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Androgen regulation of epithelial–mesenchymal transition in prostate tumorigenesis

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Abstract

Prostate cancer patient mortality is ascribed to the spread of cancerous cells to areas outside the prostate gland and the inability of current treatment strategies to effectively block progression to metastasis. Understanding the cellular mechanisms contributing to the dissemination of malignant cells and metastasis is critically significant to the generation of effective therapeutic modalities for improved patient survival while combating therapeutic resistance. In recent years, the phenomenon of epithelial–mesenchymal transitions (EMTs) has received considerable attention due to accumulating evidence indicating a role for this developmentally conserved process in tumorigenesis. Cancer cells at the invasive edges of tumors undergo EMT under the influence of contextual signals that they receive from the microenvironment, such as TGF- β . Also derived from developmental studies is the fact that EMT induction is reversible; thus, upon removal of EMT-inducing signals, cells occasionally revert to the epithelial state of their cellular ancestors via the process of mesenchymal–epithelial transition. This article discusses the current evidence supporting a central role for EMT and its reverse process, mesenchymal–epithelial transition, in the metastatic progression of prostate cancer to advanced disease and the involvement of androgen signaling in its regulation towards the development of castration-resistant prostate cancer.

Keywords

androgen axis; androgen receptor; cadherins; EMT; metastasis; prostate tumor progression; stem cells; TGF- β ; tumor microenvironment; vimentin; ZEB1/2

In 2011, prostate cancer continues to be among the most commonly diagnosed cancers among American men, with an estimated 217,730 new incidences being diagnosed in 2010 [1,2]. Approximately 299,200 American men died as a result of cancer in 2010, of which

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prostate cancer accounted for 32,050 (11%) fatalities, making it the second leading cause of cancer-related deaths among US men [1,2]. Prostate cancer mortality is ascribed to the failure of current therapies to cure metastatic forms of the disease, which often result in lymph and bone metastasis [3]. Disease incidence has consistently risen and prostate cancer is expected to become the leading cause of cancer-related deaths in men living in Western countries [4]. Curing prostate cancer requires a greater understanding of distinct biological events that differentiate indolent prostate cancer from advanced life-threatening disease.

The prostate is an androgen-dependent organ in which organogenesis, morphology and normal functioning are regulated via the androgenic/androgen receptor (AR) signaling axis [5]. In early stages of development, androgens act as the principal growth factor regulating the proliferation and differentiation of prostatic cells. Later in life, androgens ensure the maintenance and proper functioning of adult prostatic tissue. The actions of circulating androgens (testosterone and dihydrotestosterone) are elicited via interacting with the ligand-dependent AR steroid hormone receptor. In the absence of the ligand, the AR is predominately localized to the cytosol where it is maintained in an inactive complex via associating with chaperone and heat-shock proteins. Upon ligand activation, the AR dissociates from the protein complex before undergoing a conformational change to expose its nuclear localization signal, thereby allowing its translocation into the nucleus and subsequent dimerization. Once in the nucleus, the AR associates with androgen response elements located in the promoter regions of target genes to regulate the synthesis of proteins associated with numerous cell processes [6]. In the nongenomic mechanism of action, the activated AR remains within the cytosol and interacts with a number of downstream effectors associated with various signaling cascades regulating growth and apoptosis outcomes. The fundamental importance attributed to the androgenic/AR signaling axis within normal prostate tissue is relevant to pathological conditions.

Androgens act as drivers for the development of prostate carcinomas and facilitate malignant disease progression. During the initial stages of tumorigenesis, prostate cancer cells depend on androgens for growth promotion and inhibition of apoptosis; consequently, androgen blockade and/or impairing the androgenic signaling axis represents a first-line treatment for reducing the rate of androgen biosynthesis while lowering the levels of circulating androgens [7]. Appreciation of the central role of the androgenic signaling axis during prostate tumor initiation and progression to advanced disease has been driving the development of therapeutic strategies aimed at inhibiting androgen biosynthesis or inhibiting normal AR function [8]. Surgical or medicinal androgen deprivation therapy (ADT) is often administered in combination with anti-androgens for the treatment of locally advanced prostatic tumors [9]. ADT is often successful at halting tumor growth among the majority of prostate cancer patients. However, despite initial success, after a median of only 2–3 years, a number of patients relapse and develop metastatic castration-resistant prostate cancer (CRPC) [7]. The failure of ADT strategies to completely suppress androgen levels and the continued expression of functional AR within prostatic tumors sustains AR-mediated signaling, which contributes to development of CRPC [7]. While the involvement of androgen in the early stages of prostate cancer development is well established, the contribution of the androgen axis and AR signaling to the development of advanced CRPC remains to be fully defined. As opposed to the earlier stages of disease progression in which tumors are localized to the prostate organ, invasive forms spread to adjacent tissues and may ultimately metastasize to distant organs, most notably the bones and lymph nodes [9]. The development of CRPC is associated with the acquisition of genetic alterations, which allows cells to bypass their need for androgen; thus, cells are capable of surviving and proliferating despite being in androgen-depleted environments [4,9]. The emergence of hormone-independent disease is associated with distinct changes on AR-mediated signaling; thus, therapeutic blockade of the androgenic signaling axis fails to prevent metastatic disease

advancement [9]. Recent evidence has supported the notion that CRPC continues to rely on androgens via AR signaling [10]. Reduced nuclear AR staining is observed within prostate epithelial cells immediately after androgen ablation therapy; however, upon relapse, these receptors reactivate and establish nuclear levels similar to those prior to treatment within both epithelial and stromal cells occupying malignant as well as non-malignant tissues [11].

Prevailing theories concerning potential mechanisms by which androgenic/AR signaling is maintained in an androgen-depleted microenvironment and the effects of such on target gene expression, rest on firm evidence regarding AR mutations, amplification, overexpression and altered sensitivity impacting AR expression and/or function [10]. Aberrant activation of AR in the absence of hormone binding results in a differential program of target gene expression in androgen-dependent tumors compared with castration-resistant cancer [12]. Deregulated AR-associated cofactors and robust crosstalks with cytokines and growth factors can also facilitate AR activity in the advanced stages of the disease [10]. Strategies targeting the AR protein itself to inhibit its activity may ultimately effectively treat prostate carcinomas; however, a deeper knowledge pertaining to the mechanisms contributing to aberrant activation of the AR is needed to allow their development and therapeutic implementation [6].

