PROSPECTS
Journal of Cellular Biochemistry 118:1956–1964 (2017)

Journal of Cellular Biochemistry

Biochemical Changes in the Niche Following Tumor Cell Invasion

A.M. Decker,[1] F.C. Cackowski,[1,2] Y. Jung,[1] and R.S. Taichman[1]*

[1]*Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, Michigan*

[2]*Division of Hematology and Oncology, Department of Internal Medicine, University of Michigan School of Medicine, Ann Arbor, Michigan*

ABSTRACT

Metastatic cancer is the leading cause of all cancer related deaths. Prostate cancer (PCa) metastasizes preferentially to the bone marrow, specifically within the endosteal niche. Endosteal cells secrete homing molecules that may recruit PCa cells to the bone marrow. Once there, the biochemical signature of this niche regulates PCa fate including cellular dormancy or cell cycle arrest, reactivation and resistance to chemotherapeutics. Growth factors, interleukins, adhesion molecules, as well as extra-cellular matrix proteins can collectively change the phenotype of PCa cells. Understanding the biochemical signature of endosteal niche parasitism by PCa is imperative for the establishment of new and innovative therapeutic strategies. This review seeks to summarize these important niche signatures and the potential therapeutic approaches to target metastatic PCa within the bone marrow hematopoietic stem cell (HSC) niche. J. Cell. Biochem. 118: 1956–1964, 2017.

KEY WORDS: NICHE; DISSEMINATED TUMOR CELL (DTC); OSTEOBLAST; METASTASIS; PROSTATE CANCER (PCa)

Localized prostate cancer (PCa) is generally regarded with a positive prognosis; however, a significantly poorer prognosis with higher mortality is assigned to PCa that has invaded the prostate capsule and metastasized beyond the local microenvironment. In fact, the incidence of metastatic prostate cancer increased 72% between 2004 and 2013, according to a recent study, possibly due to increased detection of metastatic disease [Harryman et al., 2016; Weiner et al., 2016]. Furthermore, metastatic disease remains the primary cause of PCa cancer related-deaths [Gundem et al., 2015]. To improve these statistics, a deeper understanding is needed as to the events which surround metastatic disease, the effect of the marrow microenvironment on metastatic cells and disease progress, and the factors instigating recurrence. The aim of this work is to discuss the cues within the bone microenvironment that support metastatic PCa cell growth including systemic signaling molecules, local signaling molecules, local adhesion molecules, local extracellular matrix molecules (ECM), and current therapeutic targeting modalities regarding metastatic disseminated tumor cells (DTCs).

PCA METASTASIZES TO THE BONE MARROW

The development of clinical metastatic PCa initiates in a progression from PCa development at the primary tumor site in the prostate. Primary PCa cells then invade their surrounding environment and enter the peripheral circulation as circulating tumor cells (CTCs). CTCs can then leave circulation and enter a metastatic site as a disseminated tumor cell (DTC). Based upon animal investigations, DTCs are found in the vascular beds of all end organs, but the bone marrow (BM) is frequently the first site of conversion of occult tumor cells to frank metastasis. In fact, many men ostensibly cured of their local disease may develop clinically detectable bone metastases many years following resection or radiation of the primary tumor, suggesting that cancer cells likely escape early in the disease process, but also are able to maintain a dormant phenotype within the bone marrow prior to conversion to a proliferative phenotype years later [Pound et al., 1999; Van der Toom et al., 2016].

Microenvironment signaling factors and ECM components are thought to play a significant role in the progression of PCa from a

Grant sponsor: University of Michigan MCubed Project; Grant sponsor: National Cancer Institute; Grant numbers: CA093900, CA163124; Grant sponsor: Department of Defense; Grant numbers: W81XW-15-1-0413, W81XWH-14-1-0403; Grant sponsor: Prostate Cancer Foundation; Grant sponsor: NIH/NCI T32; Grant number: 5T32CA009357-32.

*Correspondence to: Russell S. Taichman, D.M.D., D.M.Sc., Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, 1011 North University Ave., Ann Arbor 48109-1078, MI. E-mail: rtaich@umich.edu

Manuscript Received: 12 December 2016; Manuscript Accepted: 14 December 2016

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 16 December 2016

DOI 10.1002/jcb.25843 • © 2016 Wiley Periodicals, Inc.

primary lesion to metastasis. The prostate gland itself is comprised of many defined regions surrounded by a smooth muscular stroma that is perforated by the cavernous nerve and neurovascular bundles of the pelvic plexus serving autonomic innervation to the prostate [Nagle and Cress, 2011]. The greatest innervation has been observed in the prostate's peripheral zone and perineural invasion may provide a means of cancer cell escape from the PCa capsule [Sroka et al., 2010]. Interestingly, though normal prostate tissue expresses several combinations of integrin units, PCa cells predominantly express the laminin binding integrins α6β1 and α3β1 [Schmelz et al., 2002]. Further, post-translational modification of α6β1 increases PCa cell migration and invasion as well as metastasis to laminin-rich bone [Pawar et al., 2007; Ports et al., 2009; Sroka et al., 2010]. Many cell-cell and cell-ECM interactions occur in the migration of cancer cells from the primary tumor to a metastatic site and these data suggest that biomechanical cues can be involved in cancer cell progression and metastatic site development.

Metastasis of PCa to the bone marrow microenvironment is directed through several known mediators, including the CXCL12/CXCR4 signaling axis. CXCL12 (previously described as stromal-derived factor-1 (SDF-1)) is a homeostatic chemokine that functions in health to regulate hematopoietic stem cell (HSC) and lymphocyte localization to the bone marrow. Expression of CXCL12 increases with cardiac infarctions, peripheral ischemia, excessive blood volume loss, and tissue damage related to chemotherapy [Teicher and Fricker, 2010]. CXCR4 is also widely expressed on CD34+ HSCs, T-lymphocytes, B-lymphocytes, monocytes, macrophages, neutrophils, neuronal cells, endothelial cells, and smooth muscle progenitors, allowing these cells to migrate along the CXCL12 gradients [Teicher and Fricker, 2010]. Expression of CXCR4 by PCa cells also provides a mechanism for their migration to metastatic bone marrow sites, including the HSC niche [Taichman et al., 2002; Sun et al., 2003, 2005; Shiozawa et al., 2011] (Table I). In addition to homing, CXCL12 can transiently regulate the expression of the αvβ3 integrin, which may also play a role in PCa metastatic localization to the bone marrow metastatic niche [Sun et al., 2007]. Further, annexin II receptor (ANXA2r) located on HSCs and PCa cells bind directly with annexin II (ANXA2), expressed by osteoblasts, and facilitates the anchorage of HSCs in health and PCa cells in disease conditions [Jung et al., 2007; Shiozawa et al., 2008]. Thus, many niche factors involved in PCa metastasis are increasingly relevant to the support of PCa disease progression and localization to the bone marrow metastatic site.

