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Featured Review Article

Prostate cancer progression and metastasis: potential regulatory pathways for therapeutic targeting

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Abstract: Skeletal metastasis in advanced prostate cancer (PCa) patients remains a significant cause of morbidity and mortality. Research utilizing animal models during the past decade has reached a consensus that PCa progression and distant metastasis can be tackled at the molecular level. Although there are a good number of models that have shown to facilitate the study of PCa initiation and progression at the primary site, models that mimic the distant dissemination of cancer cells, particularly bone metastasis, are scarce. Despite this limitation, the field has gleaned valuable knowledge on the underlying molecular mechanisms and pathways of PCa progression, including local invasion and distant metastasis, and has moved forward in developing the concepts of current therapeutic modalities. The purpose of this review is to put together recent work on pathways that are currently being targeted for therapy, as well as other prospective novel therapeutic targets to be developed in the future against metastatic and potentially lethal PCa in patients.

Keywords: Prostate cancer, progression, metastasis, pathways, therapeutic targeting

Introduction

Prostate cancer is the second leading cause of cancer deaths in men in the United States. The American Cancer Society has projected that 233,000 new cases and 29,480 deaths will occur in the year 2014. Mostly, men over the age of 50 are afflicted by the disease and more than 70% of the men diagnosed with prostate cancer are over 65. This high rate of mortality is primarily due to metastasis of the primary tumor. The 5 year survival rate for men diagnosed while the disease is localized is nearly 100% while only 28% of the men diagnosed with metastatic prostate cancer survive beyond 5 years. Early detection and treatment before the tumor metastasizes is critical for improving patient survival. In the past decade, the problem of progression and metastasis in prostate cancer has been increasingly studied at the molecular level. However, a major impediment in the field has been a paucity of animal models that recapitulate PCa metastasis. While there are a good number of animal models that facilitate the study of PCa initiation and progression, models that mimic the widespread clinical phenomenon of bone metastasis in advanced PCa patients are scarce. Owing to this limitation, the PCa field still lacks a thorough understanding of the mechanisms that lead PCa cells to home to the bone microenvironment. Nonetheless, research utilizing existing animal models along with clinical data has led to the identification of genes and signaling pathways that mediate various steps in the progression and to a limited extent, the mechanisms that lead particularly to the skeletal metastatic cascade. We highlight specific genes and pathways that are currently being used as therapy as well as some that have the potential to be developed as new therapeutic targets.

Wnt/β-catenin signaling

Wnts are secreted cysteine rich glycoproteins that play key roles in embryonic development and tumorigenesis. The Wnts bind to frizzled receptors leading to a cascade of signaling

events that cause the disruption of the β-catenin destruction complex, culminating in β-catenin's nuclear localization. Stabilized β-catenin leads to the activation of several factors such as MYC, MMP7 and VEGF that contain TCF/LEF1 binding sites. A number of studies have linked aberrant β-catenin expression to human prostate cancer metastases. While some studies have reported higher β-catenin nuclear levels in prostate cancer [1-3], others have found the reverse [4, 5]. There is no clear consensus that can explain the nuclear localization of β-catenin observed in some studies and in addition the clinical relevance of β-catenin is not clearly understood. However, the observation of nuclear β-catenin in both hyperplasia and advanced prostate tumors suggests that dysregulated Wnt/β-catenin signaling plays a role in the initiation and progression of prostate cancer toward castration resistance.

While Wnt signaling has been positively correlated with prostate cancer progression in several studies [4-11], few studies show its direct role in inducing bone metastasis. Several Wnt proteins have been reported to be upregulated in human prostate cancer cell lines compared with benign prostate epithelial cells [12, 13]. Autocrine Wnt/β-catenin signaling was observed in breast cancer [14, 15]. Wnt-1 and Wnt-7b have been shown to be upregulated in primary and metastatic prostate tumors [16]. In addition, Wnt-11 and Wnt-5a are frequently upregulated in prostate cancer cells [12, 17-20]. It is not clear if Wnt expression correlates with the nuclear levels of β-catenin. Another scenario that can explain β-catenin levels focuses on the paracrine nature of Wnt signals like those derived from reactive tumor stroma. Such paracrine interactions have been observed in the case of Wnt3a in a mouse model of prostate cancer [21] and in co-culture experiments where prostate cancer MDA PCa 2b cells were stimulated to proliferate through Wnt signaling by preosteoblasts [22]. In sum, despite observations of dysregulated Wnt proteins in prostate cancer, it is not clear if this is directly linked to activation of Wnt/β-catenin signaling.