Despite major advances and striking insights at the molecular and translational level, complete characterizations of the functional contributions of androgens and the AR signaling pathway during the development of CRPC are yet to be precisely established. Emerging evidence recently published by our group suggests that the epithelial–mesenchymal transition (EMT) processes activated during prostate tumorigenesis may be engaged in a functional exchange with components of the androgenic signaling axis, primarily the AR under the auspice of androgens towards the emergence of advanced androgen-independent tumors and major cytoskeletal changes characteristic of metastatic behavior [13]. Recapitulating processes associated with embryonic development are thought to underlie mechanisms of carcinogenesis. Consistent with this idea, a model of prostate organogenesis has recently been reported as a valid system for investigating prostate cancer based on the identification of prostate-specific developmental programs that arise during organogenesis and appear to be reactivated at certain stages of tumorigenesis [5]. EMT reactivation is recruited by the tumor microenvironment to facilitate prostate cancer initiation, a unique dynamic regulated by the androgen/AR signaling. The clinicopathological significance of EMT in human cancers is still very much debated. As the cellular landscape is being interrogated by proteomic analysis, EMT emerges as a process of great interest in metastatic progression. Dissection of the regulatory mechanisms through which EMT programs are elicited during normal development is important for our understanding of the contribution of tightly linked EMT processes to various stages of tumorigenesis.

EMT in normal development & its contribution to pathological events

During the early 1980s, Greenberg and Hay were among the first researchers to describe what would come to be known as EMT [14], when they discovered that fully differentiated epithelial cells could transform into mesenchymal-like derivatives [15–18]. In the simplest sense, EMT defines a complex mechanism by which epithelial cells are converted into their mesenchymal counterparts. Coordinated molecular and genetic events engendering dramatic phenotypic alterations are associated with the acquisition of mesenchymal traits leading to alterations in cell behavior [18]. Reversible or irreversible phenotypic conversions render adherent epithelial cells, with a defined apical–basal polarity axis, into spindle-like, motile mesenchymal counterparts that exhibit front–rear polarity and an enhanced invasive potential [16,18,19]. EMT activation endows cells with enhanced migratory capabilities,

invasive capacity and resistance to apoptosis, in addition to conferring stem cell-like properties [17–20]. The resulting migratory cells are capable of evading normal tissue architectural constraints. A number of extracellular signals are capable of triggering EMTs in a context-dependent manner, culminating in molecular, genetic and morphological changes that phenotypically convert epithelial cells into their mesenchymal-like counterparts [17,19–21]. The underlying stroma provides a source of stimulatory EMT signals (cytokines, growth factors and extracellular matrix [ECM] components), which activate numerous downstream signal transduction pathways [21,22]. Many of the EMT signaling pathways converge at the transcriptional level to modulate transcription factor activity to allow for EMT-associated gene-expression patterns [23]. Cooperation between diverse regulatory factors and their signaling networks is important for the temporal and spatial regulation of EMT-mediated tissue remodeling processes, which are now recognized as being critical for normal embryonic development.

The plasticity afforded to a fully differentiated epithelium, allows individual cells to dedifferentiate into mesenchymal-like derivatives. This phenotypic transformation represents a reversible process whereby several rounds of EMT and the reverse process, mesenchymal–epithelial transition (MET), allow for the formation of complex tissues in metazoans. EMT (sometimes referred to as type 1 EMT) was originally defined as an indispensable developmental program occurring throughout implantation, embryogenesis and organogenesis to generate migratory mesenchymal cells from a primitive epithelium to allow the generation of secondary epithelia upon induction of METs [19]. EMT takes central roles in tissue patterning and formation of the body plan and, without such processes, development will not proceed past the blastula stage [18]. Studies pursuing the mechanisms that control EMT activation throughout early development revealed that such processes take place later in life and are relevant to human disease. The adult epithelium harbors a dormant EMT program via maintaining factors that mediate EMT processes in a quiescent state [15]. Epithelial homeostasis is perturbed during certain pathologies, which consequentially reawakens EMT processes, yielding identical molecular and phenotypic manifestations [18]. However, one must also consider the evidence that inappropriate EMT activation is deleterious to adult organisms. Current models believed to underlie fibrosis, wound healing and tumorigenesis appear to reactivate early EMT events via identical signaling cascades [19]. Much attention has focused on understanding the involvement of recapitulated EMT processes in malignant disease progression in a variety of cancers, including prostate cancer.

Malignant cells must acquire multiple metastatic traits to assist with bypassing roadblocks necessary for metastatic disease progression [24]. Actively engaged in the metastatic cascade is a nonrandom succession of programed and distinct events escalating into the development of metastasis. Critical steps include the loss of cell adhesion, invasion through the basement membrane and into the local microenvironment, intravasation into the vasculature and extravasation upon reaching distant tissues [21,24,25]. Recently the phenomenon of EMT has emerged as a mechanism that malignant cells take advantage of in order to facilitate their dispersal throughout the body and is therefore viewed as an initial step in the metastatic cascade [21,24–26]. Carcinoma cells can induce EMT to acquire a malignant phenotype, thereby allowing benign tumors to develop into invasive and metastatic cancers exhibiting enhanced resistance to traditional anticancer therapies [18,27,28]. EMT activation endows both migratory and invasive properties to cancerous cells, allowing them to escape from the local tumor and subsequently invade the surrounding stroma [24,26]. EMT is also activated to mediate intravasation into the blood vessels, as well as extravasation upon reaching secondary organs [24,26]. In addition to these acquired capabilities, EMT confers enhanced resistance to apoptosis and stem-cell-like properties [17]. Cellular redifferentiation via MET conversions is important during the final stages of tumorigenesis as it contributes to the formation of distant metastases [26]. Upon

encountering foreign tissues, mesenchymal cells undergo MET to revert back to an epithelial phenotype for settlement and recolonization, while retaining the ability to generate new metastatic lesions. Cellular redifferentiation via MET conversions has been postulated as being an explanation for the similarity observed between primary tumors and the secondary lesions that typically arise in distant organs [24,26,29].

