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Review Article

New Insights in the Role of Androgen-to-Estrogen Ratios, Specific Growth Factors and Bone Cell Microenvironment to Potentiate Prostate Cancer Bone Metastasis

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Abstract. Prostate cancer progression to bone metastasis is an early event that remains dormant when the androgen ratio to estrogen is high. Only 40% of patients with bone metastasis and skeletal involvement survive past the first year. During andropause, changes in hormone ratios and nuclear receptor coregulator expression, in conjunction with crosstalk with fibroblast growth factors and bone stroma signaling pathways, reactivate the early metastasis. This review will provide insights into how this interplay induces changes in the osteolytic microenvironment to promote prostate cancer metastasis to the bone. While both AR and ER induce changes in the osteolytic microenvironment to promote bone metastasis, it is ERα overexpression that stimulates osteoblast differentiation, proliferation, osteoclast-mediated bone resorption, and the release of bone matrix factors. Loss of ERβ1 enhances VEGF expression and tumor cell survival through stimulation of osteoblast differentiation. Aberrant expression of FGFs and FGF receptors (FGFRs) initiates MAPK, PI3K, and PLCγ pathways, resulting in proliferation, dedifferentiation, angiogenesis and survival. The paracrine action of FGF10 may be required for bone metastasis reactivation due to interaction with bone stromal cells when E2/T ratio increases. This ratio change provides a potential mechanism for estrogen signal activation when prostate cancer cells express ERα in the presence of bone stromal cells, resulting in ERα predominance over the AR activity due to changes in coactivator/corepressor recruitment by ERα when circulating androgens are reduced during hormonal deprivation therapies.

Keywords: Prostate Cancer and Bone Metastasis; Estrogens; Androgens and Nuclear Receptors Coregulators; Growth Factors and Receptors

1. Introduction

Prostate cancer is the foremost diagnosed non-cutaneous malignancy among men and the second leading cause of male cancer death in developed Western nations [1]. Since the earliest published identification as a malignancy by J. Adams in 1853, the cause and prospective treatment of prostate cancer have been the subject of extensive research [2].

Although the connection between the function of the testes and the prostate was first recognized with John Hunter's research on the influences of castration in 1786, it was the early research of Huggins with various colleagues during the 1940s regarding castration, the use of estrogens to negate androgenic effects, and the role of the adrenal glands that proved pivotal to the development of modern protocols for combined androgen blockade therapies [3–7].

The molecular mechanisms by which androgens and estrogens promote cell cycle progression and differentiation in prostate tissue has been extensively described. Androgens, in particular dihydrotestosterone (DHT), promote prostatic morphogenesis and have been implicated in the etiology of prostate cancer. The disruption of androgen-regulated pathways in the context of stromal-epithelial cell interactions and growth factors has been demonstrated as a requirement for prostatic disease development and progression to androgen independence [8].

While androgen deprivation therapy (ADT) for locally advanced or metastatic prostate cancer has remained the standard of care for the past sixty years, the initial effectiveness in inhibiting cell growth is relatively brief, lasting between 18–24 months, and has a less-than-complete patient response of 60–80% [9–12]. Prostate cancer cells eventually acquire the ability to grow in the absence of circulating testicular androgens and few options are available to treat advanced stages, especially castration resistant prostate cancer [10, 13, 14]. Therefore, it is possible to conclude that androgen deprivation therapy is not curative and only delays progression in a high percentage of patients. Thus, while the understanding of molecular mechanisms has expanded, effective treatment has not advanced beyond Huggins and Scott's 1941 observation that although inhibition of androgenic production reduces the activity of prostate cancer, it fails to control the disease, leading to a state of androgen independence [6]. While androgens have dominated prostate cancer research, it remains but a part of a greater interplay within the prostatic molecular environment. Androgens, estrogens, nuclear receptors, coactivators, corepressors and stromal-epithelial cell interactions are integral to the normal development and homeostasis of the prostate, and provide the opportunity for genetic disruption that leads to malignancy and the development of a lethal phenotype.

2. Role of Androgens and Androgen Receptors in Prostate Cancer

Androgen synthesis in the form of testosterone is controlled by the hypothalamus-pituitary-gonads endocrine axis, and occurs in the interstitial Leydig cells of the testes when stimulated by the pituitary secretion of luteinizing hormone [9, 15]. Approximately 90% of circulating androgens are testosterone, which bind with high affinity to sex-hormone-binding globulin (SHBG) proteins [15–18]. Target cells uptake the 1–2% of free circulating androgens, not bound to SHBG, and convert the androgens into 5-alpha-dihydrotestosterone (DHT) in the prostate using steroid 5-alpha-reductase enzymes. [15, 19–21]. Peripheral synthesis of androgens also occurs in the adrenal glands, producing dehydroepiandrosterone (DHEA), androstenediol and androstenedione, which comprise the remaining 10% of circulating androgens. [15, 17, 18, 20]. Changes in the circulating androgens, including testosterone, DHT and DHEA levels, have been shown to be relevant to the increased risk of prostate cancer initiation, progression to androgen independence, degree of malignancy, and early metastasis to bone and other organs. With the progression towards andropause, beginning around age 35–40, serum concentrations of testosterone eventually decrease to 35% [22, 23]. DHEA reduction is even more significant, decreasing by 45–50% between ages 40 to 80. [24] DHT levels, however, remain fairly constant [24]. Low testosterone levels have been associated with increased risk, poor prognosis and shorter survival [25]. Additionally, lower serum testosterone levels have been related to higher Gleason scores [26]. Conversely, high levels of free serum testosterone have also been associated with increased risk of an aggressive prostate cancer in older men [25, 27].