Several studies have also focused on Wnt antagonists. It is thought that downregulation of endogenous secreted Wnt antagonists may lead to the stabilization of β-catenin. Knockdown of Dkk1, a Wnt antagonist associated with Wnt receptor in osteolytic PC3 cells, caused an osteoblastic response while overexpressing Dkk1 in osteoblastic C42B4 xenografts caused them to develop osteolytic lesions [23]. Another study showed that Dkk1 potently inhibited the osteoblastic phenotype of canine prostate cancer cells and increased bone metastasis in an intra-cardiac mouse xenograft model [24]. In addition, both canonical and non-canonical branches of Wnt signaling can mediate the osteoblastic bone response in PCa via BMP-dependent as well as independent pathways [25]. In contrast to data from xenograft models, data from genetically engineered mouse models of prostate specific Wnt activation display invasive adenocarcinoma [9, 10] but do not produce bone metastasis.

Several small molecule inhibitors that target different components of the Wnt/β-catenin pathway are being proposed as possible therapeutic agents for prostate cancer [26, 27]. Although a few advances have been made [28-30], the clinical development of Wnt inhibitors remains at a nascent stage while their potential adverse effects in patients remains unknown.

MET and VEGFR pathways

MET receptor tyrosine kinase and vascular endothelial growth factor VEGFR pathways are reported to play key roles in the progression of prostate cancer as well as the development of bone metastases. MET is expressed in the basal and luminal cells of the normal prostatic epithelium [31] and its expression is downregulated by androgen receptor [32]. MET is expressed at low levels in prostate cancer cells [31] and androgen deprivation increases MET levels in PCa cells and increases HGF expression in the tumor and stroma [32, 33]. Experimental LNCaP and ARCaP human prostate cancer cell models showed that MET expression can be dramatically upregulated by receptor activator of nuclear factor (NF)-κB ligand (RANKL), and conferred the ability of prostate cancer cells to home to bone [34]. The clinical significance of these findings is supported by the finding that gene expression profiles of RANKL and activated c-MET, or phosphorylated c-MET, in primary prostate cancer tissues predict the overall survival of prostate cancer patients [35]. Interestingly, MET and HGF levels correlate with prostate cancer metastasis and

disease recurrence [33, 36], with the highest MET levels in bone metastases compared with soft tissue and lymph node metastases [36]. Androgen deprivation, the widely used initial line of clinical therapy for prostate cancer, may accentuate HGF/MET signaling.

VEGFR signaling is important for angiogenesis, a critical component of tumor growth. Prostate cancers have a significantly higher microvessel density compared with normal prostate and high grade prostatic intraepithelial neoplasia and this increase correlates with tumor grade and pathologic stage [37]. Further, patients with metastatic prostate cancer have higher plasma levels of VEGF and these levels are independent predictors of overall survival [37, 38]. In addition, the VEGF and MET pathways interact in prostate cancer cells. VEGF promotes the expression of Mcl-1, a member of the Bcl2 anti-apoptotic family of proteins, via a MET dependent mechanism through the co-receptor neuropillin [39]. MET and VEGF signaling in prostate cancer bone metastasis provides a cogent rationale for their dual inhibition as a therapeutic strategy in patients with castration-resistant prostate cancer (CRPC) as well as bone metastases. Caboxantinib (XL184) is a small molecule tyrosine kinase inhibitor that has shown promise in clinic for metastatic CRPC [40].

Hepsin

Several studies have shown that hepsin, a type II transmembrane serine protease (TTSP), is upregulated at both the mRNA and protein levels in more than 90% of human prostate cancers, making hepsin one of the most upregulated genes in the pathophysiology of the disease [41, 42]. Hepsin levels have been correlated positively with disease aggressiveness, with the highest hepsin expression levels present in tumors of Gleason grade 4/5. Hepsin levels are indicative of poor clinical outcome and disease relapse following therapy [43-45]. Hepsin can activate through cleavage molecules such as pro-UPA, pro-HGF, Laminin-332 and pro-MSP [46-50]. *In vivo*, hepsin co-operates with c-Myc in the development and progression of prostate cancer in a mouse model [51]. In addition, bigenic mice overexpressing hepsin and SV40 large T-antigen have prostate cancer progression and metastasis to the liver lung and bone [52]. Hepsin-overexpressing LNCaP prostate cancer cells promote tumor growth and lymph node metastasis when grown orthotopically [53]. Furthermore, a small molecule hepsin inhibitor was found to block prostate cancer bone metastasis in a preclinical mouse model of prostate cancer [54].