Androgens & AR signaling in EMT

The role of androgens and the AR in invasion and metastasis during prostate cancer is yet to be completely understood. A link between invasive potential (in both androgen-dependent and -independent cancer) within cells expressing AR and signaling via the AR has recently been suggested, based on observations of enhanced AR activity corroborating with enhanced cellular invasion [30]. Accumulating evidence has recently emerged in support of the involvement of EMT processes in the deregulation of the androgen signaling axis, which occurs during the development of advanced androgen-independent carcinomas. A recent study from our laboratory demonstrated the ability of androgens to independently induce EMT patterning within prostate cancer cells, resulting in substantial changes in cellular invasion and motility, thereby suggesting a potential role for nongenomic AR signaling in dictating EMT processes [13]. Furthermore, an inverse relationship was established between AR levels and androgen-mediated EMT induction, suggesting that low levels of AR may function to promote EMT and, thus, the dissemination of malignant cells [13]. Androgen-mediated activation of β -catenin may also serve as an alternative mechanism by which androgenic signaling induces EMT of prostate tumor epithelial cells [13].

The cadherin family & 'cell bonding'

Dissolution of intracellular adhesion occurs upon EMT induction; therefore, the regulation of cell adhesion complexes is pertinent to human disease. However, the dissolution of cell-cell adhesion alone will not confer metastatic disease progression; loss of epithelial (E)-cadherin via the induction of EMT is associated with it [31]. E-cadherin is a cell adhesion molecule that is expressed on the surface of epithelial cells and is necessary for establishing intracellular contacts between neighboring cells to maintain tissue integrity and confer homeostasis. Expression of E-cadherin is downregulated or lost altogether during both developmental and pathologic EMTs, the latter of which has been well established and inversely correlated with Gleason grade. The loss of expression or dysfunction of E-cadherin-mediated cell-cell adhesion promotes invasion and metastasis [32,33]. Among the identified EMT cell surface markers, E-cadherin is considered the prototypical epithelial marker; E-cadherin has therefore been implicated to be of prognostic value in prostate cancer as an indicator of clinical disease progression. The loss of E-cadherin expression or function is considered a hallmark of EMT, which can be the result of genetic alterations, epigenetic inactivation and/or transcriptional repression of the *CDH1* gene (from which E-cadherin is transcribed) [34]. Among the most extensively studied mechanisms of *CDH1* gene repression is the transcription factor-mediated repression of gene activity. A number of transcription factors are capable of repressing the *CDH1* gene and recent evidence has uncovered a potential role for the AR that resembles two well-established EMT-associated transcriptional repressors, Snail and Twist. The activated AR has recently been shown to promote EMT activation via suppression of E-cadherin expression within breast cancer cells [35].

Exchanges in the expression of cadherin isoforms from one form to another is a process termed 'cadherin switching' [36]. Coexpression of membrane-localized neural (N)-cadherin, either via its upregulation or in place of E-cadherin, is typically associated with cadherin switching [36]. Cadherin switching is activated during development to allow cellular

segregation, whereas during tumorigenesis, this process is effectively utilized by the tumor cells for metastatic spread [36]. A rapid switch from E- to N-cadherin expression via EMT in primary prostate tumors is capable of predicting tumor recurrence and patient mortality [37]. Consequently, the phenomenon of cadherin switching has been recognized as a characteristic of EMT induction and has been associated with the development of metastasis.

Neural cadherin is a mesenchymal-associated adhesion molecule that is expressed in multiple cell types, including smooth muscle cells, myofibroblasts, endothelial cells, neurons and neoplastic cells [38]. Enhanced expression of N-cadherin results in reduced intracellular adhesiveness by allowing only transient cell–cell contacts to be established [39]. In addition to its role in cell adhesion, N-cadherin is involved in cell signaling, formation of motile structures, actin cytoskeletal remodeling, regulating EMT processes and invasive cellular behavior [40]. Aberrant expression of N-cadherin within prostate cancer cells is capable of driving EMT, invasion and metastasis [36,41,42]. N-cadherin expression is increased upon androgen deprivation and its inappropriate expression is implicated in the development of metastatic CRPC [41,42]. High levels of N-cadherin in castration-resistant prostate tumors are largely confined to poorly differentiated areas and significantly correlate with increasing Gleason grade [41]. Enhanced expression of N-cadherin results in reduced intracellular adhesiveness or transient cell contacts and can also regulate interactions occurring between stromal fibroblasts and prostate tumor epithelial cells, thus promoting cell motility, invasion and metastasis [39]. In the clinical setting, elevated N-cadherin expression has been identified as a significant predictor of clinical recurrence in prostate cancer patients following radical prostatectomy [37].

Therapeutic targeting of N-cadherin in CRPC using monoclonal antibodies has recently been shown to be a successful strategy in delaying the emergence of prostate cancer to advanced disease [42]. ADH1 is a known inhibitor of N-cadherin that has been explored for its potential therapeutic use due to its ability to inhibit angiogenesis and prostate tumor progression *in vivo* [43]; however, it was recently demonstrated that ADH1 failed to effectively block tumor growth in a PC-3 xenograft model of human prostate cancer [43]. ADH1 effects are thought to be multifaceted and complex, therefore, future studies are needed to fully evaluate the efficacy and therapeutic impact of anti-N-cadherin-based approaches [44]. Additional insights into the potential mechanisms by which AR signaling regulates N-cadherin expression will also aid future studies aimed at developing novel therapeutic targets for CRPC harboring functional AR. In addition to the two rather well-known cadherin proteins associated with the cadherin-switching phenomenon, an additional adhesion molecule that has emerged recently is cadherin-11. Aberrant expression of this mesenchymal-associated adhesion molecule has been observed in multiple cancers types, including prostate cancer. Cadherin-11, also known as osteoblast (OB)-cadherin, is not normally expressed by prostate epithelial cells but has been detected in prostate cancer cell lines derived from bone metastasis. It has been implicated in prostate cancer progression as a facilitator of the metastatic spread of tumorigenic cells to bone [45,46]. OB-cadherin is expressed in prostate cancer cells, osteoblasts and stromal cells associated with prostatic carcinomas [44]. Cadherin-11 expression is observed in the prostate stroma and membranous expression is associated with high-grade cancers [47]. Recently Lee *et al.* have demonstrated that the expression of cadherin-11 in prostate cancer cell lines is reduced by androgens, and depletion of androgens results in enhanced expression of cadherin-11 [46]. AR activity may indirectly modulate cadherin-11 gene expression at the transcriptional level via downstream regulators [46]. Targeting N- and OB-cadherins using pharmacological antagonists may effectively reduce metastatic disease progression [44].