Androgen activity is mediated by the intracellular nuclear receptor androgen receptor (AR), which is a ligand-inducible transcription factor that regulates expression of specific gene networks involved in proliferation, differentiation and cell survival [9, 19, 28]. DHT is the biologically active hormone metabolite, due to high binding affinity as it is less susceptible to metabolism, and has a slower disassociation rate from the receptor [29, 30]. In the absence of hormone, AR is maintained in an inactive state in the cytoplasm by association with heat shock proteins (Hsp) 90 and Hsp 70, among others [15]. Activation of the receptor by hormone binding to the ligand-binding domain results in structural and functional changes that allow dimerization and binding to specific DNA hormone response elements generally located upstream of target genes to activate the RNA pol II transcription complex to either increase or decrease gene expression through interaction with coactivators or corepressors respectively [28, 31–33]. In addition, corepressor recruitment to the AR complex already in the nuclear compartment, in the absence or presence of hormone, is integral to the biological response for the decrease in the transcription rate of genes that are essential for the deactivation of pathways involved in proliferation, differentiation and cell specific function. Thus coregulators, which are nuclear proteins that act as coactivators or corepressors, play a key role in AR function to either increase or decrease gene expression. Almost 200 coactivators have been identified and shown to be required for AR and other nuclear receptor function [10]. Coactivators, such as SRC-1, CBP/P300 and CARM, among others, increase gene expression through the recruitment of histone acetyl- (HATs) and methyl-transferase activities to remodel chromatin at

the RNA pol II transcription initiation complex [32, 34–36]. Corepressors, such as SMRT and NCoR, decrease gene expression due to chromatin compacting through the recruitment of histone deacetylase (HDACs) activity to the AR complex to diminish or block transcription [32, 36–38].

As CaP progresses into castration-resistance, coactivators SRC-1, TIF-2, SRC-3, p300, CBP, and ARA70, are commonly overexpressed [10, 39]. This elevated coactivator expression may increase AR transactivation in response to low levels of circulating androgens by means of intrinsic histone acetyltransferase activity [35, 40]. SRC-1, TIF-2 and SRC-3 are part of the p160 family of coactivators that recruit histone transferases p300 and CBP and methyltransferase CARM1 to enhance transactivation activity by remodeling chromatin [28, 40]. Both p300 and CBP are upregulated during ADT. Increased expression of p300 has a direct correlation with tumor grade, larger volume, increased proliferation, poor prognosis, and is part of the transition into an androgen hypersensitive state of disease, also known as androgen independence due to the low concentration of androgens required for tumor cell growth [41, 42]. The recruitment of SMRT to the AR transcriptional complex is reduced during progression to hormone resistance. The SMRT/NCoR corepressors interact directly with AR in the absence and presence of androgen antagonists to repress AR transcriptional activity in LNCaP cells [38, 43]. The decrease in SMRT expression and increase in coactivators, p300 and TIF2, may represent one of the molecular switches that changes the androgen response and alters gene expression during progression to androgen hypersensitivity [36, 43]. However, recurrency, resistence to treatment and reactivation of metastasis in bone and other distant organs, which are refractory to therapies, continues to be the target challenge in prostate cancer.

AR is present and mediates androgen biological activity in both luminal, epithelial, and stromal cells of the prostate. The presence of AR promotes differentiation of epithelial cells, and regulates coactivator and corepressor recruitment to the AR transcriptional complex for prostatic function in stromal cells [9, 28]. During progression into malignancy, the ability of epithelial cells to modulate the AR transcriptional complex becomes altered in the stromal cell microenvironment due to AR dependent corepressor recruitment to the transcription complex, generating an androgen resistance that decreases the capacity of stromal AR to activate gene expression [28]. Similarly, AR activity in epithelial cells is decreased due to corepressor recruitment in androgen dependent manner. In addition, the molecular event involved in AR mediated transcription, which is regulated by stroma-epithelial cell interaction using an unknown paracrine factor, is lost in the primary tumor. Therefore, increased AR resistance to androgens at the initial state in tumor progression may explain the reduced requirement for ligand and influcence the role of stromal AR in prostate cancer development, progression and metastasis.

