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Role of Epithelial Mesenchymal Transition in Prostate Tumorigenesis

Mohammad Imran Khan, **Abid Hamid**[1], **Vaqar Mustafa Adhami**, **Rahul K Lall**, and **Hasan Mukhtar**[*]

Department of Dermatology, School of Medicine and Public Health, University of Wisconsin, Madison, WI-53706

[1]CSIR-Indian Institute of Integrative Medicine, Jammu 18001, India

Abstract

Globally, the cancer associated deaths are generally attributed to the spread of cancerous cells or their features to the nearby or distant secondary organs by a process known as metastasis. Among other factors, the metastatic dissemination of cancer cells is attributed to the reactivation of an evolutionary conserved developmental program known as epithelial to mesenchymal transition (EMT). During EMT, fully differentiated epithelial cells undergo a series of dramatic changes in their morphology, along with loss of cell to cell contact and matrix remodeling into less differentiated and invasive mesenchymal cells. Many studies provide evidence for the existence of EMT like states in prostate cancer (PCa) and suggest its possible involvement in PCa progression and metastasis. At the same time, the lack of conclusive evidence regarding the presence of full EMT in human PCa samples has somewhat dampened the interest in the field. However, ongoing EMT research provides new perspectives and unveils the enormous potential of this field in tailoring new therapeutic regimens for PCa management. This review summarizes the role of many transcription factors and other molecules that drive EMT during prostate tumorigenesis.

Keywords

Epithelial mesenchymal transition; prostate cancer; transcription factors; endoplasmic reticulum; stress; signaling; Y Box Protein-1

INTRODUCTION

Epithelial cancers including prostate cancer (PCa) exhibit an innate property to spread to a distant organ. In PCa, metastatic potential of the primary tumor to spread the distant sites of the body is responsible for the majority of cancer related deaths [1]. The metastatic

[*]Address correspondence to this author at the Department of Dermatology, University of Wisconsin, Medical Science Center, Rm B-25, 1300 University Avenue, Madison, WI 53706; Tel: 608 263 3927; Fax: 608 263 5223; hmukhtar@wisc.edu.

CONFLICT OF INTEREST

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phenotype is not expressed by all the epithelial cells instead there are some specific populations that express it and they must escape the restraints of the primary tumor site. This process requires specialized degrading enzymes that can break the basement membranes for invasion which is prerequisite step for the metastasis [2, 3]. During invasion, the metastasis capable epithelial cells undergo a series of actin cytoskeleton reorganization that supports and facilitates both invasion and migration of cells. Cancer cells leave the primary tumor mass mainly by losing cell-cell contact that results in altered cell shape by a phenomenon of epithelial-to-mesenchymal transition (EMT) [4, 5].

1. EMT: AN ESSENTIAL DEVELOPMENTAL PROCESS

At the time of embryonic development, cells travel very long distances to reach their final destinations. To achieve this, epithelial cells rely heavily on a very fine tuned and highly regulated EMT program that converts them into a mesenchymal state [6]. Tissues arise mainly from developmental transition of epithelial cells to mesenchymal or stromal cells; a process known as EMT which most of the times is subsequently followed by a reverse process known as mesenchymal to epithelial (MET) transition [7]. In general, epithelial cells are in very close contact with their immediate environment and also with their axis of polarity via sequential arrangements of adherent junctions, desmosomes and tight junctions [8]. In contrast, mesenchymal cells are loosely structured within a three-dimensional extracellular matrix that also comprises connective tissues [9]. For communicational purposes, the gap junctions are used by epithelial cells. In general, the developmental EMT is divided into three different types [5]:

1.1. Primary EMT

The well-recognized examples of primary EMT are gastrulation and neural crest development [9, 10, 11]. Gastrulation is an evolutionary conserved process for body plan development. During this epithelial shaped epiblast gives rise to mesenchymal shaped mesoderm. The cell undergoes sequential shifts in shape, in which there is first internalization of mesendoderm, followed by convergence to midline and eventually extension along the anteroposterior axis [12–14]. It is also noteworthy that some of these elements have been found to be evolutionary conserved. There are many evolutionary conserved transcription factors that induce EMT during gastrulation. In invertebrates both Snail and Twist play key roles [15]; Snail suppresses E-cadherin transcription, helps in both ventral furrow formation and delamination of primary mesenchyme cells [16]. Snail mediated mitotic block is necessary for gastrulation to occur.