β-catenin serving as an AR activity coordinator

The Wnt signaling pathway has important roles in embryonic development, and deregulation of this signaling axis has been associated with processes involved in tumorigenesis, including EMT activation. Several lines of evidence have implicated the Wnt signaling pathway in prostate tumorigenesis in the context of androgenic signaling. Thus, components of the Wnt signaling pathway engage in crosstalk with the androgenic/AR signaling axis and are capable of modulating the transcriptional activity associated with the AR [48]. Wnt factors including β-catenin, glycogen synthase kinase (GSK3)β, the lymphoid enhancer-binding factor 1 (LEF1) and cyclin D1 have been identified as interacting with the androgenic signaling axis [49,50]. This article will focus solely on β-catenin due to its established role in induction of EMT processes throughout development and tumorigenesis via activating specific gene-expression patterns [51].

β-catenin undergoes marked changes in its expression and/or localization during prostate carcinogenesis. Noninvasive cells typically exhibit β-catenin on the membrane, whereas invasive cells that have undergone EMT changes have more diffused cytosolic and nuclear staining patterns [52]. E-cadherin localizes β-catenin to the cell membrane in fully differentiated epithelial cells and therefore acts as a negative regulator of the Wnt signaling pathway [51]. As illustrated in Figure 1, β-catenin functions as a cell–cell adhesion molecule when maintained in cadherin–catenin complexes associated with adherence junctions. β-catenin is liberated from adherence junctions upon the loss of E-cadherin and dissolution of cell–cell contacts during EMT, resulting in its cytosolic relocation. Compartmental relocation of β-catenin is frequently observed during EMT, allowing its signaling function in the cellular networks. Cytosolic β-catenin is maintained in a multiprotein complex and its levels are regulated by GSK3β-targeted proteosomal degradation in the absence of Wnt signaling. β-catenin degradation is inhibited upon activation of Wnt signaling or via inhibition of any of the components associated with its destruction (antigen-presenting cell, axin, GSK3β), resulting in its cytosolic accumulation. Eventually, β-catenin translocates into the nucleus where it acts as a coactivator for target genes, including the T-cell factor/ lymphocyte-enhancer factor (*TCF/LEF*) and *AR* genes [53].

Reports of immunohistochemical analysis of β-catenin within prostate tissue samples have produced variable results [48]. Reduced or loss of expression of β-catenin and E-cadherin have been observed in primary prostate cancer cells [54], while in striking contrast, overexpression of membranous β-catenin and E-cadherin is associated with metastatic prostate cancer cells localizing to the bone [54]. Nuclear β-catenin signaling has been linked to prostate cancer progression; however, contradictory publications demonstrate a significant reduction in the nuclear expression of β-catenin in human prostate tumors with increased Gleason grade [53].

Physical interactions between β-catenin and AR have been demonstrated experimentally [48]. β-catenin can impact AR signaling via binding to the AR and acting as a coactivator; hence, its over-expression has been associated with androgen-independent forms of prostate cancer [50,53,55]. Increased levels of both the AR and β-catenin have been observed in CRPC and are associated with enhanced protein interaction and co-localization to the nucleus [50]. Targeting these interactions may prohibit the transcriptional activation of the AR, thereby representing a novel therapeutic strategy [50]. Similar to the signaling mechanism via which β-catenin engages the AR, the AR is capable of signaling through the Wnt/β-catenin pathway affecting CRPC in a ligand-independent manner [55]. The AR has also been shown to compete with TCF/LEF for available β-catenin [48,55]. Development of pharmacological agents that specifically target AR signaling via the Wnt/β-catenin pathway may represent effective therapeutic strategies for blocking aberrant AR signaling,

functionally engaged by prostate tumor epithelial cells to overcome the low castrate-levels of androgens associated with CRPC [55].

Snail: setting the EMT pace

The zinc-finger transcription factor Snail (SNAI1) is recognized as a master regulator of EMT that binds to E-boxes in the E-cadherin promoter to silence gene expression. Slug (SNAI2) is also a member of the Snail superfamily. Snail is involved with gastrulation within developing embryos and normal development cannot proceed without it [15]. Upregulation of Snail is found in a number of human cancers and has been correlated with tumor grade. Interestingly, AR has recently been reported to function in a manner similar to that of Snail and Twist, two well-established EMT-associated transcriptional repressors of E-cadherin [35]. Activated AR was capable of suppressing E-cadherin gene expression in both metastatic prostate and breast cancer cell lines, thus giving rise to a mesenchymal-like cell morphology [35]. Overexpression of Snail has been implicated as an early event in prostate cancer progression [56]. While Snail transcription factors bind to conserved E-box consensus sequences within the E-cadherin promoter region to repress its activity, the functional involvement of AR in E-cadherin transcriptional control is being investigated. Snail factors undergo post-translational modifications to regulate their compartmental localization and fate (degradation) [18]. The 14-3-3 proteins bind directly to transcription factors including Snail in order to modulate their functions [57]. Recently, the 14-3-3 σ isoform has been implicated with the cytoplasmic relocalization of Snail following protein kinase D1-mediated phosphorylation of its Ser11 residue within prostate cancer cells [58]. Therefore, protein kinase D1 appears to function, in part, as a novel regulator of Snail-mediated EMT [58]. The ability of Snail to drive cancer cell-mediated degradation of the basement membrane, angiogenesis and intravasation has been shown to be mediated at least in part by membrane type 1 matrix metalloproteinase and membrane type 2 matrix metalloproteinase [59].

Recent evidence established an exquisite cell death regulatory ability for Snail, primarily by acting as a survival factor within prostate cancer cells via inhibiting senescence [60]. The translational significance for such a molecular action is perhaps best reflected by studies on loss of Snail expression via RNA interference, suggesting its potential therapeutic value due to its ability to induce MET reversion while reducing invasiveness *in vitro*, as well as reducing tumor growth while increasing cellular differentiation *in vivo* [61].

Twist: propagating EMT

Twist is a basic helix–loop–helix transcription factor that is highly expressed by invasive carcinoma cells and functions as a transcriptional regulator that controls the expression of multiple genes associated with EMT processes [62]. Twist transcription factors are required for normal development; however, their aberrant reactivation is frequently observed and serves as a marker for poor patient prognosis among many forms of human cancers [63]. Within prostate cancer cells, Twist is indicative of high-grade tumors, malignant disease progression and heightened resistance to anticancer therapies, all of which confer poor patient prognosis [63,64]. Twist imparts migratory and invasive capabilities to cells [62]. Twist1 is implicated in the development of therapeutic resistance to various anticancer agents, essentially by conferring resistance to apoptosis in tumor cells treated with paclitaxel and cisplatin [62,65]. A functional link between AR and Twist1 upregulation has been reported during oxidative stress in CRPC [65]. Overexpression of Twist in prostate tumors can promote EMT via mediating cadherin switching, and enhancing N-cadherin and fibronectin expression, while its expression is inversely correlated with E-cadherin levels [34,64]. Targeting Twist expression and/or impairing its function within tumors may prevent

EMT induction, while inhibiting the migratory and invasive behaviors of cancerous cells in addition to sensitizing them to chemotherapeutic agents [62].