REGULATION OF PCA CELLS WITHIN THE BONE MARROW NICHE

The niche cells include mesenchymal stem cells, progenitor osteoblasts, osteoblasts, progenitor osteoclasts, osteoclasts that are primarily involved in the formation and maintenance of this microenvironment as demonstrated in a recently published model of the niche and its microenvironment [Araujo et al., 2014]. Each of these cell types and others within the bone marrow environment actively contribute to the cytokine gradients that dictate quiescence, survival, and affect the proliferative status of the newly engaged PCa DTCs through cytokine/chemokine signaling, adhesion, and ECM remodeling.

PCa DTCs can target and engage the HSC niche following dissemination to the bone marrow [Shiozawa et al., 2011]. Similar to HSCs, when DTCs are engaged with osteoblasts within the marrow niche, PCa cells can attach to the cell surface of adjacent osteoblasts via many cell-cell interactions that regulate cell quiescence, survival, and lower proliferative capacity. Specifically, it was shown that binding of PCa cells to osteoblasts in the bone marrow induces TANK binding kinase 1 (TBK1) expression, which subsequently inhibits mTOR signaling, induces cell cycle arrest, and increases chemotherapeutic resistance (Table I).

EFFECTS OF CYTOKINE/CHEMOKINE SIGNALING WITHIN THE BONE MARROW NICHE ON PCA CELLS

GAS6/TAM RECEPTORS

GAS6 is a growth factor expressed by osteoblasts within the bone marrow microenvironment that regulates the cell cycling of HSCs. GAS6 is a ligand for the AXL (Ufo/Ark), TYRO3 (Dtk/SKY/Rse/Brt/ETK2/Tif), and MERTK (Eyk) family of tyrosine kinase receptors and binds to these receptors via tandem G domains at its C terminus [Dormady et al., 2000]. GAS6 inhibits HSC proliferation [Shiozawa et al., 2011]. Like HSCs, GAS6 inhibits PCa proliferation and appears to participate in the induction of tumor cell dormancy, such that DTCs can remain quiescent for prolonged periods in the marrow [Shiozawa et al., 2010]. GAS6, expressed by osteoblasts regulates PCa cell cycle in the bone marrow, through induction of G_1 cell cycle arrest and S cell cycle phase delay [Lee et al., 2016]. Further, GAS6 appears to also ensure cell survival by protecting PCa cell apoptosis signals through inhibition of cleavage of caspase-3 and PARP [Lee et al., 2016]. Thus, PCa engagement with the endosteal niche exposes DTCs to osteoblast-secreted GAS6, causing PCa cell cycle arrest, survival, and resistance to chemotherapeutic advances.

Interestingly, the TAM receptors may also have an effect on PCa cell phenotype within the bone marrow. The phenotype of dormant PCa DTCs include a decrease in the p-ERK/p-p38 ratio, and upregulation of associated transcription factors NR2F1, SOX2, SOX9, NANOG, and RARB [Sosa et al., 2015]. Recently, we reported that MERTK knockdown alone induced PCa cell cycle arrest via decreased p-ERK1/2 to p-p38 and increased cell cycle inhibitors/dormancy associated transcription factors p27, NR2F1, SOX2, and NANOG [Cackowski et al., 2016]. Furthermore, GAS6 overexpression activated phosphorylation of MERTK in PCa cells, leading to an increase in the number of cancer stem cells (CSCs) among DTCs recovered from the bone marrow, suggesting that activation of Mer receptor signaling by endogenous GAS6 can contribute to the establishment of PCa CSCs (CD133+/CD44+) in the bone marrow [Jung et al., 2016].

In addition, the TAM receptor ratio of AXL/TYRO3 has also been associated with PCa cell cycling. Specifically, in vivo studies demonstrated that when Axl receptor levels were more highly expressed compared to other TAM receptors, PCa cells became growth-arrested compared to PCa cells that expressed lower Axl expression [Taichman et al., 2013]. Cells that had a lower Axl/Tyro3

TABLE I. Description of Important Biochemical Mediators of PCa Entry and Survival in the Bone Marrow

Biochemical components	Description	Effect on PCa bone metastasis	Citation
Growth factors			
CXCL12	Homing molecule secreted by osteoblasts.	Induces HSC mobilization from the HSC niche and recruits PCa cells.	Taichman et al. [2002]; Sun et al. [2003, 2005]; Shiozawa et al. [2011]
GAS6	Growth factor expressed by osteoblasts.	Ligand for PCa TAM receptor reducing cell cycling and induction of PCa dormancy.	Dormady et al. [2000]; Shiozawa et al. [2010]; Mishra et al. [2012]; Taichman et al. [2013]
TGF-β	Growth regulatory factor expressed and produced by a wide-variety of cells including osteoblasts and PCa cells.	Autocrine (from PCa cells) and Paracrine (from osteoblasts) signaling reduces cell cycling, inducing a dormant state.	Robey et al. [1987]; Xu et al. [2009]; Krzeszinski et al. [2014]; Tu et al. [2014]
BMP7	TGF-β family member, secreted by stromal cells in the bone marrow.	Induces cellular senescence in PCa CSCs.	Kobayashi et al. [2011]
EGF	Endogenous growth factor that is linked with cell growth.	Present in the bone marrow, increasing PCa cell proliferation and osteoclast differentiation/promoting osteolytic events.	Chackal-Roy et al. [1989]; Braun et al. [2001]; Krampera et al. [2005]; Lu et al. [2009]
IGF	Growth factor affecting the growth and differentiation of a variety of tissues.	Promotes osteoclastogenesis and expansion of the osteoblastic niche. May promote colonization of the bone marrow in PCa cells.	Chan et al. [1998]; Hellawell et al. [2002]; Rubin et al. [2002]; Rosen et al. [2004]; Zhang et al. [2013]
VEGF	Mediates angiogenesis in various tissues both in homeostasis and cancer.	Mobilizes bone-marrow derived endothelial precursors to mobilize angiogenesis and bone resorption creating a permissive environment for disseminated tumor cells.	Kaplan et al. [2005]
IL-6	Interleukin that regulates apoptosis/cell survival and proliferation.	PCa IL-6 secretion mediates osteoblastic differentiation and osteoclastogenesis. Osteoblast secretion of IL-6 results in PCa proliferation.	Heinrich et al. [2003];Morrissey et al. [2010]
RANKL/ RANK	RANKL expressed by osteoblasts can bind RANK on osteoclast precursor cells and induce osteoclastogenesis during the normal bone remodeling process.	RANKL released from osteoblasts can increase PCa IL-6 secretion and RANK expression.	Mundy [1997];Zheng et al. [2014]
Adhesion and ECM components			
ANXA2	Protein expressed by OBs.	ANXA2r located on PCa cells bind to ANXA2 on osteoblasts to facilitate PCa anchorage within the bone marrow.	Jung et al. [2007]; Shiozawa et al. [2008]
αVβ3	Binds fibronectin, vitronectin, TSP, and other ECM proteins.	Osteoblastic CXCL12 causes upregulation of PCa αVβ3 promoting PCa adhesion to osteoblasts in the bone marrow.	Shiozawa et al. [2010]
α1β1,α2β1, α6β1	Binds collagen.	Engages PCa cells with bone and may initiate bone metastatic motility programs.	Fornaro et al. [2001]; Hall et al. [2006]
TSP1	Anti-angiogenic ECM glycoprotein produced by various cell types including.	Downregulated in progression of PCa to promote angiogenesis in the area.	Ren et al. [2006]; Venkatraman et al. [2012]
Therapeutics			
Denosumab	Anti-RANKL antibody.	Indicated for prevention of osteoporotic fracture in all prostate cancer patients treated with androgen deprivation therapy. Increased metastasis free survival in men with non-metastatic castration resistant PCa.	Smith et al. [2012]
Zoledronate	Bisphosphonate	Did not prevent PCa progression or mortality in patients with high risk localized disease.	Denham et al. [2014]

