

## **Prospects**

### **BIOCHEMICAL CHANGES IN THE NICHE FOLLOWING TUMOR CELL INVASION<sup>†</sup>**

**A.M. Decker<sup>1</sup>, F.C. Cackowski<sup>1,2</sup>, Y. Jung<sup>1</sup>, R.S. Taichman<sup>1</sup>**

<sup>1</sup>Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI, USA

<sup>2</sup>Department of Internal Medicine, Division of Hematology and Oncology, University of Michigan School of Medicine, Ann Arbor, MI, USA

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Correspondence:

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  
MI 48109-1078  
Tel.: +1-734-763-8216  
E-mail: rtaich@umich.edu

Author Manuscript

## 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.

## INTRODUCTION:

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 [Gudem 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, 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 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  $\alpha 6\beta 1$  and  $\alpha 3\beta 1$  [Schmelz et al., 2002]. Further, post-translational modification of  $\alpha 6\beta 1$  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 [Shiozawa et al., 2011; Sun et al., 2005; Sun et al., 2003; Taichman et al., 2002]. In addition to homing, CXCL12 can transiently regulate the expression of the  $\alpha v \beta 3$  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.

## **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/Brk/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 [REF]. Like HSCs, GAS6 inhibits PCa proliferation and appears to participate in the induction of tumor cell dormancy, such that they 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<sub>1</sub> 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 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- $\beta$ /TGFBR Family Molecules:* TGF- $\beta$  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- $\beta$  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- $\beta$ [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- $\beta$  2 in osteoblasts leads to bone loss [Erlebacher and Derynck, 1996], which indicated the homeostasis between osteoblasts and osteoclasts may be at least partly regulated by TGF-  $\beta$ . Cancer cells have been found to promote metastasis in the bone through secretion of TGF- $\beta$  and subsequent control of osteoblast/osteoclast differentiation[Tu et al., 2014]. The promotion of osteoclast bone resorption by TGF- $\beta$  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- $\beta$  signaling in the osteoclasts of the metastatic site. For example, posttranslational regulation of TGF- $\beta$ -induced factor 2 by miR-34a has been shown to suppress osteoclastogenesis and the formation of the bone metastatic niche [Krzyszinski et al., 2014].

TGF- $\beta$  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- $\beta$ 1 and TGF- $\beta$ 2 expression as well as increases expression of TGF $\beta$  R2 and TGF- $\beta$  R3[Yumoto et al., 2016]. Further, expression of paracrine TGF- $\beta$  (from local osteoblasts) and autocrine TGF- $\beta$  (from PCa cells) in turn can induce PCa dormancy[Yumoto et al., 2016]. TGF- $\beta$  2 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 Smad 1/5 with a resultant phenotype of TGF- $\beta$ 2<sup>high</sup>, (ERK/p38)<sup>low</sup>, DEC2<sup>high</sup>, p53<sup>high</sup>, p27<sup>high</sup> and P-H3<sup>low</sup>[Bragado et al., 2013].

BMP7 is a TGF- $\beta$  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 efferocytosis (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 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<sup>+</sup> 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 microenvironment, 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  $\alpha$  and  $\beta$  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 sequences such as Arg-Gly-Asp or Asp-Gly-Glu-Ala in the ECM protein[Felding-Habermann, 2003]. The  $\beta$  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 intermediary, 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  $\alpha v \beta 3$  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  $\alpha v \beta 3$  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 ( $\alpha 1 \beta 1$ ,  $\alpha 2 \beta 1$ , and  $\alpha 6 \beta 1$ ) for collagen appear to be important mediators in PCa metastasis to bone. Interestingly, it was recently 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 upstream regulators 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  $\alpha 2 \beta 1$  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- $\beta$ 1, 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- $\beta$  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 zoledronate 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, 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 ways 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.

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**Figure 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- $\beta$  = Transforming growth factor  $\beta$ ; 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.

**Table 1: Description of important biochemical mediators of PCa entry and survival in the bone marrow.**

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**Table 1:** Description of important biochemical mediators of PCa entry and survival in the bone marrow.

Biochemical Components	Description	Effect on PCa Bone Metastasis	Citation
<b>Growth Factors</b>			
CXCL12	Homing molecule secreted by osteoblasts	Induces HSC mobilization from the HSC niche and recruits PCa cells.	[Shiozawa et al., 2011; Sun et al., 2005; Sun et al., 2003; Taichman et al., 2002]
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- $\beta$	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.	[Xu et al., 2009] [Robey et al., 1987] [Tu et al., 2014] [Krzyszinski et al., 2014]
BMP7	TGF- $\beta$ family member, secreted by stromal cells in	Induces cellular senescence in PCa CSCs.	[Kobayashi et al., 2011]

	the bone marrow		
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.	[Krampera et al., 2005] [Chackal-Roy et al., 1989] [Lu et al., 2009] [Braun et al., 2001]
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.	[Rosen et al., 2004] [Rubin et al., 2002] [Chan et al., 1998; Hellawell et al., 2002] [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]
<b>Adhesion and ECM Components</b>			
ANXA2	Protein expressed by OBs	ANXA2r located on PCa cells bind directly ANXA2 on osteoblasts to facilitate PCa anchorage within the bone marrow.	[Jung et al., 2007; Shiozawa et al., 2008]
$\alpha V\beta 3$	Binds fibronectin, vitronectin, TSP, and other ECM proteins	Osteoblastic CXCL12 causes upregulation of PCa $\alpha V\beta 3$ promoting PCa adhesion to osteoblasts in the bone marrow.	[Shiozawa et al., 2010]
$\alpha 1\beta 1$ , $\alpha 2\beta 1$ , $\alpha 6\beta 1$	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	Downregulated in progression of PCa to	[Ren et al., 2006; Venkatraman et al.,

	various cell types including	promote angiogenesis in the area.	2012]
<b>Therapeutics</b>			
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]