The failure of ADT, which only addresses the influence of androgens on epithelial and stromal cells interaction, to successfully treat has lead to extensive investigations of mechanisms that may induce prostate cancer recurrence and castration resistance, including: AR amplification or overexpression; mutations that change specificity of hormones or reinstate AR function that differs from the original due to a change in the intracellular millieu; intracrine androgen production; increased growth factor-induced phosphorylation; and AR splice variants; AR deactivation of the M-phase cell cycle checkpoint, as well as the aforementioned overexpression of coactivators with loss of corepressors [9, 10, 15, 36, 39, 44, 45]. AR splice variants lack the AR ligand-binding domain (LBD), which is the target of ADT, and may account for lack of treatment response [46]. Intracrine androgens synthesized from cholesterol, as well as intratumoral conversion of androstanediol to DHT, maintain intraprostatic androgen at 20-30% of precastration levels, and may account for continued activation of AR in castration resistant prostate cancer [44, 47]. Overexpression of coactivators also diminishes the amount of androgen necessary for AR activation. [15] AR mutations are found in 8–25% of castration resistant prostate cancer patients [9]. AR amplification is characteristic of 20–33% of castration resistant prostate cancer tumors [10]. Wang *et al*. showed that AR upregulation of selective genes, such as the ubiquitin-conjugating enzyme F2C (UBE2C), in the absence of hormone induces deactivation of the M-phase of the cell cycle to promote progression to androgen independence. Increased methylation of H3K4 marks and the recruitment of transciption factors, such as FoxA1, to the UBE2C enhancers resulted in UBE2C overexpression and increased AR recruitment [45]. The finding that AR selectively and directly up-regulates M-phase genes has been proposed as a relevant cause for ADT failure [45].

No one mechanism has been determined to be a primary cause, and therefore may be but a part of a contributing collective of mechanisms. In recent years, the role of androgen as the predominating factor in the development and progression of prostate cancer has come into question, given the inability to successfully treat with ADT for the past 60 years, thus spurring a growing interest in the influences of other steroid hormones.

3. Role of Estrogens and Estrogen Receptors in Prostate Cancer

Since the 1950s, when the first estrogen receptor was proposed, studies have indicated that estrogen, in the form of 17β-estradiol (E2), may play a fundamental role in prostate carcinogenesis and progression. The E2 is a significant steroid hormone that exerts synergistic activity with androgen for normal prostatic development and function [19,

48]. E2 also modulates the hypothalamic-pituitary-gonadal axis reducing the expression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) through negative feedback, thereby controlling stimulation of Leydig cells in the testes for the production of testosterone that act on sertoli cells together with the FSH for sperm proliferation and differentiation [49, 50].

The aromatase enzyme p450 (CYP19 gene), which is expressed in prostatic stroma and is also active in adipose tissue, adrenal glands, and the testes, mediates the conversion of testosterone into E2 [51–54]. Overexpression of aromatase has been associated with increased serum estradiol and decreased serum testosterone [55]. The E2 biological activity is modulated by two subtypes of ligand-dependent transcription factor receptors, ER-alpha (ERα) and ER-beta (ERβ, also identified as ERβ1). ERα is expressed primarily in the stromal cells, and ERβ in the luminal epithelial cells, with some presence in the stroma [51, 54]. Both ERα and ERβ have specific and counterbalancing transcriptional responses to coregulators, and differ in ligand binding, heterodimerization, transactivation, and estrogen response element activity [56]. ERα is encoded by the ESR1 gene, and ERβ by gene ESR2; the genes are located at different chromosomal sites [56]. The complexity of the intermediary cell signals involved in the interaction between both estrogen receptor isoforms and AR are depicted in Figure 1.

ERα has been associated with cell proliferation, inflammation and prostatic malignancy [57, 58]. ERα expression, although in the stroma, is also required in the epithelial compartment for both early and postnatal development of the prostate and for tumor progression to occur [51, 59]. Salonia *et al.* has proposed that ERα may even have oncogenic activity, with overexpression during the transformation into malignancy, and that it may potentiate the carcinogenic effects of androgens [59]. ERα-induced inflammation has been associated with altered gene expression patterns in the prostate, and has been correlated with high tumor grade, particularly in castration resistant prostate cancer and metastases [22, 55]. Considered to present late in progression, ERα expression is found predominantly in Gleason grade 4 and 5 patients, increasing significantly after ADT [54]. Polymorphisms of ERα have been correlated to an increased risk of castration resistant prostate cancer [58].