SOX

The Sry-related high mobility group box (SOX) genes encode a family of transcription factors that are involved in regulating embryonic development and determination of cell fate [66]. They can be further subdivided into functionally related protein groups. The SoxE subgroup constitutes three members, SOX8, SOX9 and SOX10, which regulate developmental processes [66]. SOX9 has been implicated in the normal development and maintenance of the prostate gland. In the adult prostate, SOX9 is normally expressed by the basal epithelial cells; however, malignant epithelial cells aberrantly express this transcription factor [67]. During the early stages of organ development, SOX9 is required for differentiation of the prostate bud epithelia [68]. SOX9 has been implicated in prostate cancer, in which it appears as though the embryonic functions associated with this transcription factor are reactivated and contribute to tumorigenesis. Increased expression of SOX9 is associated with recurrent CRPC. Mechanistically, SOX9 expression in prostate cancer cells is regulated by Wnt/ β -catenin signaling, yet it is also a downstream target to AR; SOX9 is capable of directly interacting with the AR to transcriptionally regulate its expression [69].

Zinc finger E-box-binding homeobox tightly controls EMT

The zinc finger homeobox (ZFH) family of transcription factors consists of two members: zinc finger E-box binding homeobox 1 (ZEB1/TCF8/ZFH1A/AREB6/8EF1) and ZEB2 (SIP1/ZFH1B) [70]. Both ZEB1 and -2 are important during early embryonic development, however, only ZEB1 appears to be essential. The current knowledge regarding the roles and regulation of ZEB factors throughout development is lacking. ZEB1/2 transcription factors bind to paired CAGGTA/G E-boxes located in the promoter elements of target genes to activate or repress transcription [71]. The mechanisms responsible for determining whether ZEB factors act as coactivators versus corepressors have not been elucidated. The aberrant activation of ZEB1/2 factors is linked to tumorigenesis. Recent evidence has shown ZEB factors acting as activators for EMT processes by acting as transcriptional repressors downregulating the expression or organization of epithelial genes (namely *CDH1*, which encodes E-cadherin) and components of the cell polarity complexes (occluding, claudins and lethal giant larvae homolog 2) [23,51,70,72]. ZEB1 has also been demonstrated to facilitate the acquisition of invasive and migratory properties during EMT by repressing the expression of components associated with the basement membrane and polarity proteins along the invasive front of carcinomas [51,72,73].

Multiple studies have supported a central involvement for ZEB1 in the later stages of malignant disease development in multiple human carcinomas [74]. ZEB1 is implicated in the development of malignant carcinomas in many cancers, including prostate cancer, and the enhanced expression of ZEB1 has been correlated with high Gleason scores in prostate cancer [75,76]. ZEB1 has been suggested to be a marker for metastasis in prostate cancer [76]. The recent work of Drake *et al.* investigated the role of ZEB-mediated repression in the context of prostate cancer, revealing its function as an EMT regulator that is required for transendothelial migration within a subpopulation of prostate cancer cell lines [77]. At the molecular level, mutation of the *ZEB1* gene induces MET reversion [78]. Loss of ZEB1 in prostate cancer cells that have undergone EMT partially induces re-expression of E-cadherin, thus triggering reversion towards an epithelial-like morphology [77]. Thus ZEB1 emerges as a potential target for therapeutic impairing of EMT and ultimately metastasis

[77]. However, complete reversion to an epithelial-like phenotype may not be achieved by targeting ZEB transcription factors alone [79].

The signaling pathways responsible for activating and modulating ZEB factor activities are yet to be fully understood. ZEB1/2 have been recognized as being downstream targets for TGF- β -mediated EMTs. TGF- β induces the context-dependent expression of ZEB1/2 during tumorigenesis via signaling through downstream Snail and activated Smad proteins [70,80]. IGF-1 has also been reported as being a key regulator of ZEB1-mediated EMT processes that are activated during prostate carcinogenesis, in which its expression is enhanced [75]. Previous data have provided evidence for mechanisms in which ZEB factors are hormonally regulated, however, few studies have examined these interactions. ZEB1 has been shown to be capable of being regulated by steroid hormones that may be deregulated during carcinogenesis [81]. Recent work by Graham *et al.* demonstrated a reciprocal interaction taking place between the AR and ZEB1 in triple-negative breast cancer cell lines. In this model, ZEB1 regulates the AR by directly binding with the AR promoter, whereas AR-mediated regulation of ZEB1 occurs via an unknown intermediary protein [82]. Anose *et al.* reported that ZEB1 is regulated by androgen and its expression is upregulated in response to treatment with dihydrotestosterone yet is downregulated upon metastasis [76]. Furthermore, both ZEB1 and prostate-specific antigen (PSA) expression was enhanced following treatment with the antiandrogen flutamide [76]. Further studies are needed in order to determine the relevance of ZEB1 in the context of prostate tumorigenesis and to delineate the potential crosstalk with components of the androgenic/AR signaling cascade. Targeting ZEB factors in conjugation with targeted therapies against androgens/AR may represent a novel strategy for targeting processes implicated in the development of CRPC for improving patient survival.

EMT impacts the tumor microenvironment

A multitude of signals dictating cell fate, proliferation, morphogenesis, growth and differentiation are provided by the cellular microenvironment. The prostate gland consists of an epithelial compartment that is embedded in a surrounding stromal compartment that is comprised of fibroblasts, myofibroblasts and smooth muscle cells that express the AR [47]. The underlying stroma is imperative for the establishment and maintenance of normal tissue architecture. The tissue situated immediately adjacent to tumors is histologically distinct from the normal stroma and is therefore referred to as the reactive stroma [83]. A severely altered stroma significantly influences tumorigenesis [84] in the context of the tumor microenvironment. A number of nonepithelial components, including ECM, fibroblasts, smooth muscle cells, endothelial cells, immune cells, inflammatory cells and vasculature components, constitute the tumor microenvironment [83]. During tumorigenesis, the microenvironment undergoes alterations engendering a growth-promoting state that can elicit signals leading to the activation of EMT processes reminiscent of developmental programs [21].