receptor ratio were able to escape from dormancy, suggesting that in addition to the presence of GAS6 there may be an association of the receptor ratios and the ability to enter or exit dormant or proliferative states [Mishra et al., 2012; Taichman et al., 2013].

TGF-β/TGFBR FAMILY MOLECULES

TGF-ß is a growth regulatory factor that is produced by most replicating cells and has a wide range of effects on the cells within the PCa/Bone marrow niche cells. At the site of the primary tumor, TGF-β promotes transition from an epithelial to mesenchymal phenotype and subsequent escape of the tumor cell from the primary site [Xu et al., 2009]. Similar morphogenetic and phenotypic changes occur in bone metastatic sites, particularly in the context of the native osteoblasts and osteoclasts. Osteoblasts have been shown to synthesize and respond to TGF-ß [Robey et al., 1987]. In general, TGF-B signaling tends to have a suppressive effect on the cells of the bone marrow; for example, forced overexpression of TGF-β2 in osteoblasts leads to bone loss [Erlebacher and Derynck, 1996], which indicates the homeostasis between osteoblasts and osteoclasts may be at least partly regulated by TGF- β. Cancer cells have been found to promote metastasis in the bone through secretion of TGF-β and subsequent control of osteoblast/osteoclast differentiation [Tu et al., 2014]. The promotion of osteoclast bone resorption by TGF-ß aids in the bioavailability of cell-survival markers in the bone marrow, which in turn enhances proliferation and growth of the disseminated tumor cells. Other regulatory targets can have an effect on TGF-β signaling in the osteoclasts of the metastatic site. For example, posttranslational regulation of TGF-ß-induced factor 2 by miR-34a has been shown to suppress osteoclastogenesis and the formation of the bone metastatic niche [Krzeszinski et al., 2014].

TGF-β has a wide range of effects on the cells within the PCa/marrow niche cells. Recently, it was reported that GAS6 binding to the TAM receptor Axl on PCa cells induces TGF-β1 and TGF-β2 expression as well as increases expression of TGFβ R2 and TGF-β R3

[Yumoto et al., 2016]. Further, expression of paracrine TGF-β (from local osteoblasts) and autocrine TGF-β (from PCa cells) in turn can induce PCa dormancy [Yumoto et al., 2016]. TGF-β2 signaling initiates a dormant state in DTCs through up-regulation of p27, a ubiquitous cell cycle inhibitor through phosphorylation of p38 and downstream activation of Smad2 and Smad1/5 with a resultant phenotype of TGF-$\beta2^{high}$, $(ERK/p38)^{low}$, $DEC2^{high}$, $p53^{high}$, $p27^{high}$, and $P\text{-}H3^{low}$ [Bragado et al., 2013].

BMP7 is a TGF-ß family member, secreted by stromal cells within the bone marrow. BMP7 signaling through BMPR2 on PCa cells induces senescence in PCa CSCs through activation of p38 MAPK and increasing cell cycle inhibitor p21 [Kobayashi et al., 2011]. Moreover, continued growth of PCa cells following withdrawal of BMP7 both in vitro and in vivo was also observed [Kobayashi et al., 2011].

EGF

Epithelial growth factor (EGF) has a well-characterized role in primary tumor growth and eventual patient outcomes. EGF signaling proceeds through a number of receptors (EGFR, HER2, ErbB2) that have been linked to oncogenesis and metastasis. These receptors are often upregulated in the primary tumor, which can lead to uncontrolled proliferation and ultimately metastatic disease.

EGF is present in the bone marrow and contributes to tumor metastasis and growth in the niche microenvironment, where the EGF signaling cascades are important for the expansion of stem cells [Krampera et al., 2005]. ErbB2 overexpression of metastatic breast cancer cells in the bone marrow has been linked to poor clinical outcome, supporting the role of EGF signaling in promoting growth [Braun et al., 2001]. In the case of PCa, EGF has been shown to promote proliferation [Chackal-Roy et al., 1989]. EGF and similar ligand signaling from metastatic cells have been shown to suppress osteoprotegerin (OPG) expression by osteoblasts, which promotes osteoclast differentiation and subsequent osteolytic events [Lu et al., 2009]. EGF has been shown to significantly alter the effects of bone marrow macrophages on the bone marrow metastatic niche.

Macrophages have been demonstrated to support PCa growth in bone [Soki et al., 2015], and milk fat globule-EGF factor 8 has been demonstrated to initiate effrocytosis (the clearance of dead and dying cells) by macrophages which induces the expression of a gene repertoire promoting the tumor-associated macrophages that promote PCa growth [Soki et al., 2014]. EGFR inhibition has also been shown to decrease macrophage promoted invasion in osteosarcoma [Maloney et al., 2016].

IGF

Insulin-like growth factor (IGF) promotes tumor growth through signaling of the AKT pathway through IRS1/PI3K and activation of the RAS/RAF pathway through SHC. IGF promotes osteoblastic niche expansion and HSC cell engraftment [Caselli et al., 2013]. IGF has been shown to select for metastatic clones that have a predisposition to colonize and form recurrent tumors in the bone marrow [Zhang et al., 2013]. These cells are selected for high Src activity (an enhancer of PI3-K-Akt activation), which confers a predisposition to colonize bone. Interestingly, the loss of the IGF receptor has been associated with advancement of PCa to bone metastasis clinically [Chott et al., 1999]; however, numerous others report IGF levels as an enhanced risk factor for PCa [Chan et al., 1998; Hellawell et al., 2002]. IGF1 signaling from resorbed bone enhances breast cancer metastatic growth [Hiraga et al., 2012]. Osteoclastogenesis is partly regulated by IGF1 through regulation of osteoprotegerin and RANKL [Rubin et al., 2002]. Additionally, IGF signaling regulates osteoblast differentiation as well [Rosen et al., 2004], highlighting the complex role of the this signaling pathway in the native bone environment. The complex dynamics of IGF1 on osteoblasts, osteoclasts, and tumor cells within the bone marrow environment remain a topic of active research.