The activities of ERα are counterbalanced by ERβ, which is associated with apoptosis, differentiation, anti-proliferation, anti-inflammation and anti-carcinogenesis, and is the predominant subtype [22, 56, 57, 60]. Expression of ERβ is substantially decreased with prostate cancer progression, and is undetectable in approximately 10% of castration resistant prostate cancer patients [54, 56, 61]. The loss or silencing of ERβ may be induced by histone deacetylation or hypermethylation in the gene promoter region [56, 62, 63]. As a key factor in cell cycle regulation, the loss of ERβ creates an imbalance in the opposition to ERα action on cyclin D1 gene expression, thereby enhancing proliferation [64]. The ERβ is important in the regulation of Snail1 through the destabilization of hypoxia-inducible factor-1 (HIF-1), and vascular endothelial growth factor-A (VEGF-A) transcriptional repression via the estrogen response DNA-elements (EREs) [65]. Interestingly, Maneix *et al.* [66] recently showed, through the use of ERβ-Δex3 mice, that it is the non-ERE-dependent mode of transcription that is used by ERβ to regulate transcription of its target genes, with only 5% of ERβ-interacting regions including only EREs or ERE-half-sites [66]. Godoy *et al.* (2012) demonstrated that there is a decreased recruitment of SMRT/NCoR with progression to castration resistant prostate cancer [44]. In addition, loss of ERβ has also been associated with induction of epithelial-mesenchymal transition [65]. While ERβ expression decreases during prostate cancer development, it remains present in lymph nodes and bone metatstasis, and eventually resumes during metastasis [66]. Several studies have suggested that the protective activity of ERβ may be conferred to prostate cancer cells during regain of expression [41, 62]. Coactivators p300 and CBP are highly expressed in prostate cancer, have been associated with the regulation of ERβ activity, and may influence the resumed expression of ERβ in advanced stages [41]. P300, in particular, has been correlated with poor prognosis, increased tumor volume, and metastasis [67].

Recent studies have indicated that it is not only the opposing roles ERα and ERβ (also known as ERβ1 or ERβ wild type) that contribute to the development and progression of cancer, but also the unique, conflictive and sometimes synergistic actions of ERβ variants. Currently, in addition to ERβ1, three human non-ligand binding variants have been identified: ERβ2, ERβ4 and ERβ5 [65, 68]. Each variant differs greatly in helix 12, which plays an important role in the ligand-dependent interaction with coregulators. ERβ1 has a full-length helix 11 and 12, while ERβ2 has a distorted heliz 12, and ERβ4 and -5 lack helix 12 [69]. Variances also occur in exon 8 length deletions and substitutions, resulting in truncated C-terminal receptor proteins [70]. ERβ1 is considered to be the only fully functioning variant, as it forms homodimers upon ligand binding, recruits coregulators and binds to response elements [69]. Located in the nucleus, ERβ1 actions are reflective of ERβ attributes in general, namely the anti-proliferative, epithelial-mesenchymal transition (EMT)-repressive properties that check the actions of ERα. With the progression of prostate cancer, ERβ1 expression is diminished or lost. ERβ2 and ERβ5 are found in the cytoplasm, and the synergistic activity increases prostate cancer cell invasion and proliferation; the combined expression has been associated with short post-operative survival [69]. ERβ2 is the most abundant of the two, and is involved in the upregulation of Twist1 and Slug, which are important factors in EMT, as well as the bone metastasis regulator Runx2 [71–73].

