Chapter 6
Epithelial-Mesenchymal Transition (EMT) and Prostate Cancer

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Abstract Typically the normal epithelial cells are a single layer, held tightly by adherent proteins that prevent the mobilization of the cells from the monolayer sheet. During prostate cancer progression, the epithelial cells can undergo epithelial-mesenchymal transition or EMT, characterized by morphological changes in their phenotype from cuboidal to spindle-shaped. This is associated with biochemical changes in which epithelial cell markers such as E-cadherin and occludins are down-regulated, which leads to loss of cell-cell adhesion, while mesenchymal markers such as vimentin and N-cadherin are up-regulated, thereby allowing the cells to migrate or metastasize to different organs. The EMT transition can be regulated directly and indirectly by multiple molecular mechanisms including growth factors and cytokines such as transforming growth factor-beta (TGF-β), epidermal growth factor (EGF) and insulin-like growth factor (IGF), and signaling pathways such as mitogen-activated protein kinase (MAPK) and Phosphatidylinositol 3-Kinase (PI3K). This signaling subsequently induces expression of various transcription factors like Snail, Twist, Zeb1/2, that are also known as master regulators of EMT. Various markers associated with EMT have been reported in prostate cancer patient tissue as well as a possible association with health disparities. There has been consideration to therapeutically target EMT in prostate cancer patients by targeting the EMT signaling pathways.

Keywords Epithelial-Mesenchymal Transition · Prostate Cancer · Transcription Factors · Growth Factors · Cytokines

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6.1 Introduction

In most metazoans, prostate epithelial cells are in close contact to the basal membrane, held together by tight junction and adherens junction proteins. However, during development, cuboidal epithelial cells undergo morphological and biochemical changes to transition into a mesenchymal phenotype which are more elongated and spindle-shaped. This process is called epithelial-mesenchymal transition or EMT, and can be divided into three different types primary, secondary and tertiary. The primary EMT takes place during early development and is well recognized at early gastrulation and neural crest development [1]. Gastrulation is described as the early formation of the three germ line layers (ectoderm, mesoderm, and endoderm) from the initial epithelial cells [2]. The post gastrulation is considered as the secondary EMT type, leading to formation of neural crest within ectodermal zones, thus giving rise to different cells such as neurons, bone, and mesodermal cells. At this point, the cells convert into epithelial type again by the reverse process of EMT called mesenchymal-epithelial transition (MET) [3]. The tertiary type of EMT can be well explained through a successive cycle of heart formation. During cardiac development, the mesodermal cells differentiate with other cardiac progenitors into two epithelial layers; another EMT process follows to form endothelial cell linings of the heart. The endothelial cells from atrioventricular canal undergo a tertiary EMT to form the endocardial cushion and later, the cells will assemble to form atrioventricular valvuloseptal complex [4].

Mesenchymal cells exhibit a front back end polarity with loss of structured cuboidal shape, and acquisition of mesenchymal markers which make this type of cells migratory, invasive and more resistant to apoptosis [5]. Molecularly, EMT is associated with loss of epithelial markers such as E-cadherin, occludin and zonula-occludens (ZO-1), and acquisition of mesenchymal markers such as vimentin, N-cadherin, and fibronectin [6].

Most patients with prostate cancer succumb to the disease due to the primary tumor metastasizing to an organ critical for survival such as the lungs or the liver [7]. Prostate cancer also has a propensity to metastasize to the bone [7]. Cancer cells have hijacked the EMT process to become invasive, migratory and acquire the ability to breakdown the basement membrane and metastasize (Fig. 6.1). However, not all the tumor cells are able to escape the primary organ and this phenomenon appears only in a specific population of the tumor cells [1]. EMT plays a critical role in cancer progression and metastasis [8]. Although the complete evidence of how the cancer cells undergo EMT is still ambiguous, strong evidence shows this process can be reproduced in animal models, including animal models of prostate cancer [9]. EMT is not characterized by a complete change in the cell identity, but more by a transient change in the cells' mobility and behavior. In tumors, incomplete EMT occurs where the cancer cells gain the mesenchymal characters while still expressing some epithelial markers, thus, without facing the complete transition as found within the embryo [9]. The majority of the death cases with prostate cancer are due

