⁸⁵

Basically, the PI3K enzymes playing a crucial role in cancer are the class I enzymes (PI3K α , PI3K β , PI3K γ , and PI3K δ), which are heterodimeric lipid kinases encoded by a regulatory subunit and a 110-kDa catalytic subunit: PIK3R1 (p85 α /p55 α /p50 α), PIK3R2 (p85 β), or PIK3R3 (p85 γ), representing the regulatory domains, and PIK3CA (p110 α), PIK3CB (p110 β), or PIK3CD (p110 δ), representing the catalytic domains.⁸⁵ PTEN prevents the accumulation of phosphatidylinositol-3, 4, 5-triphosphate (PIP3) by dephosphorylating PIP3 to phosphatidylinositol-4, 5-bisphosphate (PIP2). The tumor suppressor *PTEN* is often aberrantly expressed/mutated or lost in prostate cancers, leading to an accumulation of phosphatidylinositol-3, 4, 5-triphosphate, which activates the AKT and ERK signaling pathways to promote migration and invasion.

In addition to amplification of the *PI3K* genes, an amplification in *NCOA2* is also observed in PC.⁸⁶ *BRCA2*, *HOXB13*, *TANGO2*, and a few others have been implicated as PC susceptibility genes; and a recent study identified *HOXB13* p.Gly84Glu, *TANGO2* p.Ser17Ter, *OR5H14* p.Met59Val, and *CHAD* p.Ala342Asp as being associated with an increased PC risk.⁸⁷

Copy number variations are changes in the number of copies of specific regions of DNA due to deletions or duplication that leads to structural variants and could involve segments of DNA >1 kb. The common copy number variations identified recently were located at the *FAT4*, *PDE5A*, and *PCP4* genes.⁸⁶

Cancer-related epigenetics is known as epimutation and includes histone modifications, DNA methylation, and associated mechanisms targeting the transcription of genes. Recent studies have demonstrated that promoter hypermethylations of the genes *RARB2*, *GSTP1*, *APC*, *CCND2*, *RASSF1a*, *PTGS2*, and *MDR1* are associated with PC, with the frequency of retinoic acid receptor β 2 (RAR β 2) methylation and multidrug resistance protein 1 (MDR1) methylation being highest.⁸⁸ Promoter hypermethylation leads to gene silencing; and the genes affected during PC progression and metastasis control various cellular functions, including the cell cycle, apoptosis, tumor suppression, and DNA repair. DNA methylations at CpG islands predict a risk of prostate cancer, with CpG islands at *GSTP1*, *APC*, *RASSF1a*, *PTGS2*, and *MDR1* being hypermethylated in most PCs. Hypermethylation of CPG islands within the *EDNRB* genomic region correlated with the grade of PC.⁸³

Thus, genetic alterations play an important role in the regulation of proliferation, survival, and metastasis in PC.

Chemokine Complexes

Interestingly, there are proteins, metabolites, and molecules released by cells under certain physiological states that act on their own or in a complex with other chemokines to activate chemokine receptors. In this study, the high mobility group box 1 protein formed a complex with CXCL12 and binds to the CXCR4 ligand to mediate its effects.³ Another potent

microbicidal peptide, β -defensin, is known to complex with CCR6 and CCR2 to modulate its effects on the innate and adaptive immune system, along with promoting its anticancer effects. For example, the PC cell line, RWPE-1, secretes β -defensins on stimulation with bacteria products and soluble mediators, like TNF- α and IL-1 β . The human β -defensin production leads to expression of *CXCL8* and *CCL2*, thereby recruiting DCs and memory T cells to mediate their effects through the innate and adaptive immune system.⁸⁹

The ligand-receptor complexes are unique, although it is now clear that these complexes with the chemokine typical and atypical receptors can be formed with other structurally similar protein/peptide moieties. To highlight this aspect of chemokine signaling, the CXCR4-mediated events could serve as a paradigm; its ligands range from the CXCL12 to the macrophage migration inhibitory factor and extracellular ubiquitin, as well as gp120 from primary HIV-1 strains.⁴⁸ The opposite situation has also been observed, in that chemokines can bind to nonchemokine receptors on membranes to induce their effects. In a study on breast cancer metastasis, it was demonstrated that CCL18, a TAM chemokine, can bind to nitrite reductase 1 (NIR-1; alias NIR1 and PITPNM3), a membrane-associated protein.⁶ An elevated expression of CSF-1 leads to a concomitant up-regulation of CSF-1 regulated genes, like *CCL18* and *IL10*. The CCL18/PITPNM3 (NIR-1) complex recruits naïve CD4⁺ T cells and patient-derived monocyte-derived macrophages promote resistance to enzalutamide therapy in AR-positive PC cells. Thus, therapeutic drugs targeting CSF-1, IL-10, and CCL18 in combination with enzalutamide are being tested as part of further studies.⁴⁵