Similarly, in vertebrates the snail is important for gastrulation to proceed. Deficiency of snail in embryos leads to failure of complete gastrulation and cells fail to migrate due to unsuccessful events of EMT [17, 18]. Additionally, EMT associated factors also help in cellular invasion mainly via contributing in the process of basal membrane degradation executed by metalloproteases [19, 20]. Post gastrulation the next step is the formation of neural crest; here precursor cells delaminate from border regions of both neural and non-neural ectodermal zones thereby give rise to different structural derivatives [21]. Apart from these main developmental processes, EMT is also involved in somitogenesis [22, 23], endocardium and endocardial cushion formation [24, 25].

1.2. Secondary EMT

During the course of development, primary EMT is followed by the differentiation events in order to generate cell types of different origins [26]. For example, post primary EMT, neural crest cells differentiate and establish into neurons, bone cells and mesodermal cells of different types [9]. Reversibly, all these cellular populations are converted back to epithelial type mainly by using the MET phenomenon. However, this is further followed by a secondary EMT event to produce mesenchymal type cells with a highly constrained differentiation potential. Similarly a secondary EMT process of both ventral and dorsal epithelia gives rise to endocardial progenitors, hematopoietic stem cells and connective tissue of body wall muscle. In addition, the endoderm, ectoderm and mesoderm give rise to other important structures that includes mammary gland [27, 28], mesothelium [29], kidney [30–32], liver [33], pancreas [34–36], etc.

1.3. Tertiary EMT

A classic example of the tertiary type of EMT is development of the heart, which requires successive cycles of EMT followed by M*Et al*so [37]. Initially, mesodermal cells at the time of gastrulation get specified for this process along with other cardiac progenitors to become a two layered epithelium. This is followed by another round of EMT, which also includes formation of endothelial cell linings of the heart. Subsequently, this process further leads to the development into a four compartment structure of the heart. Interestingly, a group of specialized endothelial cells may undergo a tertiary EMT to form endocardium that later assembles into the atrioventricular valvuloseptal complex [38–40].

2. DEVELOPMENTAL EMT HIJACKED BY CANCER CELLS

In the neoplastic environment distinct cell populations are found which encompass highly populated differentiated epithelial cells to less numbered dedifferentiated mesenchymal cells. However, in order to disseminate from the local tumor environment epithelial cells must shift transiently into a mesenchymal state for which these neoplastic epithelial cells hijack the evolutionary conserved EMT process. This transient mesenchymal cell possesses multiple features that are important for metastasis. These involve the ability to invade primary tumors in vicinity, extravasate, survival during movement and formation of micrometastasis in distant organs. The role of EMT in cancer cell invasion, migration and metastasis is now well established in multiple cancer types like breast, ovary, colon, and lung and also in prostate [41]. Mostly in all these type of cancers the expression of EMT related molecules is found to be well correlated with high grade tumors especially those that have poor prognosis [42]. Looking at the scientific data it becomes apparent that cancer cells hijack and use the EMT program for metastatic colonization.