VEGF

Vascular endothelial growth factor (VEGF) is a mediator of angiogenesis in healthy and cancerous tissues. VEGF mobilizes bone-marrow derived endothelial progenitor cells to promote a number of repair/remodeling functions (angiogenesis [Carmeliet and Jain, 2000], bone resorption [Nakagawa et al., 2000]). Tumor cells likely upregulate VEGF production for this reason, and have been shown to exploit this pathway as both a mechanism for establishing a blood supply at the primary site as well as creating a permissive environment for metastasis. $VEGFR1^{+}$ bone marrow progenitor cells have been implicated in the establishing the premetastatic niche in cancers [Kaplan et al., 2005]. While not marrow specific these findings indicate the role of VEGF in establishing a permissive environment to disseminated tumor cells and the formation of a secondary tumor.

IL-6 AND RANK/RANKL

Interleukin 6 (IL-6) is an interleukin that binds to the IL-6R and activate three major signaling pathways: the Janus-tyrosine family kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, the ERK1/2 and MAPK pathway and the PI3-K pathway. Through, these pathways, IL-6 regulates apoptosis/cell survival, and proliferation [Heinrich et al., 2003]. While IL-6 has been implicated in many stages of PCa progression and metastasis, it appears to play a key role in bone metastases specifically. IL-6 secreted from PCa cells can mediate osteoblastic differentiation and enhance osteoclastogenesis, thus inducing bone turnover and a key event in establishment of osteoblastic bone metastases [Bellido et al., 1997; Lu et al., 2004; Taichman et al., 2007]. In return, osteoblastic production of IL-6 stimulates PCa cell proliferation, initiating a "vicious cycle" whereby PCa cells stimulate osteoblastic activity, which in turn stimulates tumor growth in a paracrine fashion [Lu et al., 2004; Mundy, 1997; Nguyen et al., 2014]. Analysis of human PCa soft tissue and bone metastatic samples indicates that IL-6 is more highly expressed in the bone metastases compared to soft-tissue counterparts [Morrissey et al., 2010]. Thus, IL-6 remains a key signaling mediator in the growth of PCa metastases through action on both PCa cells as well as the bone microenvironment cells.

Receptor activator of nuclear factor kappa-B ligand (RANKL), expressed by osteoblasts and other cells within the bone micro-environement, is one of the primary factors leading to the activation of osteoclastogenesis and accelerated bone resorption. Proposed as a "vicious cycle," osteoclastogenesis is necessary to create space for the tumor, but also releases PCa growth stimulating factors

embedded in the demineralizing matrix [Mundy, 1997]. More recently, the relationship of IL-6 and RANKL has been explored. RANKL, released from local osteoblasts, can stimulate the expression of IL-6 in PCa cells and also increase RANK expression, increasing PCa sensitivity to RANKL [Zheng et al., 2014]. Conversely, in a murine model, inhibition of IL-6 signaling with tocilizumab, inhibits skeletal tumor growth and decreased RANKL serum levels, as well as RANK expression in PC3-derived bone tumors [Zheng et al., 2014].

EFFECTS OF ADHESION MOLECULES/ECM COMPONENTS WITHIN THE BONE MARROW NICHE ON PCA CELLS

INTEGRINS/RGDs

Integrins are transmembrane adhesion molecules that are comprised of noncovalently linked α and β subunits, whereby each heterodimer binds to different ECM proteins, such as collagen, laminin, vitronectin, and fibronectin. In the bone the most abundant protein is type I collagen. Integrin binding is dependent on divalent cations and specific binding RGD sequences such as Arg-Gly-Asp or Asp-Gly-Glu-Ala in the ECM protein [Felding-Habermann, 2003]. The β unit of the integrin binding pair can initiate a signal transduction pathway that is facilitated with intracellular molecules such as focal adhesion kinase (FAK), which in turn lead to ligand-mediated activation of ras/mitogen activated protein kinase (Ras/MAPK) and phosphatidylinositol 3-kinase (PI-3kinase) signal transduction pathways [Felding-Habermann, 2003]. In normal prostate, FAK expression is low or non-detectable; however, in metastatic PCa it is significantly elevated compared to both healthy, benign PCa, or low-grade adenocarcinoma [Stanzione et al., 2001]. FAK association with Src is critical for prostate cell migration; however, FAK association with PI3K activation affects proliferation, survival, differentiation, and migration through the intermediator, serine/threonine protein kinase B (AKT) [Fornaro et al., 2001]. In addition, FAK can activate the Ras proteins, a large family of GTPases that function to stimulate many signaling cascades, such as ERK, that affect cell cycle and proliferation [Fornaro et al., 2001]. In fact, these pathways are monitored continuously when evaluating PCa cellular dormancy in the bone marrow through evaluation of the p-ERK1/2 to p-p38 ratio [Sosa et al., 2015].

Integrin αvβ3 is another integrin involved in PCa cellular binding to fibronectin, vitronectin, thrombospondin (TSP), among other ECM matrix proteins. Interestingly, osteoblast secreted CXCL12 binds to CXCR4 on the resident PCa cells, upregulating αvβ3 and CD164, both adhesion molecules that bind PCa cells to osteoblasts and ECM components [Shiozawa et al., 2010]. Further, ANXA2r on PCa cells binds to osteoblastic ligand ANXA2, resulting in transcription of TAM receptor, Axl, decreasing proliferative cell cycle signaling and subsequent quiescent phenotypes [Shiozawa et al., 2010].

Integrin pairs (α1β1, α2β1, and α6β1) for collagen appear to be important mediators in PCa metastasis to bone. Interestingly, it was reported that bone metastatic PCa cells bound collagen I, whereas cells that only formed visceral metastases failed to bind collagen [Hall et al., 2006]. Since Ras mutations are uncommon in PCa, it was previously reported that chronic stimulation of Ras/MAPK pathway is most likely stimulated through alterations in upsteam regulars such as integrins, growth factors, and growth factor receptors during PCa progression [Fornaro et al., 2001]. One group reported that PCa-Collagen I attachment was mediated by α2β1 to initiate motility programs through Rho-family of small GTPases, RhoC [Hall et al., 2006].