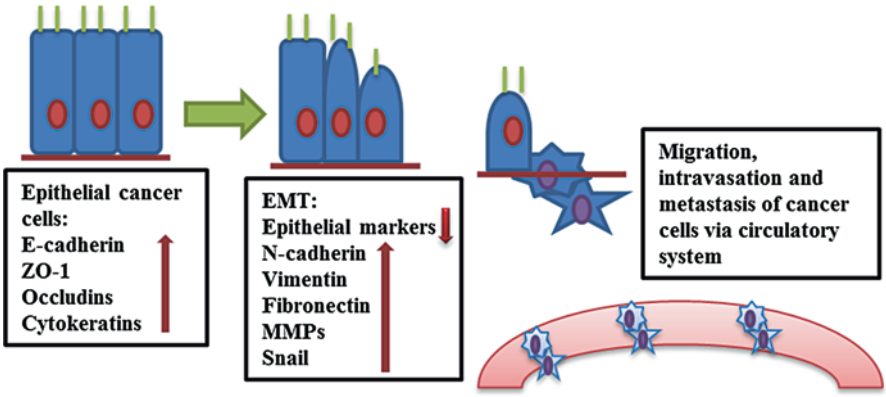

Fig. 6.1 Epithelial-mesenchymal transition (EMT) in cancer cells. Cuboidal epithelial cells can transition into spindle-shaped mesenchymal cells which is associated with downregulation of epithelial markers such as E-cadherin, zonula occludens-1 (ZO-1), occludins and cytokeratins, and metalloproteinases (MMPs) and Snail

to metastatic disease that does not respond to treatment, and that have become castration-resistant [10]. Androgenic /androgen receptor (AR) signaling plays a role not only in prostate organ development in early stages, but studies show that in the initial stages of tumorigenesis cancer cells depend on androgen to promote cell growth and inhibit apoptosis, but with androgen-deprivation therapy, some tumors with time become resistant and eventually metastatic [11]. EMT plays a critical role in the development of the metastatic castration resistant prostate cancer (mCRPC) [12]. Additionally, it has been reported that AR can repress E-cadherin and induce EMT; Liu et al., demonstrated that active AR is able to downregulate E-cadherin expression which led to loss of cell-cell adhesion and promotion of metastasis [13].

6.2 Transcription Factors that Regulate EMT

EMT can be induced by various transcription factors such as Snail, Slug and Twist [14]. Deficiency of Snail in the embryo leads to unsuccessful completion of the EMT process [15]. Snail transcription factor is a zinc finger protein, known as a master protein which regulates EMT. Snail regulates EMT by downregulating E-cadherin during both development and tumor progression [16]. Snail can regulate E-cadherin by binding to the E-box region within the E-cadherin promoter and repressing transcription in prostate cancer cells [17]. In prostate tumorigenesis, the high expression of Snail is associated with loss of E-cadherin [18]. In addition, Snail can also repress epithelial markers such as occludin and ZO-1 [17].

6.3 Growth Factors and Cytokines that Induce EMT in Prostate Cancer

Various growth factors and cytokines have been shown to contribute to the process of EMT in prostate cancer. Some of the growth factors and cytokines reported to play a role during prostate cancer progression are transforming growth factor beta (TGF-β), Insulin-like growth factors (IGF), epidermal growth factor (EGF), and CX3CL-1 [19–25]. The mechanism of action is through activation of growth factors and cytokines to their respective receptors leading to induction of signaling pathways downstream [26, 27].