2.1. EMT in Prostate Cancer

There is considerable data to suggest that EMT contributes to PCa progression and metastasis. Nauseef and Henry [43] reviewed this evidence and explored studies that have aimed to better define the role and mechanisms of EMT in PCa. Conclusive evidence to suggest a role for physiologic EMT in human PCa is still lacking, although the expression of EMT related markers in human PCa samples is now well established its association with

PCa on the basis of pathological findings is quite difficult due to the possibility of misinterpretation related to compartmental staining between the stromal and the basal layers. Also, no lineage tracking has been done so far to understand how this dynamic process moves on with the progression of age. Another aspect of this ambiguity is that majority of EMT related data is based on PCa cell lines of human origin.

3. MOLECULAR MECHANISMS OF EMT INDUCTION IN PCA

Many molecular events directly or indirectly govern the program of EMT in PCa (Fig. 1). Below we discuss some of these important and established molecular mechanisms that play an important role in EMT induction in PCa.

Growth Factor Signalings—There are different types of growth signals that include transforming growth factor beta (TGF-β), endothelial growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) that are known to contribute in EMT in various cancers including PCa.

3.1. TGF-β Signaling

The TGF-β family includes three TGF-βs, two activins, several bone morphogenetic proteins (BMPs), homodimers and heterodimers of ligands that act in a sequential manner through binary combinations of transmembrane dual specificity kinase receptors. Generally, during the period of development, the expression of all isoforms TGFβ1, TGFβ2 and TGFβ3 is associated with EMT-like events however, only TGFβ1 induces EMT in wound healing, fibrosis and cancer [44].

A role for EMT is also suggested in the development of benign prostatic hyperplasia (BPH). Hu *et al* [45] showed that treatment of BPH-1 cells with normal prostate stromal WPMY-1 cells results in accumulation of mesenchymal-like cells. To assess the role of TGF-β they treated the cells in the presence of anti-TGF-β antibody and observed upregulation of E-cadherin and CK5/8 levels and down-regulation of p-SMAD3. These results showed that stromal cell supernatant was able to induce EMT in BPH-1 cells, possibly through secreting TGF-β1 to activate Smad signaling. In addition, Slabakova *et al*. [46] also showed that TGF-β induces expression of Snai2/Slug, a well-known transcription factor involved in EMT in BPH-1 cells. In PCa TGF-β can induce the nuclear accumulation of nuclear factor-kappa B (NF-κB) along with morphological change towards a mesenchymal type. However, EMT like features have been shown to be blocked by the inhibitor of NF-κB demonstrating that NF-κB is an important mediator of TGF-β mediated EMT in PCa [47]. There is evidence for the involvement of other important molecules like STAT3, PAR-4 and NEDD9 in TGF-β induced EMT in PCa. STAT3 expression was induced by TGF-β treatment which further mediated the induction of TWIST1 and HIF1α expression in different PCa cell lines [48, 49]. Similarly Chaudhry *et al* [50] showed upregulation of prostate apoptosis response-4 (PAR-4) along with EMT related markers in PCa cell lines after TGF-β treatment. PAR-4 upregulation was also observed along with Smad2 and IκB-α in the presence of each TGF-β isoforms, suggesting PAR-4 an important target of TGF-β signaling. Disruption of TGF-β

signaling reduces the PAR-4 expression. It has been reported that the overexpression of PAR-4 results in the upregulation of vimentin and Snail expression together in simultaneous with cell migration. However, Par-4 silencing by Si-RNA resulted in decrease of these proteins and prevented also the TGF-β-induced EMT. Recently, Morimoto *et al* [51] showed critical role of NEDD9, a Crk-associated substrate (Cas) family protein in TGF-β-induced EMT in PCa. Importantly, the Knockdown of endogenous NEDD9 expression completely diminished the TGF-β-triggered tumor invasion in several PCa cell lines. In addition to the EMT inducing potential of TGF-β, other components of this signaling like TGF-β receptor type 2 (TGF-βR2) have been shown to influence EMT in TRAMP mouse model of PCa. The *In vivo* disruption of TGF-β signaling was shown to accelerate the pathologic malignant phenotype of prostate of the TRAMP mouse model by altered prostate growth and by inducing EMT [52]. Overall, current evidences related to the TGF-β induced EMT in PCa is well recognized but there are important issues that remain unanswered such as a conclusive evidence of EMT from human tissues and role of TGF-β (stromal and/or epithelial) in EMT induction in PCa.

