

REVIEW

Advanced prostate cancer: reinforcing the strings between inflammation and the metastatic behavior

G Gueron, A De Siervi and E Vazquez

Department of Biological Chemistry, School of Sciences, University of Buenos Aires, Ciudad Universitaria, Pabellón II, Buenos Aires, Argentina-CONICET

It is currently estimated that inflammatory responses are linked to 15–20% of all deaths from cancer worldwide. Although many studies point to an important role of inflammation in prostate growth, the contribution of inflammation to castration-resistant prostate cancer is not completely understood. The presence of inflammatory mediators in tumor microenvironment raises the question whether genetic events that participate in cancer development and progression are responsible for the inflammatory milieu inside and surrounding tumors. Activated oncogenes, cytokines, chemokines and their receptors, sustained oxidative stress and antioxidant imbalance share the capacity to orchestrate these pro-inflammatory programs; however, the diversity of the inflammatory cell components will determine the final response in the prostate tissue. These observations give rise to the concept that early genetic events generate an inflammatory microenvironment promoting prostate cancer progression and creating a continuous loop that stimulates a more aggressive stage. It is imperative to dissect the molecular pathologic mechanism of inflammation involved in the generation of the castration-resistant phenotype in prostate cancer. Here, we present a hypothesis where molecular signaling triggered by inflammatory mediators may evolve in prostate cancer progression. Thus, treatment of chronic inflammation may represent an important therapeutic target in advanced prostate cancer.

Prostate Cancer and Prostatic Diseases (2012) 15, 213–221; doi:10.1038/pcan.2011.64; published online 20 December 2011

Keywords: castration-resistant prostate cancer (CRPC); metastasis; inflammation; chemokines; reactive oxygen species (ROS)

Introduction

Prostate cancer is a common malignancy among men worldwide.¹ Androgens are responsible for the gland growth and are involved in the prostate carcinogenesis. Hormonal deprivation therapy, also known as hormonal ablation therapy, which reduces serum testosterone levels to $<50\text{ ng dl}^{-1}$, is a well-established form of treatment for various stages of prostate cancer. However, in some cases prostate cancer will progress and evolve to an androgen-independent state, known as castration-resistant prostate cancer (CRPC) (formerly referred as ‘hormone resistant or refractory’). CRPC is characterized by its heterogeneity clinically and molecularly, and is the main cause of prostate cancer mortality.¹ Treatment options for patients with CRPC are very limited, mainly because of the intrinsic chemoresistance acquired by cells during disease progression on therapy.

Although acute inflammation is critical for host defense, chronic inflammation contributes to tumorigen-

esis and cancer.² It has been documented that the recruitment/activation of antigen-presenting cells in treated prostate tissues may contribute to local T-cell activation. This induction of T-cell responses within prostate tissues might generate an abrogation of host tolerance to hormone-sensitive tissues triggered on hormone withdrawal.³ In addition, regressing androgen-dependent tumors after androgen ablation exhibit infiltration of leukocytes, including B cells, with a concomitant activation of IKK β (inhibitor of nuclear factor κ -kinase subunit β), cytokine burst, triggering hormone-free survival.⁴ Hence, it is reasonable to draw attention to the immune cells infiltration for the development of immunotherapeutic treatments of prostate cancer.

Effective therapeutic approaches for CRPC are extremely limited and the generation of suitable *in vivo* models is critical to better understand the processes associated with the progression of prostate cancer. A novel xenograft model was developed using locally recurrent CRPC specimens, which showcases sequential changes (wild-type androgen receptor (AR), regression soon after castration and restoration of the ability to proliferate without AR) that strongly resembles the clinical behavior of the disease.⁵ Of interest, the expression of prostaglandin E receptor EP4 subtype (EP4) was significantly increased during progression in this model and its ectopic expression in LNCaP cells enhanced PSA

Correspondence: Professor E Vazquez, Department of Biological Chemistry, School of Sciences, University of Buenos Aires, Intendente Guiraldes 2160, Ciudad Universitaria, Pabellon II, 2do Piso (1428), Buenos Aires C1428EGA, Argentina.
E-mail: elba@qb.fcen.uba.ar

Received 6 September 2011; revised 24 October 2011; accepted 5 November 2011; published online 20 December 2011

production in the absence of hormone supplementation.⁵ Given that prostaglandins and their receptors are highly associated with the inflammatory disease⁶ and cancer,⁷ EP4 antagonists emerge as a promising therapeutic strategy.

It is well documented that the AR signaling remains active even with castration levels of serum testosterone. Induction of the activity of CYP17 (cytochrome P-450c17), an enzyme that catalyzes key reactions in extragonadal androgen biosynthesis, is one way to maintain the hypersensitivity of the AR.⁸ Moreover, AR mutants are able to bind promiscuous steroids, and may convert AR antagonists to agonists.⁹ Other hormones and their receptors are involved in the abnormal growth of the gland. Particularly, estrogens and estrogen receptors defined a subclass of prostate cancer with a very aggressive clinical phenotype.¹⁰ In addition, other signaling cascades, such as cyclooxygenase 2 activation, loss of tumor-suppressor phosphatase and tensin homolog (PTEN), inhibition of the phosphatidylinositol-3 kinase (PI3K)/Akt, Bcl-2 overexpression and pro-inflammatory cytokine and chemokine burst, are switched on bypassing the androgen/AR axis, which in turn favor tumor progression.¹¹ Similarly, highly oxidative stress produced during inflammation, has a critical role in cellular processes associated with malignant transformation, such as DNA damage, proliferation, senescence and angiogenesis.

As in other malignancies; in prostate cancer, reactive oxygen species (ROS) cause oxidative damage to macromolecules in epithelial cells and can react with other cellular components initiating a free radical chain reaction, thus sustaining the prostate carcinogenic process favoring progression.¹² Moreover, ROS accumulation was shown to trigger CXCR4-mediated functions through the inactivation of PTEN in prostate cancer.¹³ The imbalance between ROS generation and elimination because of excessive production or inadequate activity of the antioxidant defense system (or both), conveys to oxidative stress and the subsequent onset of the inflammation, producing a hypoxic microenvironment, favoring the spread of the disease.

In this review, we will outline how inflammatory mediators are implicated in the progression toward the castration-resistant stage.

The strut of chemokines and their receptors in the metastatic behavior

The directed migration of a cell toward the source of a secreted protein signal, known as chemotaxis, has been commonly associated to the leukocyte trafficking triggered by infection and to secondary lymphoid organs. In addition, they also have a critical role in tumor initiation, promotion and homing of cancer cells to selected organs.¹⁴ Chemokines, the executors of chemotactic signals, are constitutively expressed in destined cell types and tissues maintaining the homeostasis of the hematopoietic and the immune system. However, inflammatory chemokines behave differently and their expressions are induced on inflammatory stimuli promoting proliferation and angiogenesis.