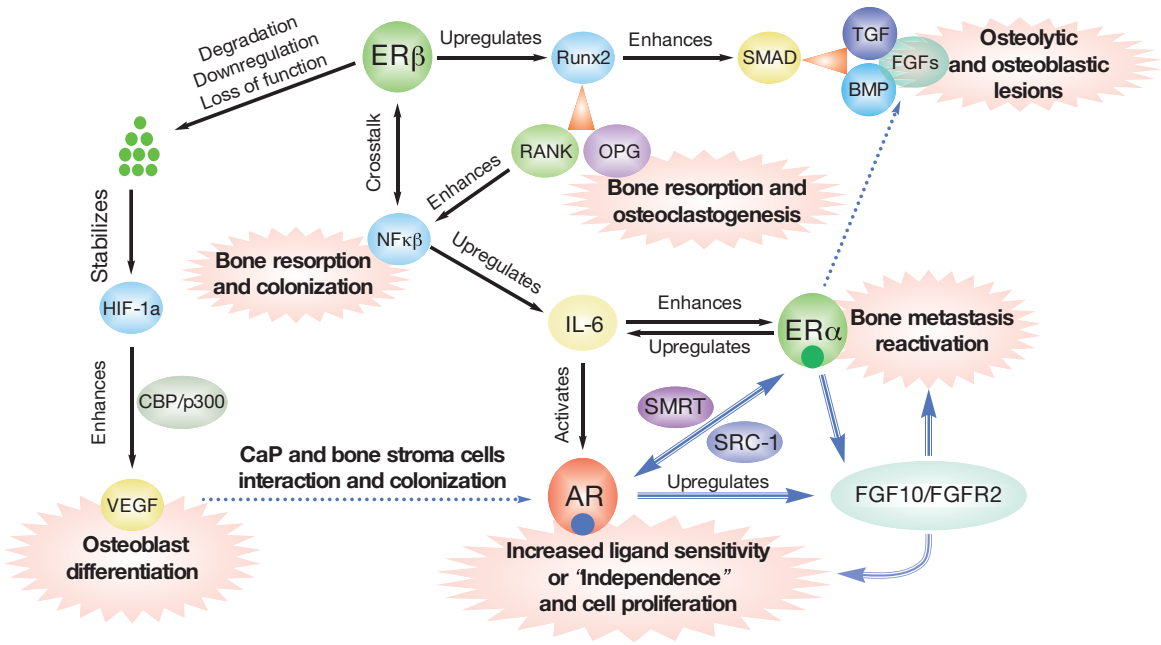

Figure 1: **Androgen and estrogen ratio changes alter prostate cancer bone metastasis microenvironment.** Elevated ratio of E2 to T, as well as ratio changes in ERα to ERβ, may initiate the process for bone stroma and prostate cancer cells to alter the bone microenvironment for the early CaP bone colony to reactivate metastasis and proliferation. Crosstalk between ERβ and NF$\kappa\beta$, and the axis between NFκB, RANK and OPG, activate osteoclastogenesis and promote bone resorption and further colonization. The Runx2, upregulated by ERβ, interacts with RANK and OPG to also promote osteclastogenesis. The transcription factor Runx2 also interacts with SMADS to mediate TGFb activation and BMP expression, which act synergistically with FGFs signals to promote osteolytic and osteoblastic lesions. Degradation or down-regulation of ERβ stabilizes HIF-1α, which, with upregulated CBP/p300 during ADT, enhances VEGF-A transcription and osteoblast differentiation. Increased ERα expression up-regulates IL-6 expression. IL-6 in turn enhances ERα expression. In addition, IL-6 regulates AR activity and may activate AR during ADT. Increased ligand sensitivity or hypersensitivity to androgens during the ADT phase of the disease increases cell proliferation. Crosstalk between AR, ER, coregulators SRC-1/p160 and SMRT/NCoR, FGF10 and growth factor receptors may be a mechanism for reactivation of early bone metastasis. Activation of ERα may mediate FGFs/BMP synthesis that, in coordination with AR, increases FGF10/FGFR2 signaling pathway to modify bone metastasis and reactivation due to osteoblastic lesions.

4. Steroid Hormone and Receptor Expression Ratio Changes

While prostate cancer clinically manifests at the onset of andropause, the actual process of carcinogenesis may span 35+ years [56]. During the aging process, serum testosterone (T) and DHEA decline significantly, to about 30%, while estradiol (E2) remains stable or increases [22]. This results in an elevated ratio of E2 to T. In castration resistant prostate cancer, there is a notable increase in AR expression, which may be a compensation for the decline in androgen levels, mediated by alterations in the function of coregulators [9, 15, 40]. The activity of coactivators SRC-1, TIF2, SRC-3, ARA70, among others become enhanced, while corepressors SMRT and NCoR are diminished, leading to increased proliferation and anti-apoptosis [10, 15, 28, 33, 43].

Chronically elevated estrogens have been associated with polymorphisms in estrogen metabolizing genes, heightened aromatase expression, increased risk of prostate cancer, and ER expression alterations during progression [56, 74]. Increased aromatase has also been associated with age-related increases in body fat mass [17]. In addition, as the E2/T ratio changes, the anti-inflammatory influences of testosterone are lost, resulting in intensified pro-inflammatory influences of ERα [55, 75]. Inflammation has been associated with pre-malignant lesions and carcinogenesis [22]. Enhanced expression of ERα has been correlated with the increase of interleukins, such as IL-6, as a response to inflammation. IL-6 regulates AR and may activate ligand-independent AR expression in castration resistant prostate cancer (Figure 1) [10, 33]. Additionally, IL-6 promotes aromatase activity associated with altered regulation in epithelial tumor cells, and may enhance ERα expression (Figure 1) [17, 51]. Also, the use of ADT may affect T-lymphocytes and elicit inflammation, as demonstrated by increased frequency of CD4+ T-cells in peripheral blood samples of treated patients [13].

In addition to the change in E2 to T ratio, there is also a ratio change in ERα to ERβ1. With the development and progression of prostate cancer, ERβ1 expression declines and is virtually non-existent in castration resistant prostate cancer patients. The reciprocal balance of ERα to ERβ1 is relevant to prostatic response to the presence of estrogen [58]. Without the homeostatic-protective modulation

of ERβ1, cell cycle progresses unchecked, permitting the continuation of proliferation of abnormal epithelial cells [56, 59, 64]. Additionally, VEGF-A transcription is enhanced without ERβ1 promotion of SNAIL1 nuclear localization and destabilization of HIF-1α, thereby promoting tumor angiogenesis and EMT (Figure 1). Not surprisingly, high Gleason score tumors exhibit significant HIF-1α and VEGF expression [65]. EMT has also been implicated in cancer metastasis and hormone resistance following ADT [76]. Although considered to be clinically significant, albeit short-term, ADT may not be appropriate for all patients. More research on steroid hormones, their respective receptors, ratio changes, and interacting coregulators needs to be conducted to better understand the ramifications of their mechanisms and their manipulation in therapeutic endeavors.