3.2. FGF Signaling

It has been reported that both FGF and its receptor FGFR1 are upregulated in PCa [53]. However, to assess the role of FGFR1 activation on PCa progression in an inducible FGFR1 model that express a prostate-specific, inducible chimeric version of FGFR lead towards the phenotypic switching of epithelial cells towards a mesenchymal phenotype along with development of 100% adenocarcinoma. Also in these mice, lymph node and liver metastasis was evident and the metastatic foci retain mesenchymal features. To identify the possible molecules that contribute to iFGFR1 induced EMT a gene expression study in tumors derived from these mice was done that showed a transcription factor SOX-9 as a possible mechanism mediating this effect [54]. This study clearly confirms EMT inducing potential of this pathway. In addition to FGFR1 other receptors of this pathway like FGFR4 were also found to be co-expressed with matrix metalloproteinase (MMP-14) in certain PCa cell lines. Overexpression of MMP-14 was also shown to induce an EMT-like state in PCa [55], however the precise mechanism for the EMT induction by MMP-14 is not well defined.

Recent literature suggests that the switching between alternatively spliced isoforms may lead towards the imbalanced FGFR signaling. In addition, the alternative splicing of the third Ig-like domain determines the ligand-binding specificity of the receptor and generates the IIIb or the IIIc isoform of the FGFRs [56]. Mostly IIIb isoforms are expressed in epithelial cells, whereas IIIc isoforms are expressed in mesenchymal cells. Exon switching in epithelial cells from the epithelial FGFR2 IIIb isoform to the mesenchymal FGFR2 IIIc isoform by the alternative splicing has been also reported in rat models of prostate [57], suggesting an additional mechanism for FGF induced EMT in Pca.

3.3. IGF1 Signaling

The IGF1 induced EMT in PCa is not extensively studied however, Graham *et al*. showed that treatment of PCa cell lines with soluble IGF1 results in the upregulation of Zinc finger E-box-binding homeobox 1 (ZEB1), a transcription factor mostly involved in EMT induction [58]. ZEB1 has been mainly known for E-cadherin repression, thereby in

enhancing the metastasis. IGF1 treatment also resulted in the upregulation of the MAPK pathway. Based on these observations, it was proposed that IGF1 activation of ZEB1 in PCa cells was MEK/ERK dependent. The evidence of involvement of IGF signalling in EMT in PCa has been recently supported by studies of Chen *et al*, who showed that EMT-related gene signature of circulating tumor cells (CTCs) included both IGF1 and IGF2 along with some other signaling molecules [59].

3.4. EGF Signaling

The aberrant expression of epidermal growth factor receptor (EGFR) in both androgen independent and metastatic PCa, that are also known to possess heightened EMT related features, is well established [60]. Many studies have shown the EGF mediated phenotypic switching in PCa. For example, the treatment of EGF alone or in combination with TGF-β results in epithelial to mesenchymal phenotype in ARCaP cells. Similarly, Gan *et al* showed that EGF treatment to PCa cell lines promotes loss in cell-cell besides the down-regulation of E-cadherin and the upregulation of the transcriptional repressor i.e Snail which forms the typical characteristics of EMT [61]. EGF induced EMT was found to be mainly dependent on Akt activation, as inhibition of Akt signaling abolished EGF driven EMT in PCa cell lines [62, 63]. In addition, EGF was shown to selectively induce the protein degradation of epithelial origins in neoplasm; or LIM domain and actin binding 1, LIMA-1 (EPLIN), a putative suppressor of EMT in PCa [64]. Further mechanistic analysis revealed that EGF activated the phosphorylation, ubiquitination, and degradation of EPLIN through an extracellular signal-regulated kinase 1/2 (ERK1/2)-dependent signaling cascade. This study highlighted a novel molecular mechanism for EGF mediated regulation of EMT in PCa. Recently Cho *et al* showed that EGF induced upregulation of both TWIST1 and STAT3 transcription factors, known to be critical regulators of EMT in PCa [48].