There are four subgroups within the chemokine family: CXC, CC, CX3C, and C chemokine ligands (X represents

any amino acid) depending on the positioning of the conserved cysteines in the amino-terminal part of these small inducible proteins.¹⁴ Chemokine binding to their corresponding seven transmembrane-domain G-protein-coupled receptors causes activation of signal transduction networks leading to chemotaxis. These receptors have been implicated in the migration of breast, prostate and lung cells to secondary sites in the bone.¹⁵ Up to date the most relevant chemokine receptors in prostate cancer dissemination, are CXCR4, CXCR7 and CXCR6.¹⁵

SDF-1 (stromal-derived factor 1, also known as chemokine (C-X-C motif) ligand 12, CXCL12) acts through CXCR4-dependent mechanism. CXCR4/SDF-1 α axis has been strongly implicated in metastasis and in several laboratory model systems.¹⁶⁻¹⁸ Prostate cancer cells with high bone mimicry, also express CXCR4 receptors, whereas SDF-1 α affects their adherence, migration and invasion.¹⁹ It was demonstrated that prostate cancer adhesion to human bone marrow endothelial cells in flow conditions was significantly reduced by a neutralizing antibody against fractalkine, enabling cells to migrate toward a medium conditioned by osteoblasts, which secreted the soluble form of this chemokine.²⁰ In addition, the adhesion mediated by this axis occurred partially through nuclear factor (NF)- κ B pathway.²¹ Also this axis causes upregulation of integrins, altering the interaction between cancer cells and the extracellular matrix.^{22,23} The blockade of CXCR4 was demonstrated to inhibit vascular endothelial growth factor (VEGF) expression and the resultant angiogenesis; even reducing significantly bone metastasis *in vivo*.²⁴ Furthermore, CXCR4 is positively regulated by AR.²⁵ It was demonstrated that androgen-induced CXCR4 expression was functional in TMPRSS2-ERG-positive prostate cancer cells, further indicating the relevance of this chemokine in prostate cancer metastasis.²⁶ However, recent publications reported that the chromosomal rearrangement TMPRSS2-ERG, was not prognostic for recurrence after radical prostatectomy, for the clinically localized prostate cancer.^{27,28} The immunohistochemical pattern of CXCR4 expression in patients with metastatic prostate cancer has shown that patients with high expression of this chemokine in tumors had poorer cancer-specific survival than those patients with low expression of CXCR4. This receptor expression has proved to be a useful prognostic factor for patients with metastatic prostate cancer treated with androgen-withdrawal therapy.²⁹

CXCR7/RDC1 functions as a chemokine receptor for SDF-1/CXCL12, which regulates a spectrum of normal and pathological processes. It was reported that PC3 cells express CXCR7 (ref. 30) and Wang *et al.*³¹ suggested that the expression of this chemokine receptor is associated with survival advantage for tumors, facilitating adhesive and invasive activities of prostate cancer cells *in vitro* and *in vivo*.³² This correlated with the signaling of CXCR7 through Akt activation on CXCL12 stimulation.³¹ Similarly, this receptor is more highly expressed in prostate metastases (specially those to bone) compared with primary tumors seen in clinical specimens.¹⁵ In the vasculature, the expression of CXCR7 is elevated in endothelial cells associated with tumors³³ and a critical role was proposed for this chemokine receptor in tumor-associated angiogenesis *in vivo*.³⁴ All the reports previously mentioned do not elaborate a mechanism by

which CXCR7 allows growth advantage to prostate tumor cells. Singh and Lokeshwar³⁵ recently found that CXCR7 function is regulated by other inflammatory mediators revealing a novel mechanism of ligand-independent growth promotion by CXCR7 and its coregulation by the pro-inflammatory factor interleukin 8 (IL-8) in prostate cancer. Furthermore, high serum levels of IL-8 have been reported in patients with metastatic prostate cancer.³⁶ Thus, the upregulation of CXCR7 induced by IL-8 emerges as a promoter of the advanced prostate disease.³⁵ Moreover, the prominent role of CXCR7 in prostate tumor is also validated by the observation that CXCR7-depleted tumors showed significantly reduced levels of cyclin D1, VEGF and p-EGFR (phosphorylated epidermal growth factor receptor), further implicating CXCR7 in prostate tumorigenesis.³⁵

The third relevant chemokine receptor for prostate cancer dissemination is CXCR6. It was demonstrated that the blockade of CXCR4 or CXCR7 only partially hindered metastatic behavior *in vivo*, suggesting that other functional chemokine/chemokine receptor pairs may be critical in prostate cancer progression.³⁷ CXCR6 is highly expressed in prostate cancer cell lines and in prostate tissues.³⁸ CXCL16 was identified as the ligand for this receptor and was found to signal through NF- κ B via heterotrimeric G proteins/PI3K/PDK-1/Akt/IKK/I κ B.³⁹ Moreover, it was also reported to signal through the Akt/mTOR pathway.⁴⁰ Interestingly, although lacking an ELR motif (ELR-CXC chemokines) in the chemokine domain, CXCL16 is proangiogenic.³⁷ It was demonstrated that CXCR6 regulated blood vessel formation by an autocrine/paracrine loop established between prostate cancer and endothelial cells and was observed that both IL-8 and IL-6 levels were altered in response to changes in CXCR6 expression.³⁷ The striking similarities between CXCL16 and both fractalkine and CXCL12 are likely to result in additive effects.⁴¹ Moreover, it has been shown that CXCL16 is enriched in tissues such as bone marrow⁴¹ and high expression levels of CXCL12 and CXCL16 were observed in tissues enriched with plasma cells and in cultured human bone marrow stromal cells.⁴¹ Thus, plasma cells are likely to be recruited to bone marrow and other target tissues via CXCR4 and CXCR6.³⁷ Interestingly, assessing the clinical significance of the expression of CXCL16/CXCR6 in prostate cancer patients, the pair was weakly detected in lung and liver tissues, whereas CXCL16 was found highly expressed in specimens of bone metastasis. Moreover, CXCL16 immunostaining was related to clinical parameters of the progressive disease, such as Gleason score, T stage, tumor volume, perineural invasion and lymph node metastasis.³⁸ All these data point to both, an association between high CXCL16/CXCR6 and a more aggressive prostate cancer phenotype and an interconnection between high CXCL16 expression and bone metastases.³⁸

Prostate cancer cells and the surrounding stroma are exposed in a milieu enriched with several interleukins and chemokines and receive their signaling stimuli, enforcing tumor-promoting functions. The expression of CXCL8 (also known as IL-8), one of the best-characterized members of the chemokine family, correlates with increased angiogenesis, tumorigenicity and lymph node metastasis *in vivo*.^{42,43} In addition, IL-8 is a transcriptional target of NF- κ B and its expression is elevated in

androgen-independent prostate cancer, contributing to the transition to a castration-resistant state.⁴⁴

IL-8 is a pro-inflammatory chemokine classically associated to neutrophils chemotaxis and degranulation. This chemokine activates multiple intracellular signaling pathways downstream of CXCR1 and CXCR2 linked to G proteins. Enhanced expression of IL-8 was detected in tumoral cells, endothelial cells, infiltrated neutrophils and tumor-associated macrophages, suggesting that this interleukin act as a modulator of the tumor microenvironment.⁴⁵

IL-8 overexpression has been detected in the serum of patients with metastatic prostate cancer.⁴⁶ Moreover, Wilson *et al.*⁴⁷ reported elevated expression of IL-8 and IL-8 receptor in biopsy tissues of prostate cancer patients, with maximum immunoreactivity in CRPC. Interestingly, the high levels of this ligand and its receptor in prostate cancer imply a continuous autocrine IL-8 signaling stimulus *in situ*.