Growth Factors and cytokines are secreted glycoproteins that act as signaling molecules to regulate various cellular functions [28]. The two words are often used interchangeably however, growth factors are assumed to have a positive role on cell proliferation whereas as cytokines can also have a negative effect on cell growth [28]. Some of the growth factors and downstream signaling pathways that regulate EMT in prostate cancer are shown in Fig. 6.2. One of the well-studied cytokines that plays a key role during tumor progression and metastasis is TGF-β. It has three family members namely, TGF-β1, TGF-β2, and TGF-β3 [29]. TGF-β has opposing roles during prostate cancer progression, as a tumor suppressor during the early disease stages and a tumor promoter in the later stages [21]. In the benign stages of prostate cancer, TGF-β binds to its receptors and activates its signaling pathway that leads to apoptosis [21]. It also mediates processes such as cell differentiation, cell proliferation

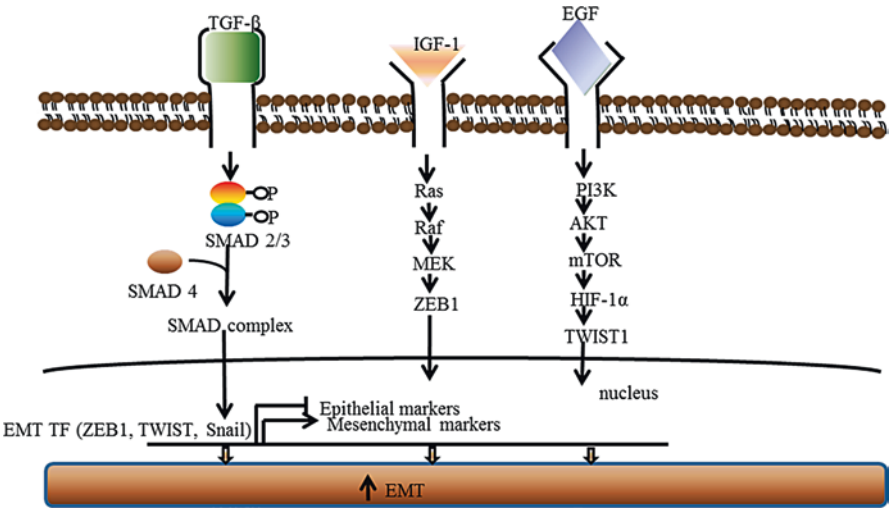

Fig. 6.2 Growth factor signaling pathways that triggers EMT in prostate cancer. Growth factors such as TGF-β, IGF-1 and EGF can trigger downstream signaling pathways such as MAPK and PI3K, that lead to activation of transcription factors (TF) such as Snail, ZEB1, TWIST. This eventually leads to downregulation of epithelial markers and upregulation of mesenchymal markers

and migration [21]. In late-stages of prostate cancer, TGF-β is shown to be up-regulated leading to increased cell invasion and metastasis [30]. It plays a role during EMT by downregulating epithelial markers such as E-cadherin and upregulating mesenchymal markers such as vimentin [31]. TGF-β cell signaling utilizes either a SMAD or non-SMAD pathway [21]. For the SMAD mediated pathway, TGF-β binds to its receptor, TGF-β type II receptor (TβRII) which leads to recruitment and activation of TβRI by phosphorylation at the serine and threonine residues [21]. The activated TβRI then recruits and phosphorylates SMAD 2 and SMAD 3 [21]. These two proteins then form complexes with SMAD4 leading to translocation into the nucleus where they regulate their target genes [21]. Examples of TGF-β signaling target genes are SMAD 7, p21, c-Jun, among others [19]. Thahur et al., showed that c-Jun binds to Snail promoter hence initiating migration and invasion of prostate cancer cells [19]. Some of the non-SMAD pathways are MAPK, mTOR, Ras, c-Src, PI3K/ AKT, RhoA, Cofilin, among others [21].

In most late stage tumors, TGF-β signaling components are lost or there are alterations in a downstream signaling component such as Ras activation [30]. One mechanism by which TGF-β signaling is altered in prostate cancer is through loss of TβRII, and this has been correlated with high grade tumors. Tu et al. did a study using transgenic mice with a TβRII mutation (DNIIR) that rendered it a dominant negative mutant [20]. They observed that the mutant mice had increased tumor metastasis compared to control mice [20], thus demonstrating that loss of TGF-β signaling is one mechanism by which it acquires its tumor promoter role in late stage prostate cancer [20]. Therapeutic treatments designed to target TGF-β signaling should seek to keep its apoptotic role while inhibiting the tumor invasion and metastasis role [30].