TSP1

Thrombospondin 1(TSP1) is a potent angiogenesis inhibitor and down-regulation TSP1 has been suggested to alter tumor growth. In wound healing, TSP1 delays neoangiogenesis via activation of the caspase death pathway in endothelial cells [Nör et al., 2000]. In tumor progression, TSP1 is upregulated by p53 and down-regulated by oncogenes, Myc and Ras [Ren et al., 2006]. Further, TSP1 activates TGF-β1, suggesting a critical role in the regulation of tumor progression [Ren et al., 2006; Venkatraman et al., 2012]. Interestingly, androgen is reported to increase VEGF-A and decrease TSP1 expression in PCa, suggesting that androgen may play an important role in the angiogenic process of cancer [Miyata et al., 2015]. Together the pro-angiogenic factors, such as VEGF, and anti-angiogenic factors, such as thrombospondin 1 (TSP1) remain important mediators of the angiogenesis balance, ECM remodeling, and cellular recruitment. TSP1 is an ECM glycoprotein is produced by many different cell types and has important roles in cell attachment, angiogenesis, inflammation, and fibrosis.

THERAPEUTIC IMPLICATIONS

Therapeutics designed to target the abnormal microenvironment induced by PCa have been proposed. As discussed above, the vicious cycle model proposes that PCa cells stimulate increased bone remodeling, which subsequently liberates IL-6, TGF-β, and other factors that further increase proliferation of tumor cells. Thus, the use of drugs which inhibit osteoclast function was proposed to halt the abnormal osteoclast activation component of the vicious cycle and thereby slow PCa progression [Vignani et al., 2016]. Both the bisphosphonate, zoledronate, and the anti-RANKL antibody, denosumab, are proven to be effective in prevention of skeletal events such as pathologic fracture, spinal cord compression, and bone pain [Fizazi et al., 2011]. The majority of positive studies have been in patients with bone metastases from castration resistant PCa (i.e., progressing despite medical or surgical castration). Either denosumab or zoledronate is standard of care in this patient population [Network, 2016]. Furthermore, denosumab is effective and indicated for prevention of osteoporotic fracture in all prostate cancer patients treated with androgen deprivation therapy, regardless of disease stage or castration resistant status [Smith et al., 2009].

It is unclear; however, if osteoclast targeted drugs have the desired effects on cancer cells in PCa patients. Despite, their useful benefits in prevention of skeletal complications, denosumab and zoldronate have not shown improvements in overall survival in any PCa patient population—as would be expected if the drugs were targeting cancer cells [Vignani et al., 2016]. Additionally, clinical data on osteoclast targeted drugs has not supported their use to prevent formation of bone metastases. Specifically,

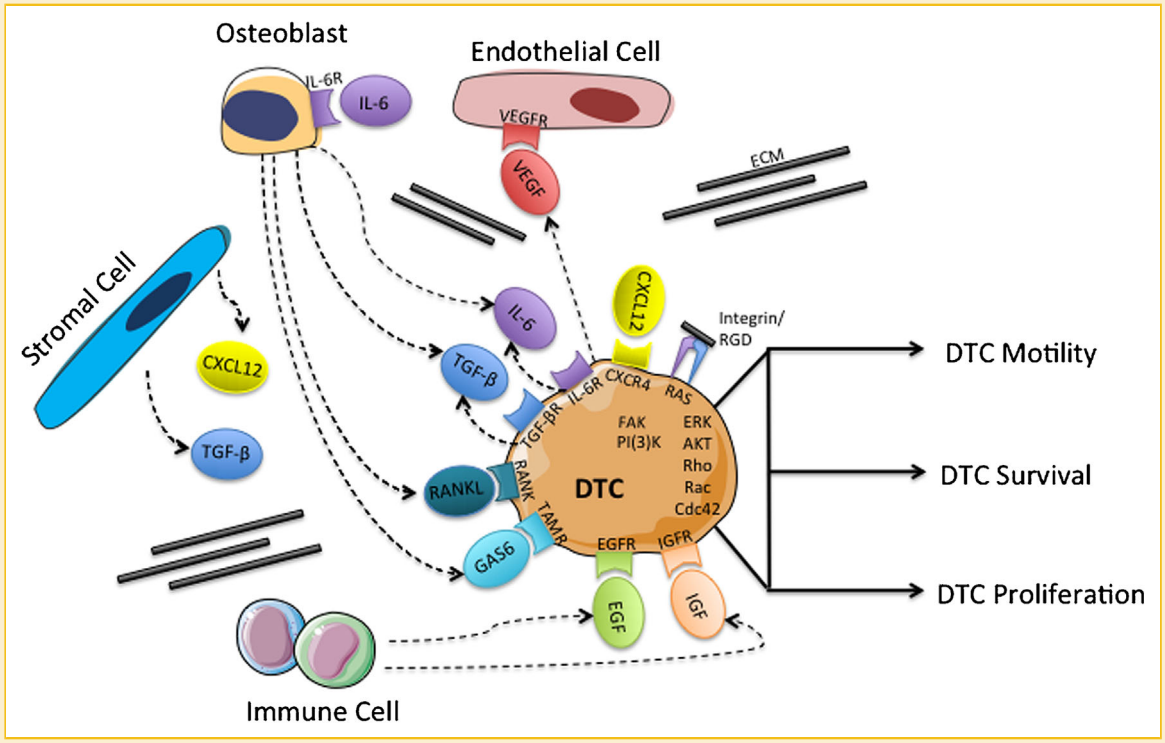

Fig. 1. Summary of the molecular interactions of PCa cells in the bone marrow microenvironment. IL-6, Interleukin 6; VEGF, Vascular endothelial growth factor; CXCL12, SDF-1 = Stromal derived factor 1; CXCR4 CXC chemokine receptor 4; TGF-β, Transforming growth factor β; RANK, Receptor activator of nuclear factor kappa-B; TAMR, TYRO3, AXL, MERTK receptor; GAS6, Growth arrest specific 6; EGF, Epithelial growth factor; IGF, Insulin growth factor; ECM, Extracellular matrix.

zoledronate did not increase the time to first skeletal related event in men with castration sensitive metastatic prostate cancer [Smith et al., 2014]. Also, while denosumab did increase metastasis free survival in men with non-metastatic castration resistant PCa (PSA rising after castration, but no gross metastases on imaging), it did not increase overall survival and is not FDA approved in this setting [Smith et al., 2012]. Similarly, in the adjuvant setting, zoledronate did not prevent PCa progression or mortality in patients with high risk localized disease [Denham et al., 2014]. Therefore, although they have prevented much morbidity from bone complications in PCa patients, osteoclast targeted drugs have not yielded all the desired beneficial effects in clinical trials. However, because of the research avenues discussed above, we are confident that targeting the bone microenvironment will continue to yield effective therapeutics in the future.