One of the most relevant inflammatory mediators clearly implicated in prostate cancer is IL-6, a multifunctional cytokine that has been associated with proliferation, apoptosis, angiogenesis and also with the modulation of tumor growth and differentiation in many cancers.⁴⁸ Many cell types including prostate cells, immune cells and osteoblasts produce this interleukin. High levels of IL-6 and its soluble receptor in the circulating plasma are observed in patients with prostate cancer, which correlate with more advanced stages of the disease, therapy resistance, poor prognosis and can be predictive of recurrence after treatment of localized cancer.⁴⁹

IL-6 foremost effect is the activation of Janus kinase signaling and of signal transducers and activators of transcription (STAT) proteins, especially STAT3; however, in certain cellular context mitogen-activated protein kinase and PI3K pathways can also be involved.^{50,51} It can be produced autocrinally in castrate-resistant prostate cells transactivating AR.⁵² However, the effect of IL-6 on ligand-independent AR activation, tumor formation and subsequent growth shifts depending on the status of the AR as well as other interacting signaling cascades.^{44,53} Additionally, IL-6 has been proposed to initiate an intracrine-signaling pathway, alternative to the AR pathway, influencing the levels of metabolic enzymes.⁵⁴ It was demonstrated that testosterone plasma levels increased when IL-6 overexpressing LNCaP cells were inoculated in castrated mice.⁵⁴ Similarly, this cytokine increased the expression of steroidogenic enzymes involved in androgen biosynthesis in these prostate cancer cells.⁵⁴

Another inflammatory mediator relevant in prostate cancer is tumor necrosis factor alpha (TNF α). This cytokine acts as a critical switch in building an elaborate association between inflammation and cancer. Its role as a key regulator of the tumor microenvironment is well recognized. This molecule, mainly released by macrophages and other cells including tumoral cells primes de tumor microenvironment. It facilitates cancer development acting directly on neoplastic cells or indirectly through endothelial and other inflammatory cells.⁵⁵ However, the mechanisms by which TNF α enables these events are not fully described. A recent publication from Davis *et al.*⁵⁶ explains the dichotomy of TNF α effect on the control of apoptosis in prostate cancer cells.

These investigators propose a physiologic role for TNF α in prostate regression after androgen withdrawal. This factor is required for castration-induced prostate regression, but membrane-bound TNF α protein and stromal cell-specific TNF α mRNA levels increase in rat prostate after castration, which is coincident with a paracrine effect of TNF α in prostate regression. However, when wild-type non-castrated mice were treated with TNF α no regression of the gland was observed.⁵⁶ All these evidences showed that this cytokine acts in the context of supplemental castration-induced signals.

TNF α is an upstream regulator of NF- κ B through TNFR1 signaling. NF- κ B is constitutively activated in human prostate adenocarcinoma and correlates with disease progression.⁵⁷ Jin *et al.*⁵⁸ showed that the activation of NF- κ B is sufficient to maintain androgen-independent growth of prostate and prostate cancer by regulating AR action. Also, it is worthy to remark that NF- κ B blockade results in decreased angiogenesis in several prostate cancer models.^{59,60}

Chemokines and cytokines signaling can also modify the sensitivity of prostate cancer cells to environmental stresses such as hypoxia, oxidative stress, DNA damage, altering several pathways crosstalk and producing hormone-refractory aggressive tumors. In addition to the classical roles in promoting invasion and metastasis, the pleiotropic effects of cytokines (specially IL-6 and IL-8) include potentiating the production of growth factors, inducing growth signals, stimulating angiogenesis, attenuating apoptosis, further linking the cytokines signaling to another 'hallmark' of cancer cells.⁶¹ These observations give support to the hypothesis that inflammation and inflammatory mediators must emerge as the seventh hallmark of cancer, as was proposed by Mantovani.⁶²

ROS and the sustained stress signaling framework in prostate cancer progression

Decreased androgen levels in elderly men are associated with increased prevalence of prostate cancer and CRPC, in which androgen downregulation might enhance androgen signaling by augmented AR expression.⁶³ As the prostate gland depends on the androgen/AR signaling for growth, androgen suppression may lead to a shift in the prooxidant-antioxidant balance toward an oxidative status, which correlates with increased risk of carcinogenesis⁶⁴ and CRPC occurrence through AR overexpression.⁶³ Intriguing, <10% of CRPCs were found to possess somatic AR gene mutations⁶⁵ and 10–20% exhibit AR gene amplification.⁶⁶

It has been appreciated that ROS production is increased in cancer cells.⁶⁷ ROS are considered to be tumor promoters given the potential for induction of DNA damage. However, little is known about the molecular machinery that mediates ROS in tumor progression. Interestingly, castration-induced oxidative stress in prostate cancer cell lines increased AR levels through Twist 1 overexpression, which might result in the gain of a castration-resistant phenotype.⁶³ Twist1, a member of basic helix-loop-helix transcription factors, has been proposed as an oncogene.⁶⁸ Recently,

its upregulation was associated with malignant transformation⁶⁹ and was recognized to be responsible of metastasis.⁷⁰ Evidently, there is a connection between oxidative stress and androgen deprivation in prostate cancer, which is also supported by previous observations of increased oxidative damage associated to the development of malignancies.⁷¹

Of interest, when comparing CRPC gene expression profile with hormonal-sensitive tumors, the antioxidant defense endogenous system is clearly repressed, in particular SOD2 (manganese superoxide dismutase).⁷² SOD2 regulates ROS production by converting superoxide to a less reactive species. Hence, SOD2 in CRPC could be mechanistically linked to AR reactivation. Sharifi *et al.*⁷³ knocked down SOD2 expression in AR-expressing LNCaP cells and assessed the gene expression changes revealing upregulation of androgen-responsive genes, such as VEGFA and FKBP5. Moreover, an array for transcription factor DNA-binding activity showed that AR binds to DNA among other transcription factors after SOD2 knocked-down. Oxidative stress produced as consequence of elevation of ROS anabolism and diminution of antioxidant detoxification enzymes was also shown *in vivo* after castration using rat models. Furthermore, androgenic regulation of NAD(P)H oxidases may be related to the physiological changes that occur during castration-induced involution and androgen-induced regeneration of the ventral prostate in the rat.⁷⁴ These findings correlate with a clear transcriptional repression of stress-related genes, including thioredoxin, peroxiredoxin 5 and MnSOD.⁷⁵

It is of particular significance that many genes that are regulated by oxidative stress are targets of NF- κ B.⁷⁶ NF- κ B is constitutively activated in human prostate carcinoma and correlates with disease progression.⁵⁷ Also, it is worthy to remark that NF- κ B blockade results in decreased angiogenesis in several PCa models.^{59,60} NF- κ B is an inducible transcription factor that belongs to the Rel/NF- κ B family.^{77,78} Increasing evidence suggests that inhibition of NF- κ B activity in prostate cancer cells can suppress the angiogenesis, invasion and metastasis by downregulating the expression of NF- κ B downstream target genes, such as VEGF, plasminogen activator type urokinase and matrix metalloproteinase 9 (MMP9),⁷⁹ however, its role in regulating the prostate metastatic disease is yet to be elucidated.

Additionally, heme-oxygenase 1 (HO-1), the rate-limiting enzyme in heme degradation, confers cytoprotection against oxidative stress and inflammation.⁸⁰ This protein exerts vital metabolic functions limiting the axis of heme degradation and maintaining the cellular homeostasis. Several signaling molecules are implicated in the cytoprotection conferred by HO-1, including NF- κ B and PI3K/Akt.⁸¹ Although classical recognized as a microsomal protein, its presence has been detected in other subcellular compartments.^{82,83} Recent studies have reported that HO-1 suffers a proteolytic degradation in its hydrophobic C-terminal domain, which would facilitate its entrance to the nucleus.⁸⁴ It has been proposed that HO-1 possesses in the nucleus a non-catalytic canonical function participating in the regulation of the activity of several nuclear transcription factors and also regulating its own transcription.^{83,84} Moreover, we have documented the nuclear HO-1 expression in human primary prostate carcinomas.⁸² We have also

reported that it impairs prostate tumor growth *in vivo* and downregulates the expression of target genes associated with inflammation.⁸⁵ Additionally, we have demonstrated that HO-1 challenges the angiogenic switch *in vivo* in prostate cancer through a mechanism partially mediated through NF- κ B.⁸⁶ However, clinical data demonstrated a statistically significant difference in HO-1 epithelial expression between benign, high-grade prostatic intraepithelial neoplasia, localized prostate cancer and CRPC, where CRPC presented the highest HO-1 expression followed by benign tissue. This work provides experimental evidence for a crosstalk between epithelial HO-1 expression and PTEN deletions, which are associated with adverse clinical outcome.⁸⁷

Taken together, these findings may indicate that the oxidative stress imbalance may cooperate with a signature of oncogenes/tumor-suppressor genes closely related to prostate cancer progression, suggesting that antioxidants may be critical to developing intervention strategies for this advanced disease.