Insulin-like Growth Factor (IGF) is a growth factor that is known to regulate differentiation, apoptosis, proliferation, and cellular metabolism [22]. It has been implicated in prostate cancer bone metastasis [22]. IGF has two family members, IGF-I and IGF-II and two receptors, IGF-IR and IGF-IIR, as well as 6 binding proteins (IGFBPs 1–6) [22]. These proteins interact with each other as well as cross-talk with other signaling pathways [22]. IGF-IR is a tyrosine kinase receptor located on the cell membrane [22]. When IGF binds to its receptor it induces downstream signaling pathways such as mitogen-activated protein kinase (MAPK) and Phosphatidylinositol 3- Kinase (PI3K) [22]. Insulin-like Growth Factor (IGF) is a growth factor that has been reported to increase EMT in prostate cancer [23]. This occurs by up-regulation of ZEB1 expression which is a transcription factor known to down-regulate E-cadherin levels [23]. In this study, they treated ARCAPE prostate cancer cells with recombinant IGF-1 and showed that ZEB1 was increased two-fold in the nucleus compared to the control, leading to increased MAPK activation and cell migration [23].

Another growth factor that plays a role in the process of EMT in prostate cancer is Epidermal Growth Factor (EGF) [32]. Lorenzo et al., did a clinical study of prostate cancer patients and assessed Epidermal Growth Factor Receptor (EGFR) expression. Their results showed that EGFR was expressed in all the patients they assessed who had metastasis [24]. EGF has been reported to induce EMT through

increased expression of transcription factors responsible for reducing E-cadherin and promoting cancer invasion [32]. They showed a mechanism in which EGF increases prostate cancer progression through a Ras/ STAT3/ HIF-1 alpha/ TWIST1/ N-cadherin signaling pathway [32].

6.4 Clinical Evidence of EMT in Prostate Cancer

Epithelial mesenchymal transition (EMT) is a distinguishable feature of aggressive tumors in prostate cancer. In prostate cancer, several transcription factors are instrumental in inducing EMT such as *Snail* and *Twist*. Following regulation by *Snail*, EMT occurs and prostate cancer cells experience reduced E-cadherin and increased/ up regulation of N-cadherin [33]. In the 2007 study aimed to determine the significance of EMT, tissue from a consecutive series of 104 men treated by radical prostatectomy for clinically localized cancer during 1988–1994 was utilized [33]. The tissue microarray was studied using immunohistochemistry techniques to analyze cell adhesion molecules including classic cadherins (E-cadherin, N-cadherin, and P-cadherin) and β-Catenin and $p120^{CTN}$ and confirmed using Western blot analysis. In this study, it was determined that the decrease of E-cadherin and subsequent up regulation of N-cadherin (E-cadherin to N-Cadherin switch suggestive of EMT) is a strong predictor of clinical recurrence after radical prostatectomy [33]. This finding is a direct indicator that cell adhesion molecules may be used as prognostic information along with histologic evaluation and also demonstrates the importance of EMT for patient prognosis of human prostate cancer [33].

In other clinically related research, tissue (archived, formalin fixed, and paraffin-embedded) containing both tumor and adjacent normal tissue was obtained from surgically resected prostate cancer specimens (10 primary and 10 prostate cancer bone metastasis). Each tissue section was immunostained using specific antibodies for EMT biomarkers E-cadherin, Nuclear factor kappa B (NF-κB), Notch-1, ZEB1, and Platelet-derived growth factor D (PDGF-D) [34]. Slides of each marker were scored and accessed by stain localization, intensity, and percentage of stained cells within the tumors. From the 20 samples of primary and bone metastasis, E-cadherin was expressed within the membrane; Vimentin and PDGF-D expression in the cytoplasm; and NF-κB, Notch-1, and ZEB1 were expressed in the nucleus [34]. Results in this study demonstrated that the upregulation of all observed EMT markers, specifically Notch-1 play a significant role in prostate cancer and bone metastasis [34].