5. ER and Prostate Cancer Bone Metastasis

Metastasis to the bone is a strong factor in most prostate cancer related deaths, with 85% of mortality cases presenting bone metastasis [77, 78]. Osteoblastic activity is characteristic of prostate cancer bone metastasis, which occurs directly adjacent to the metastatic tumor [79]. As with AR, estrogen receptors induce changes in the bone stroma osteolytic microenvironment due to changes in osteoblastic and osteoclastic activity, promoting reactivation of prostate cancer metastasis in the bone. Increased expression of ERα also increases IL-6, which exhibits both pro-tumorigenic and pro-metastatic activity, and is correlated with poor prognosis and bone metastasis (Figure 1) [71, 80–82]. Upregulation of cytokine IL-6 stimulates osteoblast differentiation, proliferation, and osteoclast-mediated bone resorption (Figure 1) [80, 83]. This produces an environment that is conducive for metastatic growth due to the release of bone matrix factors [84]. Prostate cancer metastasis is, however, a very early event in tumor progression, with disseminated cells detected in the bone marrow nitche as early as Gleason 2. [85] As such, early metastasis remains silent or dormant in the niche until reactivation at a later stage of progression. The role of changes in E2 to T ratio in circulation, in the context of bone stromal cells, together with changes in recruitment of AR coregulators due to modification of hormones, suggest that the use of ADT requires serious consideration and further investigation. Numerous studies have postulated ERβ as a potential target to inhibit proliferation and metastatsis of malignant prostate cells. However, for a patient who may have already experienced early metastasis to the bone, modulation of ERβ may promote rather than inhibit the tumorigenic state. ERα, as an oncogenic influencer, may serve as a more impactful therapeutic target. Ultimately, prognostic tools must be developed to properly determine the molecular state of the patient before application of treatment.

Crosstalk between ERβ and the nuclear factor kappa beta (NF$\kappa\beta$) transcription factor also induces bone resorption to facilitate tumor cell colonization (Figure 1) [69, 86]. The NFκB also upregulates transcription of IL-6 encoding gene, and NFκB/IL-6 dependent pathways promote anti-apoptosis for tumor cell survival (Figure 1) [69, 87]. Furthermore, the axis consisting of NFκB, RANKL expressed in osteoblast, and osteoprotegerin (OPG), activate osteoclastogenesis and promote bone resorption and metastasis (Figure 1) [88]. Osteoprotegerin, a cell-cell adhesion non-collagen molecule, which works as a decoy receptor for RANKL, is important in bone remodeling, and is often overexpressed in cancer progression [89, 90].

Transcription factor Runx2, which is also associated with ERβ2 and interacts with RANKL to regulate osteoclastogenesis, may inhibit osteoclast activation through OPG and differentiation (Figure 1) [91]. Runx2 also recruits co-regulatory factors that mediate transduction of steroid receptor coactivators, bone morphogenetic proteins (BMP) and TGFβ signaling (Figure 1) [92]. Additionally, Runx2 has been shown to interact with SMADS, which are transcription factors involved in signal translocation from membrane receptors to the nucleus to mediate TGFβ/BMP signaling, thereby promoting the formation of tumorigenic osteolytic and osteoblastic bone lesions (Figure 1) [93, 94].

Loss of ERβ1 during prostate cancer progression results in stabilization of HIF-1α transcription factor through the binding of CBP/p300 and the enhancement of VEGF expression (Figure 1) [65, 95]. The VEGF expression supports tumor cell survival in the hypoxic bone microenvironment and also regulates bone remodeling through the stimulation of osteoblast differentiation (Figure 1) [65, 96, 97]. As expected, upregulation of CBP and p300 during ADT contributes to the increased expression and activity of VEGF [98, 99]. In addition, transcription factors that regulate expression of Snail and Slug also downregulate the expression of E-cadherin resulting in induction of EMT in the presence of TGFβ [72]. The BMP ligands, which are part of the TGFβ family members, have been shown to act synergistically with fibroblast growth factors (FGFs) in LNCaP to induce and promote cell proliferation (Figure 1) [100]. As a key component of the reactive stroma environment, TGFβ has also been postulated to induce expression of FGFs to mediate prostate cancer metastasis (Figure 1) [101].