Homing to the bone: the signature of the activating metastatic genes

A strictly interplay between prostate cancer cells and the tissue microenvironment determines the capability of prostate tumors to metastasize and successfully grow in a different organ.

Prostate cancer cells that metastasize to bone express several bone matrix and signaling proteins involved in adhesion and migration contributing to the osteomimetic properties. These include osteocalcin, osteopontin (OPN), bone sialoprotein, MMP and Wnt factors.⁸⁸

The prostate metastatic disease and subsequent process of bone remodeling is a result of factors secreted by the tumor cells.⁸⁹ Among the signaling molecules, the receptor activator of NF- κ B ligand (RANKL),⁹⁰ parathyroid hormone-related protein (PTHrP) and IL-8^{91,92} promote bone resorption, while endothelin and Wnt pathway factors promote osteoblastic lesions.^{93,94}

A key question is the extent to which the expression of runt-related transcription factor 2 (Runx2) contributes to the progression of prostate cancer and is functionally related to the formation of osteolytic and osteoblastic lesions at the bone site. Pathways such as Wnt, Src, bone morphogenic proteins and TGF β signaling are targeted by Runx2 and are activated in tumor cells.^{95–97} Akech *et al.*⁸⁸ reported that Runx2-mediated gene expression is associated with increased motility and invasiveness of prostate cancer cells and with the aggressiveness of the osteolytic bone damage. Moreover, their findings suggest that the expression of Runx2, MMP and PTHrP provide a signature for responsiveness of prostate cancer cells to the bone microenvironment. Several investigations and clinical data clearly implicated MMP in metastasis (reviewed in Deryugina and Quigley⁹⁸; Morgia *et al.*⁹⁹) and was demonstrated that MMP are regulated by Runx2.^{100,101}

Other transcription factors are also involved in bone metastasis. Hypoxia-induced growth factor 1 α (HIF1 α) in tumor cells, inhibits osteoblasts differentiation, induces osteoclasts differentiation and promotes tumor growth.

Hypoxia and TGF β signaling in parallel drive the development of tumor bone metastases and regulate a common set of tumor genes stimulating the production of VEGF and CXCR4 in both tumor cells and bone microenvironment to enhance angiogenesis and tumor homing.¹⁰² VEGF, a target gene of Runx2, facilitates tumor growth and both the osteolytic and the osteoblastic disease.^{103,104} Additionally, prostate cancer cell lines express mediators of tumor growth and bone destruction, among them IL-8, IL-6 and PTHrP.¹⁰⁵ Thus, Runx2 is a key regulator of metastasis-related genes and, considering that strong Runx2 expression was documented in advanced prostate cancer compared with early neoplastic lesions, its presence in the primary tumor could be critical for the diagnosis of prostate cancer bone metastasis.⁸⁸

In nearly all patients with advanced prostate cancer, the disease metastasizes to bone and causes osteoblastic growth.¹⁰⁶ Primary tumors may govern the bone marrow through signaling cascades that direct cells in the bone niche and facilitate its colonization. Osteoblasts, bone marrow stromal cells and hematopoietic stem cells form part of the tumoral microenvironment and engage with tumoral cells through cell to cell interactions mediated by integrins such as α 4 β 1, vascular cell adhesion molecule 1, chemokines such as CXCL12/CXCR4, glycoproteins such as CD34, adhesion molecules as PECAM, cadherin superfamily, bone morphogenic proteins, Notch, nestin and OPN, among other factors. Some of these molecules proved to be relevant in the clinic and may represent interesting pharmacodynamic markers of the clinical response of CRPC patients.¹⁰⁷

Notch signaling appears to be very attractive because it is involved in a variety of cellular processes, including cell differentiation, proliferation and survival and its activation is associated with tumorigenesis. Emerging evidence suggests that Notch also has an important role in prostate development and progression.¹⁰⁸ Specifically, bone metastases from prostate cancer patients expressed Notch-1 protein in osteoblastic lesions.¹⁰⁹ Correspondingly, Notch ligand Jagged-1 was found to be highly expressed in metastatic prostate cancer compared with localized disease or benign prostate tissues, and high Jagged-1 expression in a subset of clinically localized tumors was found to be significantly associated with tumor recurrence.¹¹⁰ Although the molecular mechanism of Notch signaling is not completely understood, silencing of Notch-1 inhibits MMP9, uPA and VEGF expression, given support to the effect of Notch in invasion.^{108,111} Moreover, Wang *et al.*¹⁰⁸ recently proposed a downregulated signaling cascade downstream of Notch-1, with reduced Akt and mTOR phosphorylation and inactivated NF- κ B signaling.

Primary tumors secrete factors that predispose to bone homing. The circulating factors include: OPN,^{112–114} MMP,¹¹⁵ PTHrP⁸⁹ and the chemokine CCL2.¹¹⁶

It is noteworthy that OPN is associated with poor prognosis in prostate cancer.¹¹⁷ It is expressed not only in tumor cells but also in cellular components of the microenvironment and as part of the extracellular matrix. Moreover, tumor and normal cells express OPN receptors. Although there may be structural differences in tumor-derived versus stromal-derived OPN, there may also be functional differences in how the target cell responds, depending on the cell type and tissue context.