TGF- β

TGF- β signaling takes a lead role in tumor–stroma interactions and regulation of the prostate microenvironment [85]. Stromal expression of this connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis [85]. By virtue of the central regulatory role played by TGF- β in coordinating cell growth and apoptosis during normal prostate homeostasis, an imbalance in either the production of, and/or the response to, TGF- β results in growth perturbations that contribute to prostate tumor development and progression [86,87]. During prostatic development, androgenic action is suppressed within the smooth muscle cells via TGF- β -mediated translocation of nuclear AR into the cytoplasm [88]. Increased expression of TGF- β is found in patients with advanced prostate cancer [89] and

elevated TGF- β promotes tumor suppression, primarily through paracrine effects on stromal elements such as increased angiogenesis and decreased immune surveillance. Enhanced expression of this cytokine is associated with both stromal and epithelial compartments in prostate carcinomas [47]. TGF- β -mediated induction of EMT processes is associated with specific stages of morphogenesis and during tumorigenesis by activating downstream signaling pathways in both Smad-dependent and -independent mechanisms. TGF- β may have direct tumor-promoting properties on the epithelium in advanced tumors through the induction of EMT, leading to alterations in ECM production and adhesion molecule regulation or localization [90]. Compelling support for this concept stems from studies documenting that the stroma promotes prostate tumorigenic growth and angiogenesis via targeting TGF- β effectors: expression of the connective tissue growth factor, a key TGF- β signaling mediator in tumor-reactive stroma [85]; decreased β -catenin signaling in the early stages [91]; and TGF- β mediated increased caveolin-1 secretion into the microenvironment inhibiting prostate cancer cell apoptosis during perineural invasion [92].

TGF- β 1 exerts opposing functions during prostate tumorigenesis, that is, initially promoting tumor suppression and later enhancing tumor progression and metastasis. Eventually cells become insensitive to the growth inhibitory signals emanating from TGF- β [47]. The concept that androgens, via the AR, play a critical role in the deregulation of TGF- β 1 signaling in prostate tumorigenesis and that TGF- β 1 effectors (Smads 3 and 4) serve as negative regulators of AR-mediated transcription in prostate cancer cells has been established by several investigations. Our work on the cross-talk between the androgen axis and TGF- β 1 signaling revealed that androgens enhance the apoptotic effects elicited by TGF- β 1 in prostate cancer cells LNCaP T β R11 with a mutant AR. Cytokine signaling is blocked upon loss of the TGF- β receptor II, which acts as a promoter for metastasis formation in prostate cancer cells [93]. Studies by Cano and colleagues demonstrated that the AR-mediated transcriptional activity of prostate stromal cells is modulated by epithelium-mediated recruitment of AR-associated coregulators. Stromal cells isolated from carcinoma tissue displayed reduced AR transcriptional activity and such activity was thought to be manifested by the recruitment of AR coregulators via associated epithelial cells [94]. Thus, changes within the androgen signaling axis in prostate tumors arise due to altered stromal–epithelial cell interactions and recruitment of aberrant AR coregulators [94].

Considering that TGF- β -directed EMT is a key process driving the development of metastatic prostate cancer, it seems feasible that administering neutralizing antibodies or shRNA to inhibit cytokine signaling may represent an effective treatment strategy [95]. Novel therapeutic strategies aimed at inhibiting critical interactions associated with the AR and TGF- β signaling networks may be used to treat prostate cancer patients by administering AR antagonists in combination with TGF- β -targeted therapies [95]. Other potential EMT-based therapies include selective targeting of tumor–stroma interactions, specific stromal components, interfering with the actions of some soluble factors secreted from the stroma, as well as blockade of cell–cell communication and cell–ECM interactions [28].

Vimentin

Tumors depend on a supportive microenvironment that fosters invasion and dissemination of neoplastic cells. Microenvironmental remodeling is characteristic of EMT induction and is known to enhance tumor survival [96]. Expression of mesenchymal markers within the tumor microenvironment reflects the acquisition of traits conferring altered cellular behaviors and motility [96]. Vimentin is a well-characterized type III intermediate filament protein that is highly expressed in mesenchymal cells; thus, enhanced levels of vimentin currently serves as a marker for identifying cells that have undergone EMT [97]. Recent observations of vimentin immunoreactivity within tumor-associated microenvironments

have raised the possibility of using this mesenchymal intermediate filament as a marker of a tumor microenvironment that has undergone remodeling [96]. The switch from keratin expression to vimentin during the EMT processes is associated with diminished cell–cell adhesion, allowing enhanced invasive capacity. Vimentin expression is downregulated during MET transitions and upon silencing of Snail [61]. Expression of this atypical intermediate filament in epithelial cells is actively involved with the resulting phenotypic and behavioral alterations associated with EMT. Mendez *et al.* reported that vimentin alone was sufficient for producing a mesenchymal cell morphology in which cells appeared more flattened and exhibited enhanced motility [98]. Moreover, vimentin confers invasive properties to prostate cancer cells, with the acquisition of value in predicting aggressive metastatic forms of prostate cancer [97].

Matrix metalloproteinases

Among the most notable proteases implicated in tumorigenesis is the matrix metalloproteinase (MMP) family of zinc-dependent endopeptidases [99]. MMPs can be classified as being membrane-associated or secreted [100]. MMPs normally play important homeostatic roles within the cellular microenvironment, roles that become deregulated during carcinogenesis [99]. MMPs function in ECM remodeling, induce cellular motility and stimulate morphological changes in cells [101]. These proteases promote tumor-cell invasion and metastatic progression by activating proteolytic degradation of the ECM/ basement membrane, altering cell–cell and cell–ECM interactions [102]. Tumor-derived MMPs have also been identified as promoters of processes associated with EMT [99,101]. Elevated levels of MMPs are associated with prostate cancer progression to metastasis and poor prognosis [101]. MMPs are overexpressed and act as promoters of prostatic tumor growth and metastasis [103]. Overexpression of several MMPs has been correlated with EMT, including MMP-2, -3, -9, -13 and -14 [102]. MMP-2 and -9 were implicated as having enhanced invasive capacities expressed by both androgen-dependent and -independent cells and, therefore, may be regulated by AR signaling [30]. Two members of the tissue kallikrein family of serine proteases, PSA/kallikrein-related peptidase 3 (KLK3) and KLK4 serve as prognostic markers for hormone-refractory prostatic tumors and have been shown to induce EMT prostate carcinomas *in vitro* [104]. While both of these factors are known to be regulated by androgen, KLK4 has emerged as a prospective therapeutic target due to its potential roles related to EMT and ECM degradation, among other processes implicated in prostate tumorigenesis [105]. Attempts have been made to control the secretion, synthesis, activation and activity of MMPs via the development of MMP inhibitors [102]. Peptidomimetic inhibitors, nonpeptidomimetic inhibitors, chemically modified tetracyclines, novel mechanism-based inhibitors, off-target inhibitors and natural inhibitors of MMPs have been exploited for pharmacologic targeting of MMPs [102], but have been met with severe challenges, hindering their therapeutic value.