PTEN, NF- κ B, and Other Signaling Molecules

Inflammation and the associated signaling pathways lead to the initiation and progression of PC. The NF- κ B signaling pathway, which is constitutively activated in PC, is crucial as it binds and activates the E26 transformation-specific (ETS) factor epithelium-specific ETS transcription factor 1 (ESE1)/ETS-specific related transcription factor 3 (ELF3).⁹⁰ Some of the other transcription factors involved in AR-mediated PC progression are the PI3K/AKT, MAPK/ERK, mTOR, and hedgehog signaling pathways.⁹¹ Infiltrating mast cells are another hallmark of inflammation in PC, and this leads to a phosphorylation of p38 that leads to proliferation in PC. In a recent study, inhibition of p38 led to reversal in therapeutic resistance in PC.⁹²

PTEN is down-regulated in PC; and hypoxic conditions lead to an up-regulation of VEGF, HIF-1 α , NF- κ B, and PTEN.⁷⁴ The PI3K-AKT-mTOR group of transcription factors is activated on stimulation through chemokines. WNT5A promotes chemokine production by cells in the TME through the MAPKs, PI3K/AKT, and NF- κ B.⁴⁶ The PI3K/AKT pathway is activated by CCR10 and CCR7 binding to its ligand, which leads to evasion from an immune attack in PC.

The CXCL13: CXCR5 axis modulates PC proliferation, invasion, and progression through the PI3K/AKT and stress-activated protein kinase/c-jun kinase pathways.²⁹ The activation of the CXCL13: CXCR5 pathway is mediated via NF- κ B. Thus, targeting CXCL13 and/or CXCR5 led to an impairment in the migratory and tumorigenic potential of PC cells.⁹³ Studies demonstrating an association of the levels of CXCL13 to PC progression have been contradictory, although it is associated with cell invasion and adhesion in a CXCR5-dependent manner.²⁹ CXCL13 is an AR responsive/target gene with androgen response element sites within its enhancer and is up-regulated at the mRNA as well as protein levels and involved in cell migration and invasion.⁸² A recent study identified the CXCL13: CXCR5 pathway as a direct effector of protein kinase C ϵ over-expression and/or loss of PTEN in a mouse PC model.⁹³

Loss of PTEN subsequently leads to an up-regulation of CXCL8, CXCR1, and CXCR2 and thereby leading to sustained proliferation and survival of PTEN-deficient cells.²⁸ CCL2 and CXCL12 secretion from stromal cells was derived from an enhancement in CXCL8 derived from the tumor site, which supports proliferation and invasion in PC cells depleted of PTEN.⁵⁵ Basically, CXCL8 regulates PTEN deficiency; and in these tumors, this leads to macrophage infiltration enhancing TNF- α signaling and NF- κ B—dependent survival of PC cells by regulating cIAP-1, the antiapoptotic gene.²⁸ A dual cellular-inhibitor of apoptosis (cIAP-1) and XIAP blocker, AT-IAP (Astex Pharmaceuticals, Cambridge, UK), presents as an effective therapy in a proportion of PTEN-deficient PC patients. A proteasome inhibitor, bortezomib, which functions by inhibiting NF- κ B, also inhibited CXCL8 production and proliferation in PC cell lines.⁹⁴

In PC, the CXCL8: CXCR1/2 axis leads to phosphorylation of AKT473, and this serves as a poor prognostic factor.⁹⁵ In metastatic PC after ADT, activation of the PI3K/AKT pathway has been demonstrated, which is suppressed in the normal prostate by inositol polyphosphate 4-phosphatase type II and PTEN, with PTEN being lost in approximately 40% of metastatic PC patients.⁹⁶ Another study demonstrated that AR regulates inositol polyphosphate 4-phosphatase type II and inositol polyphosphate 4-phosphatase type II, in turn, suppresses protein kinase C signaling, which further down-regulates CXCL8 and its target COX-2.⁹⁷