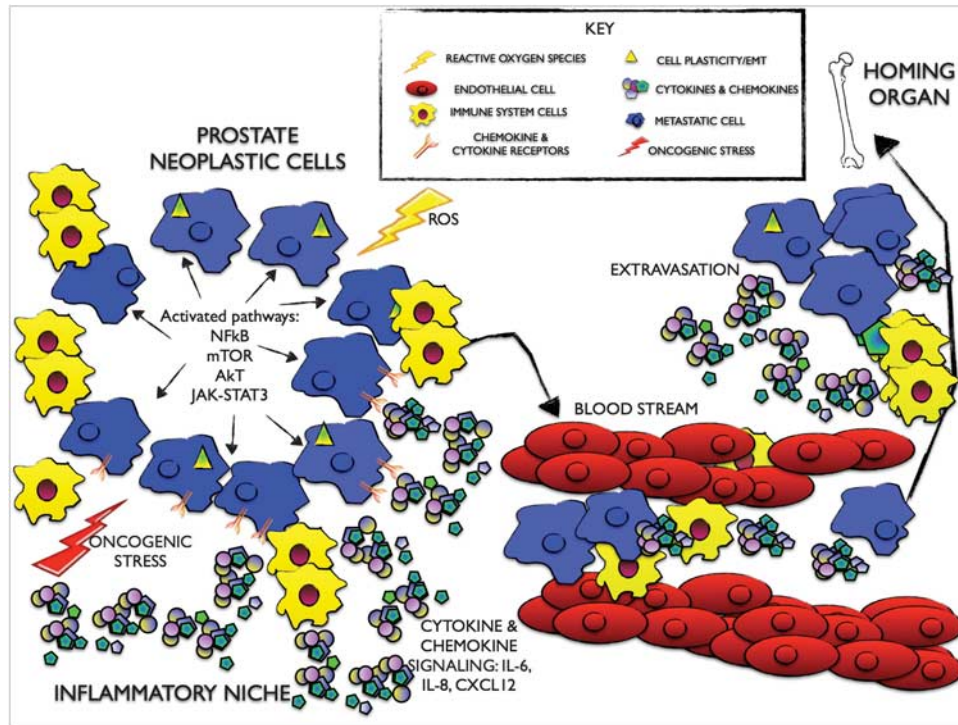


Figure 1 Schematic representation of the prostate neoplastic cells and the inflammatory milieu in advanced prostate cancer. Molecular signaling triggered by inflammatory mediators may generate a persistent inflammatory niche in advanced prostate cancer. Signaling cascades are switched on bypassing the androgen/AR axis. Among them, loss of tumor-suppressor phosphatase and tensin homolog (PTEN), with the concomitant inhibition of the phosphatidylinositol-3 kinase (PI3K)/Akt, mTOR and Janus kinase (JAK)/signal transducers and activators of transcription (STAT3) activation and sustained nuclear factor (NF- κ B) activity. All these pathways result in the burst of pro-inflammatory cytokines, chemokines and their receptors and other growth factors production. In addition, highly oxidative stress, oncogenic stress and antioxidant imbalance, generate an inflammatory microenvironment, which promotes the advanced stages of the disease by increasing cell motility, invasiveness and handling the shear stress in the vasculature, creating a continuous loop that stimulates a more aggressive stage, contributing to the acquisition of castration-resistant prostate cancer (CRPC) phenotype and favoring the ultimate bone homing. IL-8, interleukin 8.

Thus, OPN produces a complex scenario as a future cancer therapy.¹¹⁴

Altogether, the crosstalk of the several signaling pathways involved in the interaction between cancer cells and microenvironment conform an adequate niche for CRPC cells to grow and modify bone proliferation and differentiation.

Perspectives

It is clear that genetic and environmental factors contribute to the development of prostate cancer, and growing evidence suggests a role for chronic inflammation in its subsequent progression. In our opinion, it is necessary to identify those biochemical and molecular events associated to inflammation that control the acquisition of the castrate-resistant phenotype, in order to develop novel and more effective therapeutic approaches, which implicate modifiers of host tissue response as means of controlling metastatic prostate cancer growth. As therapies of cancer headway into individualized medicine, the need to elucidate the intricacy of CRPC becomes eminent. Prostate cancer progression is highly associated with several physiological/pathological processes such as inflammation. Although there are substantial clinical data supporting these findings, the influence of these inflammatory and

oxidative mediators on the prostatic disease has yet to be determined. Therefore, it is reasonable to draw attention into the molecular signaling pathways involved in extravasation, survival in the distant organ and the establishment of persistent growth. Therapy of CRPC should be focused not only on the intrinsic growth and survival properties of tumor cells, but also on the homeostatic factors that control the tumor niche. Figure 1 provides a schematic representation of the crosstalk between prostate neoplastic cells and the inflammatory environment in CRPC. In this scenario, targeting the inflammatory and oxidative network emerges as a promising target in CRPC, impairing the sustained inflammatory orchestration and shedding light into new promising avenues for the treatment of the hormone refractory prostate disease.

Conflict of interest

The authors declare no conflict of interest.

References

- 1 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69–90.