Linking EMT to stem-cell signatures

Stem cells typically constitute a small percentage of the cellular population within adult organs and are necessary for regenerating the epithelial compartment by differentiating into one of the specialized progeny cells comprising the tissue. Stem cells inhabit a niche (or specialized microenvironment) that in the prostate represents the proximal region of murine prostatic ducts and is the area from which carcinomas originate [106]. Evidence suggests that prostatic stem cells exist in both the basal and luminal cell populations [107,108]. Specific stem-cell subpopulations have been associated with normal and diseased prostate tissue [107]. Recent studies suggest that prostate cancer arises from a subpopulation of tumor-initiating cells that display a stem cell-like signature and are capable of seeding new tumors and are self-renewing [109–111]. These so-called cancer stem cells (CSCs) have been implicated in tumor recurrence and progression to metastatic disease in a number of

solid tumors, including CRPC [109,110]. Similar to normal prostatic stem cells, CSCs also possess self-renewing capabilities and are capable of giving rise to terminally differentiated cell types [112].

Epithelial–mesenchymal transitions have been mechanistically linked with the generation and maintenance of stem-like cell populations during tumorigenesis [20,111]. Prostate cancer cells that have undergone EMT appear to be phenotypically and genomically similar to stem cells [110,113]. Thus, yet another consequence of EMT processes is the generation of CSCs and the development of therapeutic resistance [20,112]. Effective elimination of CSCs may dramatically improve the therapeutic efficacy of future anticancer approaches [28]. Promoting differentiation of cancer stem cells towards a more epithelial-like state via treatment with miRNAs, histone deacetylase inhibitors and TGF- β inhibitors, among other components, has recently emerged as an attractive strategy for eliminating cancer stem-like cells [112]. This concept gains direct support from independent studies demonstrating that elimination of cancer stem-like cell populations via selectively targeting them to undergo MET may have therapeutic value in the treatment of CRPC [110]. Understanding the interactions between the epithelial stem-cell niche and the adjacent stromal mesenchyme will enhance our understanding of the complex signaling dynamic of cellular and molecular events activated during oncogenesis, as elegantly demonstrated by Blum *et al.* [106].

A functional link between CSCs, EMT and miRNAs has been suggested, which contributes to tumor metastases [114]. Kong *et al.* have shown that the miRNAs *miR200* and *let-7* are involved in linking the EMT phenotype with features of CSCs [110]. miRNAs are capable of regulating both normal stem cells and CSCs [115]. Markers capable of identifying CSCs have been established and include the cell adhesion protein CD44. CSCs in various human cancers, including prostate cancer, express the CD44 adhesion molecule. A recent publication by Liu and colleagues illustrated the ability of the miRNA, *miR34a*, to suppress CD44 expression within CSCs for effectively blocking tumor progression and the subsequent development of metastasis in a prostate cancer xenograft model. CD44-positive CSCs are associated with reduced *miR34a*, which facilitates prostatic tumorigenesis. Thus, *miR34a* represents not only a promising agent that can be exploited for therapeutic targeting to combat the tumor-promoting effects of prostatic CSCs, but in the context of controlling CSCs, this miRNA may also have potential value as a molecular marker of prostate cancer dissemination and progression to metastasis [115]. miRNAs have been identified to regulate specific stages associated with tumorigenesis, including driving the EMT program, which can facilitate malignant progression upon dysregulation [116]. The *miR-200* family, *miR-205* and *miR-155* have emerged as primary regulators of EMT [114,116]. Thus, RNA interference has emerged as a novel strategy aimed at targeting EMT [28,114]. The *miR-200* family is comprised of *miR-200a*, *miR-200b*, *miR-200c*, *miR-141* and *miR-429*, all of which have been discovered as being capable of reverting EMT and inducing cellular differentiation [71]. The *miR-200* family and *miR-205* are reported to induce epithelial differentiation via targeting the ZEB1 and silencing its expression [117]. The *miR-200* family of miRNAs appears to be engaged in a negative-feedback loop with ZEB factors in which miRNA-mediated repression of ZEB1 triggers an increase in E-cadherin expression [77]. The ability of drugs to manipulate the direction of this loop has yet to be fully evaluated or understood [71]. Interactions between miRNAs and the AR have also emerged, suggesting a link between miRNA expression and AR status and consequently an opportunity for therapeutic intervention strategies [118]. Recent elegant studies by Kallioniemi's group utilized a systematic analysis approach to reveal several miRNAs that appear to post- transcriptionally regulate the AR by interacting with the 3'UTR and therefore offer strategies for future therapeutic development [119]. It is crucial for future research efforts to uncover the complex mechanisms by which specific miRNAs affect gene expression in order to facilitate the development of miRNA-based therapies.

Expert commentary

Interrogation of EMT regulator networks emerges as an attractive and innovative avenue for therapeutic discoveries in cancer patients. The failure of current treatment options to efficiently control metastatic disease progression has led to the idea of gating the metastatic potential of cancer cells by blocking their acquisition of invasive and migratory capabilities as a viable treatment approach aimed at reducing patient mortality without actually curing the disease. Induction may be stimulated by certain anticancer treatments; thus, administering EMT inhibitors in conjunction with other anticancer agents may reduce tumor recurrence and impair metastasis [120]. Therefore, one can easily argue that the potential of EMT to contribute to patient mortality via the development of therapeutic resistance and tumor recurrence can no longer be ignored [20]. Induction of EMT may revert cancerous cells toward a dedifferentiated state, exhibiting stem cell-like characteristics that endow cells with self-renewing capabilities [20]. Cells can acquire a stem cell-like phenotype during pathological processes including tissue injury and tumor development [112]. The significance of EMT in human cancers is still being investigated in both the prognostic and therapeutic settings. Metastatic disease progression and therapeutic resistance result from EMT induction, both of which are attributed to patient mortality. The clinical relevance of this enticing process in tumor progression to metastasis must be addressed in future studies. The need for improved prognostic markers has generated momentum in the evaluation of clinically useful EMT biomarkers capable of indicating the stage, grade and metastatic potential of prostate tumors. Therefore, future studies are necessary in order to highlight the principle components of EMT networks that are capable of serving as markers for improving patient prognosis and to facilitate the development of targeted therapies for preventing or reversing metastasis.