SUMMARY

There are many was to address the problems arising from metastatic disease, one of which is through targeting the microenvironment in which these cells colonize, survive, and proliferate. There are many molecular signals that direct the homing of PCa cells to the bone marrow and regulate DTC proliferative activity, as summarized in Figure 1. Identification of these players has been increasingly a point of interest to the research setting; however, translation of these findings to the clinic remains limited. Future efforts need to be made to identify how these molecular players distinctly regulate PCa cell survival, dormancy, and re-activation to determine more effective clinically relevant therapeutic targets that can not only increase the life-span of these patients but also improve the quality of a cancer patient's life.

ACKNOWLEDGMENTS

Select vector images were provided by Servier Medical Art. This work is directly supported by the University of Michigan MCubed Project, the National Cancer Institute (CA093900, CA163124), the Department of Defense (W81XW-15-1-0413 and W81XWH-14-1-0403), and the Prostate Cancer Foundation. F. Cackowski receives support from the NIH/NCI T32 training grant 5T32CA009357-32 to The University of Michigan Division of Hematology and Oncology. R.S. Taichman receives support as the Major McKinley Ash Collegiate Professor.

REFERENCES

Araujo A, Cook LM, Lynch CC, Basanta D. 2014. An integrated computational model of the bone microenvironment in bone-metastatic prostate cancer. Cancer Res 74:2391–2401.

Bellido T, Borba VZ, Roberson P, Manolagas SC. 1997. Activation of the Janus Kinase/STAT (Signal Transducer and Activator of Transcription) signal transduction pathway by interleukin-6-type cytokines promotes osteoblast differentiation 1. Endocrinology 138:3666–3676.

Bragado P, Estrada Y, Parikh F, Krause S, Capobianco C, Farina HG, Schewe DM, Aguirre-Ghiso JA. 2013. TGF-β2 dictates disseminated tumour cell fate in target organs through TGF-β-RIII and p38α/β signalling. Nat Cell Biol 15:1351–1361.

Braun S, Schlimok G, Heumos I, Schaller G, Riethdorf L, Riethmüller G, Pantel K. 2001. ErbB2 overexpression on occult metastatic cells in bone marrow predicts poor clinical outcome of stage I-III breast cancer patients. Cancer Res 61:1890–1895.

Cackowski F, Eber MR, Rhee J, Decker A, Yumoto K, Berry JE, Lee E, Shiozawa Y, Jung Y, Aguirre-Ghiso JA. 2016. Mer tyrosine kinase regulates disseminated prostate cancer cellular dormancy. J Cell Biochem. doi: 10.1002/jcb.25768

Carmeliet P, Jain RK. 2000. Angiogenesis in cancer and other diseases. Nature 407:249–257.

Caselli A, Olson TS, Otsuru S, Chen X, Hofmann TJ, Nah HD, Grisendi G, Paolucci P, Dominici M, Horwitz EM. 2013. IGF-1-mediated osteoblastic niche expansion enhances long-term hematopoietic stem cell engraftment after murine bone marrow transplantation. Stem Cells 31:2193–2204.

Chackal-Roy M, Niemeyer C, Moore M, Zetter B. 1989. Stimulation of human prostatic carcinoma cell growth by factors present in human bone marrow. J Clin Invest 84:43.

Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M. 1998. Plasma insulin-like growth factor-I and prostate cancer risk: A prospective study. Science 279:563–566.

Chott A, Sun Z, Morganstern D, Pan J, Li T, Susani M, Mosberger I, Upton MP, Bubley GJ, Balk SP. 1999. Tyrosine kinases expressed in vivo by human prostate cancer bone marrow metastases and loss of the type 1 insulin-like growth factor receptor. Am J Pathol 155:1271–1279.

Denham JW, Joseph D, Lamb DS, Spry NA, Duchesne G, Matthews J, Atkinson C, Tai KH, Christie D, Kenny L, Turner S, Gogna NK, Diamond T, Delahunt B, Oldmeadow C, Attia J, Steigler A. 2014. Short-term androgen suppression and radiotherapy versus intermediate-term androgen suppression and radiotherapy, with or without zoledronic acid, in men with locally advanced prostate cancer (TROG 03.04 RADAR): An open-label, randomised, phase 3 factorial trial. Lancet Oncol 15:1076–1089.

Dormady SP, Zhang X-M, Basch RS. 2000. Hematopoietic progenitor cells grow on 3T3 fibroblast monolayers that overexpress growth arrest-specific gene-6 (GAS6). Proc Natl Acad Sci USA 97:12260–12265.

Erlebacher A, Derynck R. 1996. Increased expression of TGF-beta 2 in osteoblasts results in an osteoporosis-like phenotype. J Cell Biol 132: 195–210.

Felding-Habermann B. 2003. Integrin adhesion receptors in tumor metastasis. Clin Exp Metastasis 20:203–213.

Fizazi K, Carducci M, Smith M, Damiao R, Brown J, Karsh L, Milecki P, Shore N, Rader M, Wang H, Jiang Q, Tadros S, Dansey R, Goessl C. 2011. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: A randomised, double-blind study. Lancet 377:813–822.

Fornaro M, Manes T, Languino LR. 2001. Integrins and prostate cancer metastases. Cancer Metastasis Rev 20:321–331.

Gundem G, Van Loo P, Kremeyer B, Alexandrov LB, Tubio JM, Papaemmanuil E, Brewer DS, Kallio HM, Högnäs G, Annala M. 2015. The evolutionary history of lethal metastatic prostate cancer. Nature 520:353–357.

Hall CL, Dai J, van Golen KL, Keller ET, Long MW. 2006. Type I collagen receptor (α2β1) signaling promotes the growth of human prostate cancer cells within the bone. Cancer Res 66:8648–8654.

Harryman WL, Hinton JP, Rubenstein CP, Singh P, Nagle RB, Parker SJ, Knudsen BS, Cress AE. 2016. The cohesive metastasis phenotype in human prostate cancer. Biochim Biophys Acta 1886(2):221–231.

Heinrich PC, Behrmann I, Serge H, Hermanns HM, Müller-Newen G, Schaper F. 2003. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374:1–20.

Hellawell GO, Turner GD, Davies DR, Poulsom R, Brewster SF, Macaulay VM. 2002. Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. Cancer Res 62:2942–2950.

Hiraga T, Myoui A, Hashimoto N, Sasaki A, Hata K, Morita Y, Yoshikawa H, Rosen CJ, Mundy GR, Yoneda T. 2012. Bone-derived IGF mediates crosstalk between bone and breast cancer cells in bony metastases. Cancer Res 72:4238–4249.

Jung Y, Decker AM, Wang J, Lee E, Kana LA, Yumoto K, Cackowski FC, Rhee J, Carmeliet P, Buttitta L. 2016. Endogenous GAS6 and Mer receptor signaling regulate prostate cancer stem cells in bone marrow. Oncotarget 7(18): 25698–25771.