6.5 EMT in Prostate Cancer Health Disparities

Among men, prostate cancer is the most diagnosed cancer as well as the second leading cause of death [35]. African American men have a two-fold increase in mortality due to prostate cancer as compared to Caucasian men [35]. Some have

suggested that this health disparity could be due to biological factors. To date it has been difficult to find data on EMT in prostate cancer health disparities. However, research has been conducted on Kaiso, a transcriptional factor that is a member of the BTB/POZ zinc finger protein family and can induce EMT. Localization of Kaiso in the cell is characterized by a methylation-dependent silencing of E-cadherin [36] . As with regulation of EMT by *Snail* [33], down regulation of E-cadherin by Kaiso is associated with increased cell migration invasiveness and tumor aggressiveness [36]. Specifically, it has been observed that a shift in localization from the cytoplasm to the nucleus in cells causes methylation-dependent silencing of E-cadherin, which promotes cell migration and aggressiveness [36]. Experimentation was conducted to determine the relationship between Kaiso and miR-31 in a panel of cells: normal cell line (PREC), immortal normal epithelial cell line (RC-77 N/E), and Caucasian human prostate cancer lines LNCaP, DU-145, C4-2B and PC-3 [36]. MiR-31 is a microRNA that plays a role in cell proliferation, and EMT. Quantitative real-time polymerase chain reaction (qRT-PCR) revealed that Kaiso expression was low in PREC and RC-77 N/E but higher in prostate cancer cell lines with expression increasing in more aggressive cells like PC-3 and C4-2B cells. In the panel of prostate cancer cells, Kaiso levels were negatively/inversely correlated with miR-31 expression [36]. These results were supported in the observation that patients with high Kaiso levels and low miR-31 expression experienced the most significant decrease in survival compared to patients who exhibited low mRNA Kaiso levels with high miR-31 expression, and that the expression of Kaiso was higher in African American patient tissue as compared to Caucasian American patient tissue [36]. More studies are needed in the area of EMT in prostate cancer health disparities.

6.6 Therapeutic Targeting of EMT in Prostate Cancer

Biomarkers including Snail, E-cadherin, N-cadherin, Vimentin, ZEB1, TWIST have been demonstrated to play a role in the upregulation of EMT. Other EMT regulatory factors include castration, and androgen deprivation [37]. An N-cadherin antagonist, Alcohol dehydrogenase-1 (ADH-1), is a targeted therapy for EMT that has been proposed [38]. However, larger studies must be done to validate their findings. Therapeutic strategies that intervene the EMT process or reverse EMT phenotypes may be alternatives for cancer therapy. Specific N-cadherin antibodies can suppress the up regulation of EMT simultaneously decreasing tumor growth invasion and migration and blocking the progression to castration-resistance [37].

Small molecule inhibitors are also being tested as possible therapies that target EMT. For example, one compound, DZ-50, was shown to inhibit EMT in prostate cancer cells by targeting the TGF-β and IGF axis [39]. Another potent small-molecule compound, BMS-345541, was identified as a highly selective IKKα and IKKβ inhibitor that could inhibit EMT in prostate cancer cells and induce apoptosis [40].

Targeting the growth factors that promote EMT have also been studied pre-clinically and in clinical trials. However, results for IGF-1R inhibitors as single agents in prostate cancer clinical trials have not been promising [41]. Neutralizing antibodies, antisense oligonucleotides and small molecule inhibitors have also been tested in pre-clinical studies to target tumor-promoting activities of TGF-β [42].

Natural products have also been proposed as potential therapies for prostate cancer EMT. Studies have shown that muscadine grape skin extract (MSKE) that has strong anti-oxidant activity, can inhibit EMT in prostate cancer cells and promote apoptosis without affecting normal cells [43, 44]. This product is also being tested in clinical trials in prostate cancer patients [45].

6.7 Conclusions

Prostate cancer cells have hijacked the EMT process to become invasive, migratory and metastatic. This EMT can be induced by various growth factors, cytokines and downstream signaling leading to activation of various transcription factors. Evidence of EMT has also been shown in prostate cancer patients. Therefore, some of these growth factor- and cytokine-mediated pathways provide excellent targets for therapeutic interventions for treatment of prostate cancer patients *via* antagonizing EMT.

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