- 2 Karin M. Nuclear factor-kappaB in cancer development and progression. *Nature* 2006; **441**: 431–436.
- 3 Mercader M, Bodner BK, Moser MT, Kwon PS, Park ES, Manecke RG *et al*. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. *Proc Natl Acad Sci USA* 2001; **98**: 14565–14570.
- 4 Ammirante M, Luo JL, Grivnenikov S, Nedospasov S, Karin M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* 2010; **464**: 302–305.
- 5 Terada N, Shimizu Y, Kamba T, Inoue T, Maeno A, Kobayashi T *et al*. Identification of EP4 as a potential target for the treatment of castration-resistant prostate cancer using a novel xenograft model. *Cancer Res* 2010; **70**: 1606–1615.
- 6 Matsuoka T, Narumiya S. The roles of prostanoids in infection and sickness behaviors. *J Infect Chemother* 2008; **14**: 270–278.
- 7 Hull MA, Ko SC, Hawcroft G. Prostaglandin EP receptors: targets for treatment and prevention of colorectal cancer? *Mol Cancer Ther* 2004; **3**: 1031–1039.
- 8 Attard G, Reid AH, Olmos D, de Bono JS. Antitumor activity with CYP17 blockade indicates that castration-resistant prostate cancer frequently remains hormone driven. *Cancer Res* 2009; **69**: 4937–4940.
- 9 Bonkhoff H, Berges R. From pathogenesis to prevention of castration resistant prostate cancer. *Prostate* 2010; **70**: 100–112.
- 10 Bonkhoff H, Berges R. The evolving role of oestrogens and their receptors in the development and progression of prostate cancer. *Eur Urol* 2009; **55**: 533–542.
- 11 Dutt SS, Gao AC. Molecular mechanisms of castration-resistant prostate cancer progression. *Future Oncol* 2009; **5**: 1403–1413.
- 12 Khandrika L, Kumar B, Koul S, Maroni P, Koul HK. Oxidative stress in prostate cancer. *Cancer Lett* 2009; **282**: 125–136.
- 13 Chetram MA, Odero-Marah V, Hinton CV. Loss of PTEN permits CXCR4-mediated tumorigenesis through ERK1/2 in prostate cancer cells. *Mol Cancer Res* 2011; **9**: 90–102.
- 14 Vandercappellen J, Van Damme J, Struyf S. The role of CXC chemokines and their receptors in cancer. *Cancer Lett* 2008; **267**: 226–244.
- 15 Thobe MN, Clark RJ, Bainer RO, Prasad SM, Rinker-Schaeffer CW. From prostate to bone: key players in prostate cancer bone metastasis. *Cancers (Basel)* 2011; **3**: 478–493.
- 16 Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME *et al*. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001; **410**: 50–56.
- 17 Wang J, Loberg R, Taichman RS. The pivotal role of CXCL12 (SDF-1)/CXCR4 axis in bone metastasis. *Cancer Metastasis Rev* 2006; **25**: 573–587.
- 18 Chinni SR, Sivalogan S, Dong Z, Filho JC, Deng X, Bonfil RD *et al*. CXCL12/CXCR4 signaling activates Akt-1 and MMP-9 expression in prostate cancer cells: the role of bone micro-environment-associated CXCL12. *Prostate* 2006; **66**: 32–48.
- 19 Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res* 2002; **62**: 1832–1837.
- 20 Shulby SA, Dolloff NG, Stearns ME, Meucci O, Fatatis A. CX3CR1-fractalkine expression regulates cellular mechanisms involved in adhesion, migration, and survival of human prostate cancer cells. *Cancer Res* 2004; **64**: 4693–4698.
- 21 Kukreja P, Abdel-Mageed AB, Mondal D, Liu K, Agrawal KC. Up-regulation of CXCR4 expression in PC-3 cells by stromal-derived factor-1alpha (CXCL12) increases endothelial adhesion and transendothelial migration: role of MEK/ERK signaling pathway-dependent NF-kappaB activation. *Cancer Res* 2005; **65**: 9891–9898.
- 22 Engl T, Relja B, Marian D, Blumenberg C, Muller I, Beecken WD *et al*. CXCR4 chemokine receptor mediates prostate tumor cell adhesion through alpha5 and beta3 integrins. *Neoplasia* 2006; **8**: 290–301.
- 23 Sun YX, Fang M, Wang J, Cooper CR, Pienta KJ, Taichman RS. Expression and activation of alpha v beta 3 integrins by SDF-1/CXCL12 increases the aggressiveness of prostate cancer cells. *Prostate* 2007; **67**: 61–73.
- 24 Xing Y, Liu M, Du Y, Qu F, Li Y, Zhang Q *et al*. Tumor cell-specific blockade of CXCR4/SDF-1 interactions in prostate cancer cells by hTERT promoter induced CXCR4 knockdown: A possible metastasis preventing and minimizing approach. *Cancer Biol Ther* 2008; **7**: 1839–1848.
- 25 Frigo DE, Sherk AB, Wittmann BM, Norris JD, Wang Q, Joseph JD *et al*. Induction of Kruppel-like factor 5 expression by androgens results in increased CXCR4-dependent migration of prostate cancer cells *in vitro*. *Mol Endocrinol* 2009; **23**: 1385–1396.
- 26 Cai J, Kandagatla P, Singareddy R, Kropinski A, Sheng S, Cher ML *et al*. Androgens induce functional CXCR4 through ERG factor expression in TMPRSS2-ERG fusion-positive prostate cancer cells. *Transl Oncol* 2010; **3**: 195–203.
- 27 Gopalan A, Leversha MA, Satagopan JM, Zhou Q, Al-Ahmadie HA, Fine SW *et al*. TMPRSS2-ERG gene fusion is not associated with outcome in patients treated by prostatectomy. *Cancer Res* 2009; **69**: 1400–1406.
- 28 Toubaji A, Albadine R, Meeker AK, Isaacs WB, Lotan T, Haffner MC *et al*. Increased gene copy number of ERG on chromosome 21 but not TMPRSS2-ERG fusion predicts outcome in prostatic adenocarcinomas. *Mod Pathol* 2011; **24**: 1511–1520.
- 29 Akashi T, Koizumi K, Tsuneyama K, Saiki I, Takano Y, Fuse H. Chemokine receptor CXCR4 expression and prognosis in patients with metastatic prostate cancer. *Cancer Sci* 2008; **99**: 539–542.
- 30 Begley LA, MacDonald JW, Day ML, Macoska JA. CXCL12 activates a robust transcriptional response in human prostate epithelial cells. *J Biol Chem* 2007; **282**: 26767–26774.
- 31 Wang J, Shiozawa Y, Wang Y, Jung Y, Pienta KJ, Mehra R *et al*. The role of CXCR7/RDC1 as a chemokine receptor for CXCL12/SDF-1 in prostate cancer. *J Biol Chem* 2008; **283**: 4283–4294.
- 32 Burns JM, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z *et al*. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J Exp Med* 2006; **203**: 2201–2213.
- 33 Raggio C, Ruhl R, McAllister S, Koon H, Dezube BJ, Fruh K *et al*. Novel cellular genes essential for transformation of endothelial cells by Kaposi's sarcoma-associated herpesvirus. *Cancer Res* 2005; **65**: 5084–5095.
- 34 Miao Z, Luker KE, Summers BC, Berahovich R, Bhojani MS, Rehemtulla A *et al*. CXCR7 (RDC1) promotes breast and lung tumor growth *in vivo* and is expressed on tumor-associated vasculature. *Proc Natl Acad Sci USA* 2007; **104**: 15735–15740.
- 35 Singh RK, Lokeshwar BL. The IL-8-regulated chemokine receptor CXCR7 stimulates EGFR signaling to promote prostate cancer growth. *Cancer Res* 2011; **71**: 3268–3277.
- 36 Lehrer S, Diamond EJ, Mamkin B, Stone NN, Stock RG. Serum interleukin-8 is elevated in men with prostate cancer and bone metastases. *Technol Cancer Res Treat* 2004; **3**: 411.
- 37 Wang J, Lu Y, Koch AE, Zhang J, Taichman RS. CXCR6 induces prostate cancer progression by the AKT/mammalian target of rapamycin signaling pathway. *Cancer Res* 2008; **68**: 10367–10376.
- 38 Ha HK, Lee W, Park HJ, Lee SD, Lee JZ, Chung MK. Clinical significance of CXCL16/CXCR6 expression in patients with prostate cancer. *Mol Med Report* 2011; **4**: 419–424.
- 39 Chandrasekar B, Bysani S, Mummidi S. CXCL16 signals via Gi, phosphatidylinositol 3-kinase, Akt, I kappa B kinase, and nuclear factor-kappa B and induces cell-cell adhesion and aortic smooth muscle cell proliferation. *J Biol Chem* 2004; **279**: 3188–3196.
- 40 Deng L, Chen N, Li Y, Zheng H, Lei Q. CXCR6/CXCL16 functions as a regulator in metastasis and progression of cancer. *Biochim Biophys Acta* 2010; **1806**: 42–49.
- 41 Nakayama T, Hieshima K, Izawa D, Tatsumi Y, Kanamaru A, Yoshie O. Cutting edge: profile of chemokine receptor expression on human plasma cells accounts for their efficient recruitment to target tissues. *J Immunol* 2003; **170**: 1136–1140.