3.5. PI3K-AKT/RAS/MAPK Signaling

Unlike the TGFβ-induced EMT, receptor tyrosine kinases (RTKs) mediated EMT requires other important signalling nodes like AKT, mechanistic target of rapamycin (mTOR) and Mitogen-activated Protein (MAP) Kinases (MAPKs). Lim *et al* showed that BMP7 induced EMT in PCa cell lines requires both AKT and Extracellular Signal-Regulated Kinase (ERK) signaling [65]. Similarly, Mulholland *et al* showed that Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis in PCa. Mice with prostate conditional Pten deletion with conditional activatable K-ras (G12D/WT) model showed accelerated PCa progression, accompanied by EMT and macrometastasis with complete penetrance. Also, a progenitor subpopulation with mesenchymal properties from the Ras/AKT activated mice prostate was found to be highly metastatic upon orthotopic transplantation [66]. This study not only underlined the significance of Ras signaling in metastatic PCa but also uncovered the importance of EMT in these events during PTEN loss. In addition PI3K-AKT/mTOR/MAPK signaling was also involved in induction of EMT during PCa radiotherapy. EMT in association with cancer stem cells (CSCs) was found to be an important mechanism for radioresistance during PCa treatment [67]. Ma *et al* recently showed that both p38 MAPK and ERK are involved in hepatocyte-mediated phenotypic switching in PCa [68].

4. EXTRACELLULAR SIGNALS TO REGULATE EMT

Many of the extracellular signals like Wnt, Hedgehog and Notch are well known to support EMT in a variety of cancers including PCa. However, we briefly discuss here Wnt signaling as there are strong evidences to support Wnt role in the induction of EMT in PCa.

4.1. Wnt Signaling

It is well established that the wingless-type (Wnt) pathway has developmental role in tissues and organisms. Both canonical and noncanonical Wnt signaling is involved in the development of prostate gland [69]. However, the aberrant activation of the Wnt pathway has been found to be involved in the progression of PCa. Jiang *et al* first reported that activation of the Wnt/beta-catenin signaling pathway correlates with the characteristic of EMT and also positively influences invasiveness and proliferation in PCa [70]. Similarly knockdown of an essential Wnt signaling component, β-catenin in PCa cells results in dramatic reversal of EMT induced by hypoxia inducible factor-1α [71]. Additionally Wnt signaling was shown to be involved in EMT induced by SOX2. SOX2 promotes metastasis of breast and PCa cells by promoting EMT mainly through WNT/β-catenin, but not TGF-β or Snail1 signaling [72]. On the contrary, forced expression of naturally-occurring Wnt inhibitor, WIF1 in PCa cells is associated with reduced expression of EMT transcription factors e.g. Slug and Twist and morphological transitions from mesenchymal to epithelial features [73]. All these studies strongly establish a role for Wnt signaling in either inducing or supporting EMT in PCa.