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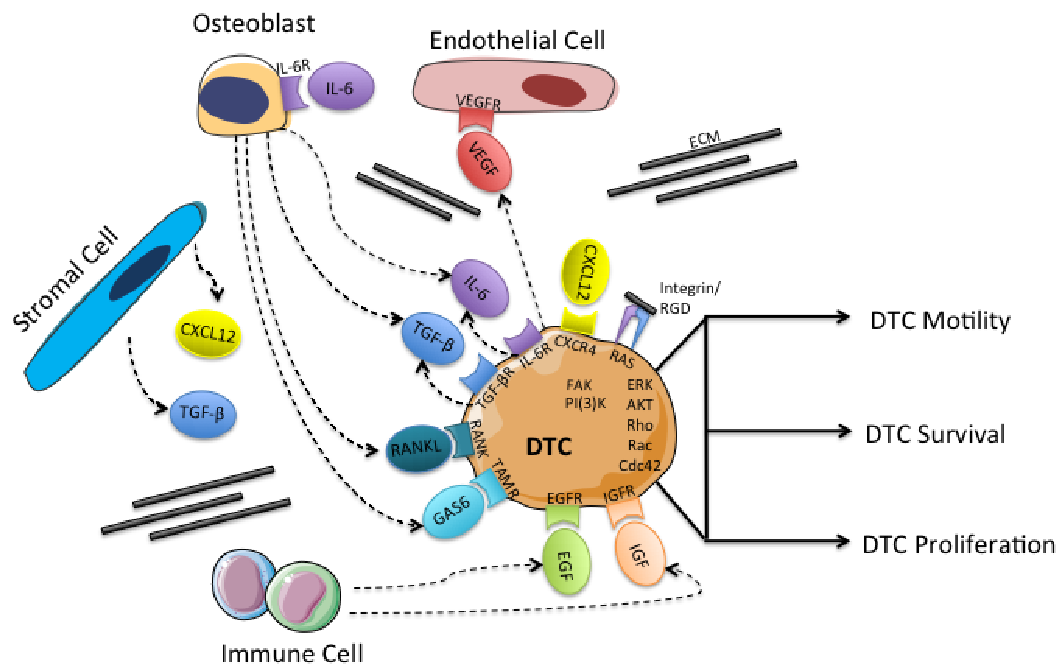


Figure 1 .

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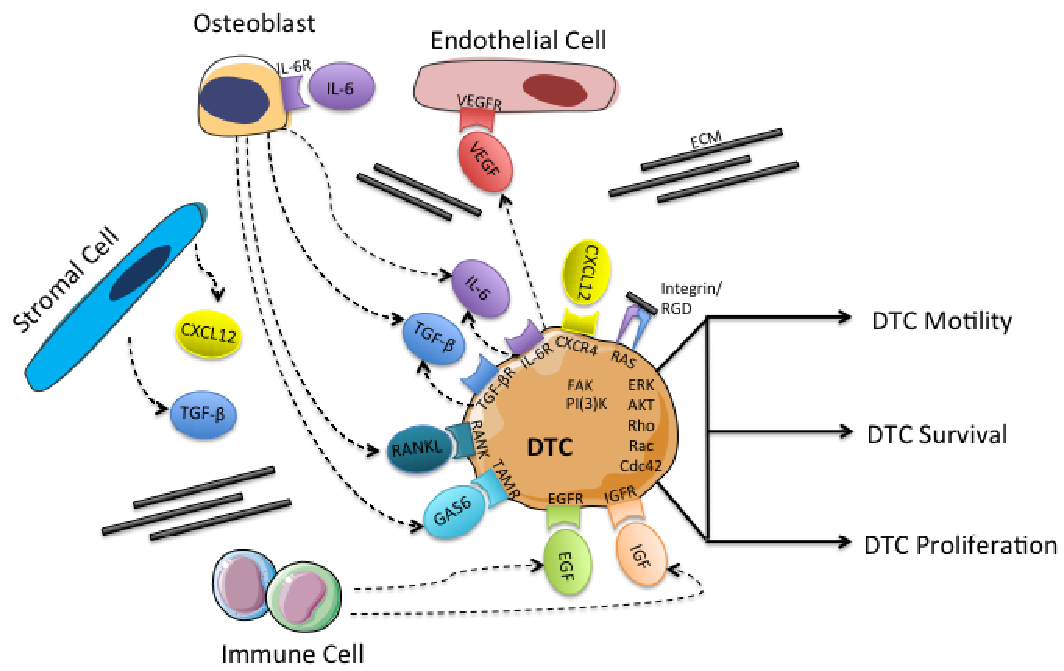


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