Androgen receptor pathway

Androgen ablation therapy remains the primary clinical treatment for patients with early stage of prostate cancer. However, despite androgen ablation, nearly all patients with advanced stages of the disease develop CRPC. The progression to CRPC takes place over a period of about 18 months, with the median survival period being 1-2 years. AR signaling under castrate levels of androgens has been described by several groups that suggested multiple escape mechanisms resulting in the phenomenon of CRPC [55-57]. In essence, it is now increasingly understood that despite suppression of circulating androgens, residual androgens produced within the tumor play a key role in mediating progression to CRPC. It is now understood that lethal prostate cancer progresses from an endocrine driven phase to a paracrine or microenvironment driven phase, and that castration does not eliminate androgens produced within the tumor microenvironment. Intra-tumoral levels of androgens are sufficient to activate AR and subsequent AR-mediated gene expression [58-60]. Therefore, therapeutic strategies that target androgens in the tumor microenvironment will be more effective. Novel AR axis inhibitors designed to target both adrenal and tumoral androgens include abiraterone acetate (Zytiga), which block endogenous androgen biosynthesis by inhibiting Cyp17α1, a steroid 17α-monooxygenase that has both 17α-hydrolase and 17, 20 –lyase activities, and enzalutamide (Xtandi, or MDV3100), a potent new AR antagonist that inhibits the transcriptional activity of AR supporting prostate cancer growth and differentiation. These two new agents have revolutionized the hormonal treatment of men with CRPC [61]. Abiraterone has emerged as an attractive line of therapy in men with metastatic CRPC due to its ease of administration and relatively low toxicity. However, despite impressive clinical responses, resistance to abiraterone or enzalutamide has been noted in the clinic; not all men respond to the drug and the improvement of survival in

patients with mCRPC was only a 4-5 month extension of life. Continued androgen production and AR activation are expected in abiraterone- and enzalutamide-resistant tumors. However, this may pave the way to combinatorial therapeutic strategies utilizing other targeted agents and modalities such as radiation, chemotherapy and immune-based therapeutics.

RANKL

RANKL and its associated receptor RANK are known to play key roles in osteoclastogenesis [62]. Since increased osteoclast activity is associated with increased bone remodeling in skeletal metastasis, targeting RANKL is an attractive therapeutic option for the prevention and treatment of bone metastases in prostate and breast cancers. Several studies have shown that pharmacological inhibition of RANKL can prevent tumor-associated bone destruction in bone metastasis models of prostate, breast, lung, renal and colon cancers [63]. In addition, experimental models have also shown that pharmacological blockade of RANKL can prevent skeletal metastases [64], indicating that RANKL plays a seminal role in mediating early tumor colonization and bone metastasis progression. In a clinical study, it was found that denosumab, a fully humanized IgG2 monoclonal antibody that binds human RANKL with high affinity, is superior to zoledronic acid in preventing or delaying the complications associated with skeletal metastases in bone metastatic patients [65]. Interestingly, in another study, compared to placebo, denosumab prolonged bone metastasis-free survival in prostate cancer patients with non-metastatic castration resistant PCa [66]. This clinical study has successfully shown for the first time that preventive targeting of the bone microenvironment can delay the metastatic establishment of tumor cells by making the microenvironment less conducive to colonization. Our lab has been actively involved in studying the mechanisms that lead to bone metastasis and we recently found that RANKL, either from the tumor cells or the host, plays a crucial role provoking a feed-forward mechanism upregulating downstream transcriptional factor (TF) targets, c-MET, RANKL, neuropilin-1, and HIF-1α, via upregulated TFs, c-MYC, MAX and AP-4, resulting in the homing of PCa cells to the bone. Our results support a new paradigm where a population of metastasis-initiating PCa cells gains mesenchymal, stem cell, neuroendocrine, and bone cell properties leading to the recruitment and reprogramming of bystander "dormant" cells that participate in soft-tissue and bone colonization [34].

Pathways studied by utilizing transgenic mice

Research using Genetically-Engineered Mouse (GEM) models have over the years made it possible to identify specific molecular alterations that take place in prostate cancer progression and study them in a pathophysiological context. Although modeling PCa with GEM is complicated by fundamental differences in anatomy, biology and tumorigenesis between mouse and human prostate, there are inherent advantages in the system including the ability to study the disease in an immune-competent setting that is genetically homogenous, and to control gene expression in a temporal manner. Although no single model over the years has been found to recapitulate the entire spectrum of pathological changes seen in human prostate cancer, a good model should mimic the fundamental features of human PCa progression in the closest possible manner. These include a primary tumor that progresses to invasive adenocarcinoma and responds to androgen ablation, and the ability to achieve visceral or bone metastases. Of these features, bone metastases have been rare in the models developed so far.