Five-year view

The contribution of EMTs at specific stages of carcinogenesis represents a novel mechanism that dictates metastatic behavior and is ultimately responsible for the lethal developments associated with advanced disease. While this theory has generated extensive debate regarding the 'authenticity' of the natural EMT as a pathological phenomenon, the striking frequency at which reports of EMT process in relation to human disease have emerged in the literature in recent years provides strong evidence in support of this argument. That being said, many fundamental questions regarding the hormonal regulation of pathologic EMTs remain to be answered. In the coming years, efforts in defining the functional significance of EMT in human malignancies should focus on addressing the uncertainties associated with the EMT process in the metastatic cascade of tumor progression as a causative feature of, rather than a consequence of, the metastatic phenotype. Initial studies linking stem cells and EMT as the differentiation program point to the possible existence of plasticity between stem cells and their more differentiated derivatives. Functional exploitation of EMT regulators should be directed at identifying their contribution to the existence of circulating tumor cells towards seeding new tumors at distant sites, and the development of castration-resistant prostate tumors, regardless of their AR status. The current excitement that surrounds EMT calls for a rigorous mechanistic insight into the EMT program and its ability to interact with components of the androgenic signaling axis. Therefore, in the coming years, it is imperative to elucidate the underlying mechanisms that contribute to the emergence of CRPC by promoting EMT. Ongoing pursuit of the dynamic cross-talk between androgens and TGF- β signaling as emerging regulators of EMT will undoubtedly lead to the development of a new platform for the identification of novel therapeutic targets.

Numerous studies investigating the mechanisms via which EMT enables the escape of individual malignant cells from localized tumors are driven by solid experimental

foundations on the role of EMT in the process of extravasation and intravasation during metastasis. However, the efforts invested in understanding the contributions of EMT in the development of drug resistance and therapeutic failure of cancer patients have been considerably less concerted. In fact, some of the controversy surrounding EMT in cancer seems to arise from the possible notion that EMT induction may actually be stimulated by certain anticancer agents; thus, the development and use of agents acting as inhibitors of the EMT signaling network should be studied in combination with traditional cancer therapies for reducing tumor recurrence and improving patient survival. The failure of current treatment options to effectively control metastatic disease progression has generated the notion that gating the metastatic potential of malignant cells by blocking their acquisition of invasive and migratory capabilities is a viable treatment approach aimed at reducing patient mortality without actually curing the disease. Insights into the mechanisms by which rounds of EMT/MET conversions facilitate the progression of localized prostate carcinomas to advanced metastatic disease appear to provide an attractive platform for drug development. Targeting individual cells actively engaged in EMT interconversions, including CSCs, may potentially halt the dissemination of cancerous cells and ultimately reduce lethality associated with malignant disease progression. Appropriate *in vivo* experimental models, improved molecular probes and proteomic-based studies must be pursued in order for us to move towards unraveling the complexity of EMT processes at the molecular level and defining their significance at the translational level.

Key issues

- Epithelial–mesenchymal transition (EMT) is a developmental program that is relevant to human disease upon being aberrantly reawakened within adult tissues.
- The process of metastasis involves multiple rounds of EMT/mesenchymal–epithelial transition towards the development of tumor lesions in distant regions of the primary tumor.
- An emerging role for EMT in the development of castration-resistant prostate cancer has recently been identified; however, additional mechanistic insights are required to fully appreciate the significance of EMT prostate tumor progression to metastasis.
- Cross-talk between many growth factor signaling cascades required for normal and pathologic prostate functions and components of androgen axis/androgen receptor (AR) signaling pathway, contribute to the emergence of androgen-independent disease.
- The androgenic/AR signaling axis has been demonstrated to promote EMT-associated events during pathological changes that are associated with prostate cancer progression to metastasis. Understanding the dynamics of such interactions may provide insight into the mechanisms by which the androgenic/AR signaling axis is sustained or deregulated in the emergence of castration-resistant prostate cancer.
- Dissecting the role of EMT processes during prostate tumorigenesis may allow the development of targeted therapies to enable improved patient prognosis and survival.
- Future studies should also aim to identify additional biomarkers for EMT processes, with better reliability and sensitivity and that are associated with prostate carcinogenesis.

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Figure 1. Regulation of epithelial-mesenchymal transition in prostate cancer

Reawakening of the epithelial-mesenchymal transition (EMT) program in prostate cancer epithelial cells engages signaling interactions among different growth factor pathways and the AR signaling axis. Individual components associated with each signaling cascade are color-coordinated. Major signaling pathways are shown, including the Wnt/β-catenin pathway (yellow), genomic and nongenomic androgen/AR signaling pathway (blue), cytokines grouped for simplicity (pink), and the TGF-β pathway (green). EMT-associated transcription factors relevant to prostate tumorigenesis via EMT regulation are shown in red. The signaling cascades converge on the EMT-associated transcription factors to control their activities. Activation of Twist, Snail and/or the ZEB factors leads to the initiation of EMT-associated gene transcription, which allows for mesenchymal gene expression. E-cadherin is silenced by the EMT-associated transcription factors. The loss of E-cadherin from the cell membrane is a hallmark of EMT-induction, which allows β-catenin to relocate to the cytosol where it interacts with AR and key EMT transcription factors. TGF-β signaling produces Smad-mediated responses, as well as Smad-independent phosphorylation of Par6. In response to TGF-β and/or androgens, Smads can interact with the AR, Snail, ZEB factors

and Twist towards EMT activation. Other growth factor pathways can crosstalk with the AR and EMT transcription factors to trigger a mesenchymal gene-expression pattern.

AR: Androgen receptor; LEF1: Lymphoid enhancer binding factor 1; P: Phosphorylation; RhoA: Ras homolog gene family member A; TCF: T-cell factor; ZEB: Zinc finger E-box-binding homeobox 1; ZO-1: Zona occludens 1.