Jung Y, Wang J, Song J, Shiozawa Y, Wang J, Havens A, Wang Z, Sun Y-X, Emerson SG, Krebsbach PH. 2007. Annexin II expressed by osteoblasts and endothelial cells regulates stem cell adhesion, homing, and engraftment following transplantation. Blood 110:82–90.

Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA. 2005. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 438:820–827.

Kobayashi A, Okuda H, Xing F, Pandey PR, Watabe M, Hirota S, Pai SK, Liu W, Fukuda K, Chambers C. 2011. Bone morphogenetic protein 7 in dormancy and metastasis of prostate cancer stem-like cells in bone. J Exp Med 208: 2641–2655.

Krampera M, Pasini A, Rigo A, Scupoli MT, Tecchio C, Malpeli G, Scarpa A, Dazzi F, Pizzolo G, Vinante F. 2005. HB-EGF/HER-1 signaling in bone marrow mesenchymal stem cells: Inducing cell expansion and reversibly preventing multilineage differentiation. Blood 106:59–66.

Krzeszinski JY, Wei W, Huynh H, Jin Z, Wang X, Chang T-C, Xie X-J, He L, Mangala LS, Lopez-Berestein G. 2014. MiR-34a blocks osteoporosis and bone metastasis by inhibiting osteoclastogenesis and Tgif2. Nature 512: 431–435.

Lee E, Decker AM, Cackowski FC, Kana LA, Yumoto K, Jung Y, Wang J, Buttitta L, Morgan TM, Taichman RS. 2016. Growth Arrest-Specific 6 (GAS6) promotes prostate cancer survival by G1 Arrest/S phase delay and inhibition of apoptotic pathway during chemotherapy in bone marrow. J Cell Biochem 117(12):2815–2824.

Lu X, Wang Q, Hu G, Van Poznak C, Fleisher M, Reiss M, Massagué J, Kang Y. 2009. ADAMTS1 and MMP1 proteolytically engage EGF-like ligands in an osteolytic signaling cascade for bone metastasis. Genes Dev 23: 1882–1894.

Lu Y, Zhang J, Dai J, Dehne LA, Mizokami A, Yao Z, Keller ET. 2004. Osteoblasts induce prostate cancer proliferation and PSA expression through interleukin-6-mediated activation of the androgen receptor. Clin Exp Metastasis 21:399–408.

Maloney CW, Symons M, Steinberg BM, Soffer SZ. 2016. Epidermal growth factor receptor inhibition decreases macrophage-promoted invasion in osteosarcoma. J Am Coll Surg 223:S140–S141.

Mishra A, Wang J, Shiozawa Y, McGee S, Kim J, Jung Y, Joseph J, Berry JE, Havens A, Pienta KJ. 2012. Hypoxia stabilizes GAS6/Axl signaling in metastatic prostate cancer. Mol Cancer Res 10:703–712.

Miyata Y, Mitsunari K, Asai A, Takehara K, Mochizuki Y, Sakai H. 2015. Pathological significance and prognostic role of microvessel density, evaluated using CD31, CD34, and CD105 in prostate cancer patients after radical prostatectomy with neoadjuvant therapy. Prostate 75:84–91.

Morrissey C, Lai JS, Brown LG, Wang YC, Roudier MP, Coleman IM, Gulati R, Vakar-Lopez F, True LD, Corey E. 2010. The expression of osteoclastogenesis-associated factors and osteoblast response to osteolytic prostate cancer cells. Prostate 70:412–424.

Mundy GR. 1997. Mechanisms of bone metastasis. Cancer 80:1546–1556.

Nagle RB, Cress AE. 2011. Metastasis update: Human prostate carcinoma invasion via tubulogenesis. Prostate Cancer 2011:1–10.

Nakagawa M, Kaneda T, Arakawa T, Morita S, Sato T, Yomada T, Hanada K, Kumegawa M, Hakeda Y. 2000. Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts. FEBS Lett 473:161–164.

Nguyen DP, Li J, Tewari AK. 2014. Inflammation and prostate cancer: The role of interleukin 6 (IL-6). BJU Int 113:986–992.

Network NCC. 2016. NCCN clinical practice gudielines in oncology: Prostate cancer version 3.2016editor^ editors.

Nör J, Mitra RS, Sutorik MM, Mooney DJ, Castle VP, Polverini PJ. 2000. Thrombospondin-1 induces endothelial cell apoptosis and inhibits angiogenesis by activating the caspase death pathway. J Vasc Res 37:209–218.

Pawar SC, Demetriou MC, Nagle RB, Bowden GT, Cress AE. 2007. Integrin α6 cleavage: A novel modification to modulate cell migration. Exp Cell Res 313:1080–1089.

Ports MO, Nagle RB, Pond GD, Cress AE. 2009. Extracellular engagement of α6 integrin inhibited urokinase-type plasminogen activator-mediated cleavage and delayed human prostate bone metastasis. Cancer Res 69: 5007–5014.

Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC. 1999. Natural history of progression after PSA elevation following radical prostatectomy. JAMA 281:1591–1597.

Ren B, Yee KO, Lawler J, Khosravi-Far R. 2006. Regulation of tumor angiogenesis by thrombospondin-1. Biochim Biophys Acta 1765:178–188.

Robey PG, Young MF, Flanders KC, Roche NS, Kondaiah P, Reddi AH, Termine JD, Sporn MB, Roberts AB. 1987. Osteoblasts synthesize and respond to transforming growth factor-type beta (TGF-beta) in vitro. J Cell Biol 105:457–463.

Rosen CJ, Ackert-Bicknell CL, Adamo ML, Shultz KL, Rubin J, Donahue LR, Horton LG, Delahunty KM, Beamer WG, Sipos J. 2004. Congenic mice with low serum IGF-I have increased body fat, reduced bone mineral density, and an altered osteoblast differentiation program. Bone 35:1046–1058.

Rubin J, Ackert-Bicknell C, Zhu L, Fan X, Murphy T, Nanes M, Marcus R, Holloway L, Beamer W, Rosen C. 2002. IGF-I regulates osteoprotegerin (OPG) and receptor activator of nuclear factor-κB ligand in vitro and OPG in vivo. J Clin Endocrinol Metab 87:4273–4279.

Schmelz M, Cress AE, Scott KM, Burger F, Cui H, Sallam K, McDaniel KM, Dalkin BL, Nagle RB. 2002. Different phenotypes in human prostate cancer: α6 or α3 integrin in cell-extracellular adhesion sites. Neoplasia 4:243–254.

Shiozawa Y, Havens AM, Jung Y, Ziegler AM, Pedersen EA, Wang J, Wang J, Lu G, Roodman GD, Loberg RD. 2008. Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. J Cell Biochem 105:370–380.