- 42 Inoue K, Slaton JW, Eve BY, Kim SJ, Perrotte P, Balbay MD *et al*. Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. *Clin Cancer Res* 2000; **6**: 2104–2119.
- 43 Kim SJ, Uehara H, Karashima T, McCarty M, Shih N, Fidler IJ. Expression of interleukin-8 correlates with angiogenesis, tumorigenicity, and metastasis of human prostate cancer cells implanted orthotopically in nude mice. *Neoplasia* 2001; **3**: 33–42.
- 44 Lamont KR, Tindall DJ. Minireview: alternative activation pathways for the androgen receptor in prostate cancer. *Mol Endocrinol* 2011; **25**: 897–907.
- 45 Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. *Clin Cancer Res* 2008; **14**: 6735–6741.
- 46 McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A *et al*. Influence of cytokine gene polymorphisms on the development of prostate cancer. *Cancer Res* 2002; **62**: 3369–3372.
- 47 Wilson C, Purcell C, Seaton A, Oladipo O, Maxwell PJ, O'Sullivan JM *et al*. Chemotherapy-induced CXC-chemokine/CXC-chemokine receptor signaling in metastatic prostate cancer cells confers resistance to oxaliplatin through potentiation of nuclear factor-kappaB transcription and evasion of apoptosis. *J Pharmacol Exp Ther* 2008; **327**: 746–759.
- 48 Tassidis H, Culig Z, Wingren AG, Harkonen P. Role of the protein tyrosine phosphatase SHP-1 in Interleukin-6 regulation of prostate cancer cells. *Prostate* 2010; **70**: 1491–1500.
- 49 Shariat SF, Andrews B, Kattan MW, Kim J, Wheeler TM, Slawin KM. Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. *Urology* 2001; **58**: 1008–1015.
- 50 Blaszczyk N, Masri BA, Mawji NR, Ueda T, McAlinden G, Duncan CP *et al*. Osteoblast-derived factors induce androgen-independent proliferation and expression of prostate-specific antigen in human prostate cancer cells. *Clin Cancer Res* 2004; **10**: 1860–1869.
- 51 Culig Z, Steiner H, Bartsch G, Hobisch A. Interleukin-6 regulation of prostate cancer cell growth. *J Cell Biochem* 2005; **95**: 497–505.
- 52 Ishiguro H, Akimoto K, Nagashima Y, Kojima Y, Sasaki T, Ishiguro-Imagawa Y *et al*. aPKClambda/iota promotes growth of prostate cancer cells in an autocrine manner through transcriptional activation of interleukin-6. *Proc Natl Acad Sci USA* 2009; **106**: 16369–16374.
- 53 Malinowska K, Neuwirt H, Cavarretta IT, Bektic J, Steiner H, Dietrich H *et al*. Interleukin-6 stimulation of growth of prostate cancer *in vitro* and *in vivo* through activation of the androgen receptor. *Endocr Relat Cancer* 2009; **16**: 155–169.
- 54 Chun JY, Nadiminty N, Dutt S, Lou W, Yang JC, Kung HJ *et al*. Interleukin-6 regulates androgen synthesis in prostate cancer cells. *Clin Cancer Res* 2009; **15**: 4815–4822.
- 55 Wu Y, Zhou BP. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. *Br J Cancer* 2010; **102**: 639–644.
- 56 Davis JS, Nastiuk KL, Krolewski JJ. TNF is necessary for castration-induced prostate regression, whereas TRAIL and FasL are dispensable. *Mol Endocrinol* 2011; **25**: 611–620.
- 57 Shukla S, MacLennan GT, Fu P, Patel J, Marengo SR, Resnick MI *et al*. Nuclear factor-kappaB/p65 (Rel A) is constitutively activated in human prostate adenocarcinoma and correlates with disease progression. *Neoplasia* 2004; **6**: 390–400.
- 58 Jin RJ, Lho Y, Connelly L, Wang Y, Yu X, Saint Jean L *et al*. The nuclear factor-kappaB pathway controls the progression of prostate cancer to androgen-independent growth. *Cancer Res* 2008; **68**: 6762–6769.
- 59 Huang S, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. *Oncogene* 2001; **20**: 4188–4197.
- 60 Gasparian AV, Yao YJ, Kowalczyk D, Lyakh LA, Karseladze A, Slaga TJ *et al*. The role of IKK in constitutive activation of NF-kappaB transcription factor in prostate carcinoma cells. *J Cell Sci* 2002; **115**: 141–151.
- 61 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646–674.
- 62 Mantovani A. Cancer: inflaming metastasis. *Nature* 2009; **457**: 36–37.
- 63 Shiota M, Yokomizo A, Tada Y, Inokuchi J, Kashiwagi E, Masubuchi D *et al*. Castration resistance of prostate cancer cells caused by castration-induced oxidative stress through Twist1 and androgen receptor overexpression. *Oncogene* 2010; **29**: 237–250.
- 64 Maynard S, Schurman SH, Harboe C, de Souza-Pinto NC, Bohr VA. Base excision repair of oxidative DNA damage and association with cancer and aging. *Carcinogenesis* 2009; **30**: 2–10.
- 65 Taplin ME, Rajeshkumar B, Halabi S, Werner CP, Woda BA, Picus J *et al*. Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663. *J Clin Oncol* 2003; **21**: 2673–2678.
- 66 Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. *Cancer Res* 2001; **61**: 3550–3555.
- 67 Murakami S, Noguchi T, Takeda K, Ichijo H. Stress signaling in cancer. *Cancer Sci* 2007; **98**: 1521–1527.
- 68 Gort EH, van Haaften G, Verlaan I, Groot AJ, Plasterk RH, Shvarts A *et al*. The TWIST1 oncogene is a direct target of hypoxia-inducible factor-2alpha. *Oncogene* 2008; **27**: 1501–1510.
- 69 Hoek K, Rimm DL, Williams KR, Zhao H, Ariyan S, Lin A *et al*. Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. *Cancer Res* 2004; **64**: 5270–5282.
- 70 Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C *et al*. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004; **117**: 927–939.
- 71 Bostwick DG, Alexander EE, Singh R, Shan A, Qian J, Santella RM *et al*. Antioxidant enzyme expression and reactive oxygen species damage in prostatic intraepithelial neoplasia and cancer. *Cancer* 2000; **89**: 123–134.
- 72 Best CJ, Gillespie JW, Yi Y, Chandramouli GV, Perlmutter MA, Gathright Y *et al*. Molecular alterations in primary prostate cancer after androgen ablation therapy. *Clin Cancer Res* 2005; **11**: 6823–6834.
- 73 Sharifi N, Hurt EM, Thomas SB, Farrar WL. Effects of manganese superoxide dismutase silencing on androgen receptor function and gene regulation: implications for castration-resistant prostate cancer. *Clin Cancer Res* 2008; **14**: 6073–6080.
- 74 Tam NN, Gao Y, Leung YK, Ho SM. Androgenic regulation of oxidative stress in the rat prostate: involvement of NAD(P)H oxidases and antioxidant defense machinery during prostatic involution and regrowth. *Am J Pathol* 2003; **163**: 2513–2522.
- 75 Pang ST, Dillner K, Wu X, Pousette A, Norstedt G, Flores-Morales A. Gene expression profiling of androgen deficiency predicts a pathway of prostate apoptosis that involves genes related to oxidative stress. *Endocrinology* 2002; **143**: 4897–4906.
- 76 Eliceiri BP. Integrin and growth factor receptor crosstalk. *Circ Res* 2001; **89**: 1104–1110.
- 77 Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, Miyamoto S. Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. *Genes Dev* 1995; **9**: 2723–2735.
- 78 Karin M, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* 2002; **2**: 301–310.
- 79 Kong D, Li Y, Wang Z, Banerjee S, Sarkar FH. Inhibition of angiogenesis and invasion by 3,3'-diindolylmethane is mediated by the nuclear factor-kappaB downstream target genes MMP-9 and uPA that regulated bioavailability of vascular endothelial growth factor in prostate cancer. *Cancer Res* 2007; **67**: 3310–3319.
- 80 Ryter SW, Choi AM. Heme oxygenase-1: molecular mechanisms of gene expression in oxygen-related stress. *Antioxid Redox Signal* 2002; **4**: 625–632.