Resistance to apoptosis is another hallmark of cancer, and a few chemokines, like CCL2, function by up-regulating the survivin (*BIRC5*) gene through the PI3K/AKT pathway to protect PC3 PC cells from autophagic death.⁷⁸ Chemokine pathways also activate the MAPK/ERK pathway, along with the JAK/STAT5, RAS/RAF, and PI3K/AKT pathways, and a few others, leading to homeostatic cell proliferation.²³ The AR/CCL4/STAT3 pathway has also been implicated in PC progression through the up-regulation of SNAIL and down-regulation of two major tumor suppressors (p53 and PTEN), thereby supporting EMT and tumorigenesis.⁹⁸ Basically, CCL4 is expressed at significantly higher levels

in PC. *PTEN* deletion often occurs with TP53 mutations, and a large proportion of PC and CRPC cases with both PTEN and TP53 alterations possess an AR-dependent TMPRSS2-ERG fusion that results in an overexpression of *ERG*. Almost half of all PCs possess the *TMPS2-ERG* gene fusion, and this phenomenon predominantly occurs because of inflammation-induced oxidative stress that promotes DNA breaks and, thus, fusion genes.⁹⁹ The TMPRSS2-ERG fusion in cancer cells regulates *CXCR4* gene expression through the binding and activation of ERG to upstream elements in the *CXCR4* gene.^{70,71} CXCR4 further leads to regulation of PI4KIII α activity and mediates metastatic activity. Blocking PI4KIII α may have therapeutic benefits in advanced PC patients.⁷⁰

Thus, cancer cells are successful in thriving in their new environment, by interacting with the microenvironment to generate favorable conditions by modulating the signaling pathways and immune response to sustain a domain rich in chemokines, their receptors, and complexes with other proteins and phospholipids.

Prostate Cancer and Ethnic Diversity

Ethnic diversity is a major factor contributing to differences in the incidence and severity of PC. Epidemiologic studies have demonstrated a higher incidence of mortality in men of Afro-Caribbean origin (NIH, National Cancer Institute, https://seer.cancer.gov/csr/1975_2016/sections.html, last accessed February 8, 2019). Several evolutionary and compensatory mechanisms, originating because of diet and climate, along with several other factors, including the effects of endemic diseases, determine the cause of such ethnic diversity in PC occurrence, metastasis, and diminished survival.

The chemokine receptor *CXCR4* is up-regulated in patients of African American origin and could possibly be associated with aggressive phenotypes.⁷¹ However, with CXCR4 being a promiscuous receptor with several possible known ligands, including CXCL12, SDF-1, and ubiquitin, the mechanistic role of each ligand and associated severity requires elucidation. The present PC marker, prostate-specific antigen, is higher in African Americans when compared with whites presenting with locally advanced PC.¹⁰⁰ At the molecular level, studies have defined several other differential changes between the profiles in African Americans, Asians, and whites. The TMPRSS2-ERG fusion, which is present in a large proportion of PC patients, is differentially present in men with differential ancestry; and it has been observed that men of European (49%) descent are prone to the fusion event occurring when compared with men of African (25%) or Asian (27%) descent.¹⁰¹ Another study also demonstrated similar findings in that the *ERG* rearrangement was more frequent in white (42.5%) men when compared with African American (27.6%) men with PC.¹⁰⁰ Again, the tumor suppressor *PTEN*, which inhibits the PI3K/AKT/mTOR pathway and is

often deleted in PC and CRPC patients, was more commonly deleted in white (19.8%) men than in African American (6.9%) men.¹⁰⁰ In a smaller study on association studies of PC in African Americans versus whites, a few loci within the genome of the PC tissue were identified that were differentially identified/up-regulated in the African American populations: i) The genes *SNRPN*, *SHANK2*, *MST1R*, and *ABCG5* were preferentially methylated at the promoter region, and this observation was associated with a down-regulation of these genes at the mRNA level.¹⁰² ii) Significantly higher methylation of *NKX2-5* and *TIMP3* is observed. iii) The motor neuron and pancreas hemeobox1 (*MNX1*) is up-regulated.