Shiozawa Y, Pedersen EA, Havens AM, Jung Y, Mishra A, Joseph J, Kim JK, Patel LR, Ying C, Ziegler AM. 2011. Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. J Clin Invest 121:1298–1312.

Shiozawa Y, Pedersen EA, Patel LR, Ziegler AM, Havens AM, Jung Y, Wang J, Zalucha S, Loberg RD, Pienta KJ. 2010. GAS6/AXL axis regulates prostate cancer invasion, proliferation, and survival in the bone marrow niche. Neoplasia 12:116–127.

Smith MR, Egerdie B, Hernandez Toriz N, Feldman R, Tammela TL, Saad F, Heracek J, Szwedowski M, Ke C, Kupic A, Leder BZ, Goessl C, Denosumab HPCSG. 2009. Denosumab in men receiving androgen-deprivation therapy for prostate cancer. N Engl J Med 361:745–755.

Smith MR, Halabi S, Ryan CJ, Hussain A, Vogelzang N, Stadler W, Hauke RJ, Monk JP, Saylor P, Bhoopalam N, Saad F, Sanford B, Kelly WK, Morris M, Small EJ. 2014. Randomized controlled trial of early zoledronic acid in men with castration-sensitive prostate cancer and bone metastases: Results of CALGB 90202 (alliance). J Clin Oncol 32:1143–1150.

Smith MR, Saad F, Coleman R, Shore N, Fizazi K, Tombal B, Miller K, Sieber P, Karsh L, Damiao R, Tammela TL, Egerdie B, Van Poppel H, Chin J, Morote J, Gomez-Veiga F, Borkowski T, Ye Z, Kupic A, Dansey R, Goessl C. 2012. Denosumab and bone-metastasis-free survival in men with castration-resistant prostate cancer: Results of a phase 3, randomised, placebo-controlled trial. Lancet 379:39–46.

Soki FN, Cho SW, Kim YW, Jones JD, Park SI, Koh AJ, Entezami P, Daignault-Newton S, Pienta KJ, Roca H. 2015. Bone marrow macrophages support prostate cancer growth in bone. Oncotarget 6:35782.

Soki FN, Koh AJ, Jones JD, Kim YW, Dai J, Keller ET, Pienta KJ, Atabai K, Roca H, McCauley LK. 2014. Polarization of prostate cancer-associated macrophages is induced by milk fat globule-EGF factor 8 (MFG-E8)-mediated efferocytosis. J Biol Chem 289:24560–24572.

Sosa MS, Parikh F, Maia AG, Estrada Y, Bosch A, Bragado P, Ekpin E, George A, Zheng Y, Lam H-M. 2015. NR2F1 controls tumour cell dormancy via SOX9-and RARβ-driven quiescence programmes. Nat Commun 6:6170.

Sroka IC, Anderson TA, McDaniel KM, Nagle RB, Gretzer MB, Cress AE. 2010. The laminin binding integrin α6β1 in prostate cancer perineural invasion. J Cell Physiol 224:283–288.

Stanzione R, Picascia A, Chieffi P, Imbimbo C, Palmieri A, Mirone V, Staibano S, Franco R, De Rosa G, Schlessinger J. 2001. Variations of proline-rich kinase Pyk2 expression correlate with prostate cancer progression. Lab Invest 81:51–59.

Sun YX, Fang M, Wang J, Cooper CR, Pienta KJ, Taichman RS. 2007. Expression and activation of αvβ3 integrins by SDF-1/CXC12 increases the aggressiveness of prostate cancer cells. Prostate 67:61–73.

Sun YX, Schneider A, Jung Y, Wang J, Dai J, Wang J, Cook K, Osman NI, Koh-Paige AJ, Shim H. 2005. Skeletal localization and neutralization of the SDF-1 (CXCL12)/CXCR4 axis blocks prostate cancer metastasis and growth in osseous sites in vivo. J Bone Miner Res 20:318–329.

Sun YX, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA, Pienta KJ, Taichman RS. 2003. Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo. J Cell Biochem 89:462–473.

Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. 2002. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res 62:1832–1837.

Taichman RS, Loberg RD, Mehra R, Pienta KJ. 2007. The evolving biology and treatment of prostate cancer. J Clin Invest 117:2351–2361.

Taichman RS, Patel LR, Bedenis R, Wang J, Weidner S, Schumann T, Yumoto K, Berry JE, Shiozawa Y, Pienta KJ. 2013. GAS6 receptor status is associated with dormancy and bone metastatic tumor formation. PLoS ONE 8:e61873.

Teicher BA, Fricker SP. 2010. CXCL12 (SDF-1)/CXCR4 pathway in cancer. Clin Cancer Res 16:2927–2931.

Tu B, Peng Z-X, Fan Q-M, Du L, Yan W, Tang T-T. 2014. Osteosarcoma cells promote the production of pro-tumor cytokines in mesenchymal stem cells by inhibiting their osteogenic differentiation through the TGF-β/Smad2/3 pathway. Exp Cell Res 320:164–173.

Van der Toom EE, Verdone JE, Pienta KJ. 2016. Disseminated tumor cells and dormancy in prostate cancer metastasis. Curr Opin Biotechnol 40:9–15.

Venkatraman L, Chia S-M, Narmada BC, White JK, Bhowmick SS, Dewey CF, So PT, Tucker-Kellogg L, Yu H. 2012. Plasmin triggers a switch-like decrease in thrombospondin-dependent activation of TGF-β1. Biophys J 103: 1060–1068.

Vignani F, Bertaglia V, Buttigliero C, Tucci M, Scagliotti GV, Di Maio M. 2016. Skeletal metastases and impact of anticancer and bone-targeted agents in patients with castration-resistant prostate cancer. Cancer Treat Rev 44:61–73.

Weiner A, Matulewicz R, Eggener S, Schaeffer E. 2016. Increasing incidence of metastatic prostate cancer in the United States (2004–2013). Prostate Cancer Prostatic Dis 19:395–397.

Xu J, Lamouille S, Derynck R. 2009. TGF-β-induced epithelial to mesenchymal transition. Cell Res 19:156–172.

Yumoto K, Eber M, Wang J, Cackowski F, Decker A, Lee E, Nobre A, Aguirre-Ghiso J, Jung Y, Taichman R. 2016. Axl is required for TGF-β2-induced dormancy of prostate cancer cells in the bone marrow. Sci Rep 6:36520.

Zhang XH-F, Jin X, Malladi S, Zou Y, Wen YH, Brogi E, Smid M, Foekens JA, Massagué J. 2013. Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. Cell 154: 1060–1073.

Zheng Y, Basel D, Chow S-O, Fong-Yee C, Kim S, Buttgereit F, Dunstan CR, Zhou H, Seibel MJ. 2014. Targeting IL-6 and RANKL signaling inhibits prostate cancer growth in bone. Clin Exp Metastasis 31: 921–933.