6. FGFs, ER/AR Ratios and CaP Metastasis to Bone

FGFs are a family of 23 polypeptide growth factor ligands that are divided into seven sub-families 1, 4, 7, 8, 9, 11, and 19, based on their sequence and function similarities [102, 103]. The binding of FGFs to cell-surface high-affinity tyrosine kinase receptors (FGFRs) initiates several cellular processes that involve proliferation, migration, cell survival and diferentiation [104]. Dimerization of the receptors occurs upon the binding of the FGF ligands and initiates activation of the FGFR, resulting in transphosphorylation of the intracellullar tyrosine kinase domain [102]. The FGFRs family

of proteins consists of four primary isoforms, with subvariants occurring through alternative splicing in the third of the three immunoglobulin-like loops present in the receptors [106]. This produces additional isoforms, IIIb and IIIc, which determine FGF ligand specificity for each of the FGFRs, with IIIb ligand binding activity more restrictive than IIIc [105, 106]. The IIIb isoforms have been described as primarily epithelial, with IIIc isoforms primarily mesenchymal [104]. In addition to splicing, alterations in FGFR occur through mechanisms that include gene amplification, chromosomal translocation, and specific mutations [104].

In the prostate cancer and the cellular environment, aberrant expression of FGF and FGFR initiates the activation of several downstream pathways, including mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), and phospholipase Cγ (PLCγ), resulting in proliferation, dedifferentiation, angiogenesis and tumor cell survival [107, 108]. The FGFR signaling pathways has both autocrine functions within the tumor cell, and paracrine functions among tumor cells and the microenvironment of stromal cells, serving to promote metastasis [108]. The signaling is increased with overexpression of the associated ligands [109]. Among the four primary isoforms of FGFRs, only FGFR3 has no demonstrated association with prostate cancer [110]. Upregulation of FGFR1 has been associated in about 40% of poorly differentiated prostate adenocarcinomas, EMT and distant metastasis [111]. The switch from FGFR2-IIIb to FGFR2-IIIc has been associated with the induction of EMT through the disruption of crosstalk between stromal and epithelial cells [112]. The FGFR4 inhibits NFκB action, resulting in pro-survival signaling, and has been associated with aggressive progression of prostate cancer [113, 114].

Several FGF family members have been associated with prostate cancer. The FGF2 regulates differentiation and promotes angiogenesis, increasing the rate of progression to prostate cancer metastasis [101]. Interestingly, FGF2 overexpression occurs in the fibroblast and endothelial stromal cells, but not in the tumor cells [108]. The FGF8 crosstalk with FGF17 have been postulated to increase production of tumor-secreted bone resorptive factors that induce release of bone matrix growth regulatory factors to promote survival in the bone metastatic environment [115]. The FGF9 has been correlated to EMT and VEGF-A in prostate cancer [116]. The FGF19 functions as an autocrine growth factor in both primary and metastatic prostate cancer to promote growth, invasion, adhesion and colony formation [118]. The paracrine action of FGF10 that is relevant for prostate organogenesis has been associated with the induction of prostate intraepithelial neoplasia (PIN) [118]. The paracrine FGF10 signaling has also been shown to promote proliferation and survival in an androgen-independent prostate environment [118]. As the role of FGF10 in the prostatic environment has been focused primarily on early development and branching, further investigations are required to understand the significance in prostate cancer initiation, progression, and metastasis. Independent of the complexity of the initiation and progression, prostate cancer cells that migrate from the primary site at an early stage of development, presumably at Gleason score 2+2, remain inactive in the bone metastastic niche for years before reactivation. Therefore, as FGF10 expression in the prostate epithelial cell, which is required for the initiation of the prostate cancer and functions in an autocrine fashion in the transformed cell, it is possible to propose that FGF10 may also be required for reactivation of bone metastasis and the adaptation process to occur, together with the changes in the estrogen to androgen ratios that affect tumor cell interactions with osteoblast and osteoclast present in the bone derived stromal cell compartment.

FGF10 is a member of the FGF subfamily that includes FGF 3, 7, and 22, and is one of the growth factor ligands for FGFR1, FGFR2, and FGFR4. The epithelial-mesenchymal paracrine signaling action of FGF10 is required for normal prostate growth and development [119]. Both FGFR1 and FGFR2 function to maintain prostate homeostasis by directing the epithelial/stromal crosstalk between FGF7 and FGF10 [108, 120]. In particular, the signaling pathway between FGF10 and FGFR2IIIb regulates the expression of morphoregulatory genes, including Shh, Bmp4, Bmp7 and Nkx3.1 (Figure 1) [121]. The change from FGFR2IIIb to FGFR2IIIc caused by a shift from exon 8 to exon 9 may account for a disruption in the crosstalk that results in EMT that potentiates invasion and metastasis. In addition to initiation and PIN, overexpression of FGF10 has also been associated with cell migration and invasion [119]. Stromal ERα has been proposed to mediate FGF synthesis, in coordination with AR activation (Figure 1) [122]. ERα, in particular, has been associated with the induction of FGF10 in mice [123]. Chen *et al*. determined through the use of ACTB-ERαKO mice with defects in prostatic branching morphogenesis that ERα was essential for proliferation and that loss of stromal ERα resulted in reduced expression of FGF10 [124]. Crosstalk between AR, ER, FGF10 and growth factor receptors are proposed to influence tumorigenic progression (Figure 1) [122, 125]. Estrogen induced transcriptional activity, along with loss of ERβ that facilitates the uncontrolled ERα function, including upregulation of FGF10, may play an integrated role (FGF10-ER-AR axis) in EMT and metastasis reactivation that is induced by ADT in advanced prostate cancer (Figure 1) [126].