The pancreatic serine peptidase inhibitor, Kazal type 1 or SPINK1, encodes a 56-amino acid serine protease inhibitor; and its expression is associated with the occurrence of aggressive PC in approximately 10% of all PC patients. Basically, SPINK1 shares structural similarity with the EGF, thereby mimicking this ligand and sharing occupancy for the receptor EGFR, present on the surface of PC cells.¹⁰⁰ SPINK1 overexpression is more common in African Americans than in whites (23.4% versus 8.2%).¹⁰⁰ Mutations in the gene encoding SPOP are one of the most common in PC.¹⁰⁰ SPOP is an E3-ubiquitin ligase adaptor protein that stabilizes the AR and the complex of proteins associated with it. *SPOP* mutations are more common in whites than in African Americans (10.3% versus 4.5%).¹⁰⁰

These observations of lower TMPRSS-ERG fusion, *PTEN* deletion, differential methylation of genes (*SNRPN*, *SHANK2*, *MST1R*, and *ABCG5*), and up-regulation of *MNX1* in men of African descent indicates a protective role for these events that is abolished on their deletion. The functional role for the deletion of *PTEN* is in line with its role as a tumor suppressor.

DARC is another such atypical chemokine receptor studied extensively in various ethnic populations. The DARC protein is expressed on red blood cells, on renal endothelial cells, on epithelial cells, and in the Purkinje cells of the cerebellum. A large proportion of individuals of African origin do not express DARC on erythroid cells or red blood cells; and with its predominant function as a sink, a decoy receptor that promotes the clearance of both CXC and CC chemokines from the PC TME, the absence of DARC from the surface of erythrocytes may play a contributory role in the higher incidence of and mortality due to PC in this population. P.K.'s laboratory recently studied the role of DARC mutations and its possible association to PC severity.¹⁰³ Thus, there could be several genetic factors responsible for PC occurrence, severity, and metastasis.

Viruses and Prostate Cancer

Although there are several factors promoting the incidence and occurrence of PC, viruses, including those hormonally regulated/dysregulated, have been reported to be associated with an increased risk of the disease. An association has been observed between the occurrence of PC and human

papillomavirus as well as PC and mouse mammary tumor virus.¹⁰⁴ The Epstein-Barr virus has also been studied in PC; and initial studies did not present with any observed oncogenic potential, although further studies are required to establish a functional role of the viral proteins influencing any viral coinfection.¹⁰⁵ Although yet to be developed significantly, advances in immunotherapy using oncotropic and oncolytic viruses could serve as potential anticancer agents.

Thus, although to date not enough has been done to study the role of each chemokine in prostate cancer, their role in acute/chronic inflammation and associated cancer remains undisputed with the pivotal role they play in each stage of cancer development, progression, EMT, migration, and metastasis. More needs to be done to understand the role of each chemokine and how they influence and activate other cells in the cancer microenvironment to secrete/release an indicative amount of the chemokine, which leads to a chain of chemokine and cellular activity, dictating the phenotype of the PC.

Conclusions

Prostate cancer is a solid malignancy triggered by an environment of chronic inflammation, generated because of inappropriate stimulation of hormones, chemicals, radiation, and various partially known and unknown causes, like viral infections. It also leads to an intrinsic inflammatory response, which dictates the recruitment of leukocytes and release of cytokines and chemokines. The inflammation and inflammatory response (acute/chronic), in turn, can dictate progression or shrinkage/elimination, depending on the effectiveness of a robust/appropriate trafficking of immune cells into the tumor microenvironment. The differential effects of the varied immune population of cells are dependent on the type of immune cell, the site of tumor/cancer origin, and the cause of tumor initiation.

The chemokine/receptor axis plays a crucial and differential role in PC and promotes tumor cell proliferation, epithelial-mesenchymal transition, angiogenesis, and metastasis. The caveat to the functionality of each chemokine/receptor complex is that chemokines bind to more than one receptor and each unique complex defines unique/redundant procancer/anticancer functions. There is also the aspect of redundancy between chemokine functions and, thus, therapeutic strategies aimed at one chemokine are mostly ineffective in clinical trials. Each complex has varied implications and differs between cell types and physiological states. A specific chemokine or its receptor may have proinflammatory effects under certain circumstances and anti-inflammatory effects under others. An example is CXCR4, which is up-regulated in PC, although one or more of its ligands (SDF-1 and ubiquitin) promote antiapoptotic effects. Ethnicity and race also play a vital role in the risk of PC, and DARC is one such protein of considerable significance in this context. A better understanding of the chemokine/receptor regulation in PC requires further studies and elucidation of the

cascades, the stimulation/triggers of chemokine production by specific cells in the TME, and the associated control/proliferative mechanisms.

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