TRAMP (transgenic adenocarcinoma of the mouse prostate) model

The first generation of models focused on creating a tumor in the prostate by using a "sledgehammer" approach or using whatever oncogenic means necessary. The TRAMP model was created by using a prostate specific probasin promoter to target the SV40 early region comprising the large T and small t antigens, and by selecting a higher transgene expressing line. This model rapidly progresses to prostatic neoplasia by 28 weeks, with 67% penetrance to pulmonary metastases and 100% lymph node metastases [67]. Bone metastases have also been reported in the TRAMP model on the FVB background, but not on C57Bl/6 [67]. Further, the tumors and androgen-dependent and upon castration develop poorly differentiated and metastatic lesions as compared with uncastrated controls [68]. The castrated or andro-

gen-independent primary tumors are 100% synaptophysin positive, and the metastases are 67% positive for synaptophysin, indicating that these tumors are neuroendocrine (NE) in nature [69]. The TRAMP model has been utilized extensively in PCa research as a tool to validate genes in the progression of the disease [70], and in the development of chemopreventive strategies and novel therapeutics [71]. This model, however, leaves much to be desired considering that most human PCa exhibits adenocarcinoma and not the neuroendocrine phenotype.

Pten model

Numerous reports have shown that the phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a significant tumor suppressor [72]. It is lost in approximately 69% of human PCa [73] and 86% of metastatic CRPC patients [74]. Conditional prostate specific Pten knockout mice develop PIN at 6 weeks and adenocarcinoma with 100% penetrance at 9-29 weeks [75]. The adenocarcinomas formed respond to surgical castration at 16 weeks, with an increase in apoptosis [75]. The castrated prostates show an increase in NE differentiation [76] as well as metastasis to the lung and lymph nodes [75, 76]. Pten conditional knockout mice have been used by several groups for application-based studies. The focus broadly has been to investigate if a given gene of interest is involved in prostate cancer progression. The Pten model has been used in drug studies to provide translational and pre-clinical data that can potentially be used to treat PCa. For example, a surgically castrated Pten conditional model treated with a combination of enzalutamide and PI3 kinase inhibitors showed significantly reduced tumor volumes [77]. The Pten model however, has limitations since the phenotypes have been found to be variable in nature. Also, when Pten conditional mice are back crossed into a C57/BL6 background they do not progress beyond PIN lesions [78]. Additionally, metastasis in PB-Cre4 Pten flox/flox mice has not been observed in a reproducible manner. Therefore the Pten model is more ideally suited for therapeutic studies designed to attenuate disease progression rather than studies that look at the mechanisms of metastasis.

Myc model

c-myc is a proto-oncogene that is over-expressed in human prostate cancer. Its expression correlates with disease progression [79, 80]. Myc overexpression in tumor cells takes place via numerous mechanisms that include gene amplification, loss of foxp3, and aberrant activation of Wnt/β-catenin pathway [81]. It is therefore physiologically relevant to target myc expression in the mouse prostate as a way to model human PCa. Myc expression was targeted in the mouse prostate utilizing the small PB promoter or the stronger ARR2PB promoter, resulting in Lo-myc and Hi-myc mice respectively [82]. While Lo-myc mice progress slowly, Hi-myc mice progress from PIN to adenocarcinoma in 13 weeks, and local invasion is seen at 26 weeks [82]. Recapitulating human PCa, Nkx3.1 expression decreases in the Hi-myc model with the onset of PIN as observed in human PIN cases [83]. However, unlike human PCa, though the tumors regress with castration they do not become castrate resistant, underscoring the disadvantage of using an androgen-regulated promoter in castration experiments. In addition, myc transgenic mice do not progress to metastatic disease [82]. The myc model has been used in several studies, the majority of which investigated the coupling of myc over-expression with a gene of interest. One study created a bigenic mouse with concomitant myc and hepsin overexpression showing accelerated adenocarcinoma progression, with the primary tumor developing in 12 weeks instead of 24 weeks [51]. In another study, constitutively activating the NF-κB pathway in Hi-myc mice resulted in a tumor resistant to castration; this study suggested that the NF-κB pathway may play a role in the progression to CRPC [84]. Another group created c-myc over-expressing mice by employing an alternative strategy using Z-myc mice whereby expression of myc is silent until recombination takes place [85]. Recombination of the Z-myc mice with PB-Cre4 mice produced invasive tumors in all four lobes of the prostate with 100% penetrance in animals aged 33-46 weeks [86]. This model circumvents the need for androgens for transgene expression and therefore is an excellent model for castration studies. Overall, the major pitfall with the myc model is that the tumors do not progress to CRPC or develop

metastatic disease. However, since myc overexpression is an early event in human PCa, the myc model is ideal for studying additional genetic changes that drive and co-operate with each other in PCa progression.

Conclusions

Bone metastasis causes a significant clinical burden for prostate cancer patients and is therefore the focus of therapeutic prostate cancer research. Currently drugs that block c-MET and RANKL pathways, in addition to androgen ablation therapy, are predominantly being used as targets in the clinic. Clinical management of advanced prostate cancer patients is often effectively achieved by a combination of therapies that target the bone and the primary tumor. Research is needed to develop the therapeutic potential of new targets as well as to design strategies for the optimal use of current therapies.

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Disclosure of conflict of interest

None.

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