7. Conclusion

Newly synthesized ERα in metastatic prostate tumor cells is presumably due to interactions with paracrine grow factors signals derived from the bone marrow stromal cells, supported by an environment of decreasing circulating androgens and stable or increasing estrogens induced by age and ADT (Figure 2). Reactivation of the ERα signaling pathways in metastasis, in presence of changed E2/T ratios and the decreased androgen activity, changes the already increased

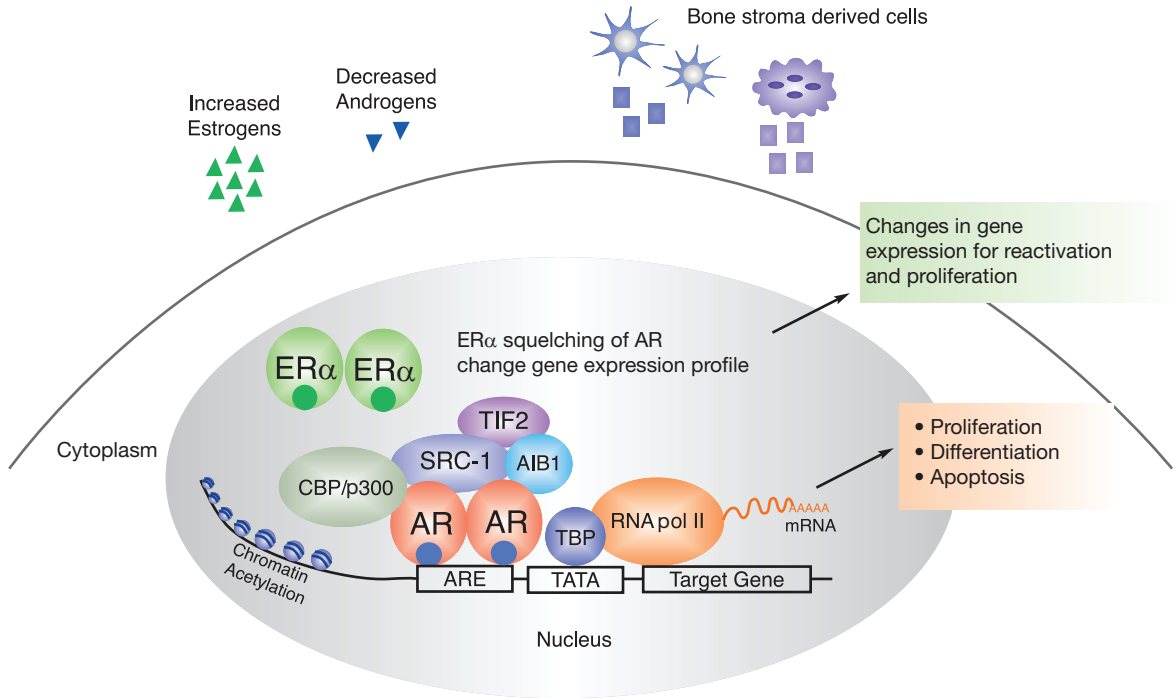

Figure 2: **Newly expressed ERα in prostate cancer cells that disseminate to bone metastasis may reactivate proliferation due to modified AR function through changes in coactivator/corepressor recruitment in the presence of low circulating androgens.** Prostate cancer cells alter the bone homeostasis by secreting paracrine factors that regulate proliferation and osteoblast differentiation. These factors include bone morphogenetic protein (BMP), transforming growth factor beta (TGFβ), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), endothelin-1, bone metastasis-related factor (MDA-BF-1), plasminogen activator (μPA) and prostate specific antigen (PSA). These growth factors modulate the function of osteoblasts to promote the deposition of a new extracellular matrix and consequently osteoclast activation that induces bone remodeling of low mechanical strength.

CoAs$^{(SRC\text{-}1/TIF2/AIB1/CBP/p300)}$ and decreased CoRs$^{(SMRT/NcoR)}$ observed in tumor cells, to promote progression to an androgen hypersensitive state of the disease. Prostate cancer cells in the metastasis site that are exposed to ADT also exhibit lost of corepressors SMRT/NCoR and increased coactivator SRC-1/TIF2/RAC3 to gain androgen driven gene expression under low concentration of androgens (Figure 2). Early metastasis of prostate cancer cells can gain ERα activity due to reduced androgen levels in the context of interactions with osteoblast and osteoblast precursors in the bone marrow tumor cell microenvironment.

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