- 81 Min KJ, Lee JT, Joe EH, Kwon TK. An IkappaBalpha phosphorylation inhibitor induces heme oxygenase-1(HO-1) expression through the activation of reactive oxygen species (ROS)-Nrf2-ARE signaling and ROS-PI3K/Akt signaling in an NF-kappaB-independent mechanism. *Cell Signal* 2011; **23**: 1505–1513.
- 82 Sacca P, Meiss R, Casas G, Mazza O, Calvo JC, Navone N *et al*. Nuclear translocation of haeme oxygenase-1 is associated to prostate cancer. *Br J Cancer* 2007; **97**: 1683–1689.
- 83 Lin Q, Weis S, Yang G, Weng YH, Helston R, Rish K *et al*. Heme oxygenase-1 protein localizes to the nucleus and activates transcription factors important in oxidative stress. *J Biol Chem* 2007; **282**: 20621–20633.
- 84 Lin QS, Weis S, Yang G, Zhuang T, Abate A, Dennery PA. Catalytic inactive heme oxygenase-1 protein regulates its own expression in oxidative stress. *Free Radic Biol Med* 2008; **44**: 847–855.
- 85 Gueron G, De Siervi A, Ferrando M, Salierno M, De Luca P, Elguero B *et al*. Critical role of endogenous heme oxygenase 1 as a tuner of the invasive potential of prostate cancer cells. *Mol Cancer Res* 2009; **7**: 1745–1755.
- 86 Ferrando M, Gueron G, Elguero B, Giudice J, Salles A, Leskow FC *et al*. Heme oxygenase 1 (HO-1) challenges the angiogenic switch in prostate cancer. *Angiogenesis* 2011; **14**: 467–479.
- 87 Li Y, Su J, DingZhang X, Zhang J, Yoshimoto M, Liu S *et al*. PTEN deletion and heme oxygenase-1 overexpression cooperate in prostate cancer progression and are associated with adverse clinical outcome. *J Pathol* 2011; **224**: 90–100.
- 88 Akech J, Wixted JJ, Bedard K, van der Deen M, Hussain S, Guise TA *et al*. Runx2 association with progression of prostate cancer in patients: mechanisms mediating bone osteolysis and osteoblastic metastatic lesions. *Oncogene* 2010; **29**: 811–821.
- 89 Guise TA, Mohammad KS, Clines G, Stebbins EG, Wong DH, Higgins LS *et al*. Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. *Clin Cancer Res* 2006; **12**: 6213s–6216s.
- 90 Armstrong AP, Miller RE, Jones JC, Zhang J, Keller ET, Dougall WC. RANKL acts directly on RANK-expressing prostate tumor cells and mediates migration and expression of tumor metastasis genes. *Prostate* 2008; **68**: 92–104.
- 91 Bendre MS, Margulies AG, Walsler B, Akel NS, Bhattacharya S, Skinner RA *et al*. Tumor-derived interleukin-8 stimulates osteolysis independent of the receptor activator of nuclear factor-kappaB ligand pathway. *Cancer Res* 2005; **65**: 11001–11009.
- 92 Araki S, Omori Y, Lyn D, Singh RK, Meinbach DM, Sandman Y *et al*. Interleukin-8 is a molecular determinant of androgen independence and progression in prostate cancer. *Cancer Res* 2007; **67**: 6854–6862.
- 93 Li ZG, Yang J, Vazquez ES, Rose D, Vakar-Lopez F, Mathew P *et al*. Low-density lipoprotein receptor-related protein 5 (LRP5) mediates the prostate cancer-induced formation of new bone. *Oncogene* 2008; **27**: 596–603.
- 94 Clines GA, Mohammad KS, Bao Y, Stephens OW, Suva LJ, Shaughnessy Jr JD *et al*. Dickkopf homolog 1 mediates endothelin-1-stimulated new bone formation. *Mol Endocrinol* 2007; **21**: 486–498.
- 95 Hall CL, Kang S, MacDougald OA, Keller ET. Role of Wnts in prostate cancer bone metastases. *J Cell Biochem* 2006; **97**: 661–672.
- 96 Kingsley LA, Fournier PG, Chirgwin JM, Guise TA. Molecular biology of bone metastasis. *Mol Cancer Ther* 2007; **6**: 2609–2617.
- 97 Li ZG, Mathew P, Yang J, Starbuck MW, Zurita AJ, Liu J *et al*. Androgen receptor-negative human prostate cancer cells induce osteogenesis in mice through FGF9-mediated mechanisms. *J Clin Invest* 2008; **118**: 2697–2710.
- 98 Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Rev* 2006; **25**: 9–34.
- 99 Morgia G, Falsaperla M, Malaponte G, Madonia M, Indelicato M, Travali S *et al*. Matrix metalloproteinases as diagnostic (MMP-13) and prognostic (MMP-2, MMP-9) markers of prostate cancer. *Urol Res* 2005; **33**: 44–50.
- 100 Selvamurugan N, Jefcoat SC, Kwok S, Kowalewski R, Tamasi JA, Partridge NC. Overexpression of Runx2 directed by the matrix metalloproteinase-13 promoter containing the AP-1 and Runx/RD/Cbfa sites alters bone remodeling *in vivo*. *J Cell Biochem* 2006; **99**: 545–557.
- 101 Pratap J, Lian JB, Javed A, Barnes GL, van Wijnen AJ, Stein JL *et al*. Regulatory roles of Runx2 in metastatic tumor and cancer cell interactions with bone. *Cancer Metastasis Rev* 2006; **25**: 589–600.
- 102 Dunn LK, Mohammad KS, Fournier PG, McKenna CR, Davis HW, Niewolna M *et al*. Hypoxia and TGF-beta drive breast cancer bone metastases through parallel signaling pathways in tumor cells and the bone microenvironment. *PLoS One* 2009; **4**: e6896.
- 103 Yang Q, McHugh KP, Patntirapong S, Gu X, Wunderlich L, Hauschka PV. VEGF enhancement of osteoclast survival and bone resorption involves VEGF receptor-2 signaling and beta3-integrin. *Matrix Biol* 2008; **27**: 589–599.
- 104 Dai J, Kitagawa Y, Zhang J, Yao Z, Mizokami A, Cheng S *et al*. Vascular endothelial growth factor contributes to the prostate cancer-induced osteoblast differentiation mediated by bone morphogenetic protein. *Cancer Res* 2004; **64**: 994–999.
- 105 Weilbaecher KN, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. *Nat Rev Cancer* 2011; **11**: 411–425.
- 106 Drake JM, Danke JR, Henry MD. Bone-specific growth inhibition of prostate cancer metastasis by atrasentan. *Cancer Biol Ther* 2010; **9**: 607–614.
- 107 Fontana A, Galli L, Fioravanti A, Orlandi P, Galli C, Landi L *et al*. Clinical and pharmacodynamic evaluation of metronomic cyclophosphamide, celecoxib, and dexamethasone in advanced hormone-refractory prostate cancer. *Clin Cancer Res* 2009; **15**: 4954–4962.
- 108 Wang Z, Li Y, Banerjee S, Kong D, Ahmad A, Nogueira V *et al*. Down-regulation of Notch-1 and Jagged-1 inhibits prostate cancer cell growth, migration and invasion, and induces apoptosis via inactivation of Akt, mTOR, and NF-kappaB signaling pathways. *J Cell Biochem* 2010; **109**: 726–736.
- 109 Zayzafoon M, Abdulkadir SA, McDonald JM. Notch signaling and ERK activation are important for the osteomimetic properties of prostate cancer bone metastatic cell lines. *J Biol Chem* 2004; **279**: 3662–3670.
- 110 Santagata S, Demichelis F, Riva A, Varambally S, Hofer MD, Kutok JL *et al*. JAGGED1 expression is associated with prostate cancer metastasis and recurrence. *Cancer Res* 2004; **64**: 6854–6857.
- 111 Bin Hafeez B, Adhami VM, Asim M, Siddiqui IA, Bhat KM, Zhong W *et al*. Targeted knockdown of Notch1 inhibits invasion of human prostate cancer cells concomitant with inhibition of matrix metalloproteinase-9 and urokinase plasminogen activator. *Clin Cancer Res* 2009; **15**: 452–459.
- 112 Pazolli E, Luo X, Brehm S, Carbery K, Chung JJ, Prior JL *et al*. Senescent stromal-derived osteopontin promotes preneoplastic cell growth. *Cancer Res* 2009; **69**: 1230–1239.
- 113 McAllister SS, Gifford AM, Greiner AL, Kelleher SP, Saelzler MP, Ince TA *et al*. Systemic endocrine instigation of indolent tumor growth requires osteopontin. *Cell* 2008; **133**: 994–1005.
- 114 Anborgh PH, Mutrie JC, Tuck AB, Chambers AF. Role of the metastasis-promoting protein osteopontin in the tumour microenvironment. *J Cell Mol Med* 2010; **14**: 2037–2044.
- 115 Lynch CC, Hikosaka A, Acuff HB, Martin MD, Kawai N, Singh RK *et al*. MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL. *Cancer Cell* 2005; **7**: 485–496.
- 116 Li X, Loberg R, Liao J, Ying C, Snyder LA, Pienta KJ *et al*. A destructive cascade mediated by CCL2 facilitates prostate cancer growth in bone. *Cancer Res* 2009; **69**: 1685–1692.
- 117 Zhang Y, Foroootan SS, Kamalian L, Bao ZZ, Malki MI, Foster CS *et al*. Suppressing tumourigenicity of prostate cancer cells by inhibiting osteopontin expression. *Int J Oncol* 2011; **38**: 1083–1091.