4.2. Androgen Related Signaling

Prostate is an androgen-dependent tissue and requires androgenic and androgen receptor (AR) signaling axis for normal functioning. However, deregulated androgen signaling is one of the most important factors in PCa progression. Initial evidences suggested an inverse relationship between AR levels and androgen-mediated EMT induction [74]. In another study, it was reported that androgen deprivation induces N-cadherin expression and N-cadherin increased in castration-resistant human tumors with established metastases [75]. This was further demonstrated by Sun *et al* who showed that androgen deprivation induces EMT in both normal prostate and PCa [76]. Additionally, targeting AR in PCa with siRNA promoted PCa cell migration and invasion mediated by CCL2-dependent STAT3 activation and EMT pathways [77]. Though it is now well established that AR signaling axis is a potent modulator of EMT but the interplay between AR signaling and expression of EMT related transcription factors needs to be further explored. Very recently a study suggested AR as a critical regulator of ZEB2 expression during EMT in PCa [78]. Apart from AR, the AR splice variants are found to be expressed in higher amounts in castration-resistant PCa which also showed increased EMT related events [79]. It is therefore speculative to suggest any correlation between AR splice variants and EMT in these settings. In line with this a recent study showed that overexpression of AR splice variants 3 (AR3) modulates the expression of TGF-β and IGF1 pathways, both well-known EMT related signaling pathways. Some EMT-associated genes were also up-regulated in AR splice variants 3 transgenic (AR3Tg) mice prostates [80]. Owing to the role of AR signaling axis in PCa it is

not surprising that EMT is heavily modulated AR signaling axis, however, this field still lacks the mechanistic explanations how AR modulate EMT in PCa.

4.3. Heat Shock Proteins

Molecular chaperones including heat shock proteins (HSPs) are important components of core stress response machinery and involved in protein homeostasis, prosurvival signaling and transcriptional networks. HSP27 and 90 are the two most well established proteins that are known to be involved in metastatic PCa. Hance *et al* provided preliminary evidence that secretory form HSP90 (referred here as extracellular HSP90) induces EMT like events in PCa cells. In support of their concept, they showed that metastatic PCa cells exhibited increased eHsp90 expression relative to their lineage-related nonmetastatic counterparts. Treatment of PCa cells with extracellular Hsp90 promoted cell proliferation and shifted cellular morphology towards a mesenchymal phenotype [81]. Conversely, inhibition of eHsp90 attenuated motility, blocked migration, and reversed cell morphology towards an epithelial phenotype. Shiota *et al* showed a role for HSP27 mediated regulation of EMT in PCa. To confirm the EMT inducing potential of Hsp27, two epithelial cell lines and $ARCaP_E$ were tailored to stably overexpress Hsp27. Hsp27 overexpression in both these cell lines downregulated E-cadherin and increase in vimentin and fibronectin expression. On the contrary, HSP27 knockdown using siRNA in the PCa cell line DU145 resulted in increased E-cadherin and decreased vimentin and fibronectin expression. Hsp27 overexpressing cells also showed upregulated mesenchymal markers such as p-GSK-3β, vimentin, fibronectin, Twist, and N-cadherin at both protein and mRNA levels [82]. Though the evidence correlating HSPs with EMT in PCa is few, it is clear that HSPs are important players during EMT. However, it would be of great interest to examine how HSPs can influence the EMT related events in other conditions like androgen deprivation and drug resistance since HSPs are known to play an important role in therapy resistance.

4.4. Epigenetic Factors

Genetic mutations are a known and one of the major causes in oncogenesis; however, rapidly increasing and overwhelming evidence and experimentation shows the alterations also occur at the epigenetic level. The main epigenetic events related to genetic processes (transcription, replication, recombination, and segregation) are DNA methylation, histone modifications, chromatin remodeling and microRNAs [83]. Recent literature supports the involvement of epigenetics in EMT in a variety of cancers including PCa [41]. The histone methyltransferase MMSET/WHSC1 (Multiple Myeloma SET domain), was recently identified as one of the important epigenetic factors, capable of inducing an EMT like state in PCa cells. Forced expression of MMSET in normal prostate RWPE-1 cells induced TWIST1 expression. Chromatin immunoprecipitation (CHIP) analysis showed that MMSET binds directly to TWIST1 locus, suggesting a direct role of MMSET in the regulation of TWIST1 expression [84]. The classical epithelial marker E-cadherin is often found to be silenced in PCa due to promoter methylation [85–86]. It is intriguing to note that methylation of E-cadherin is a priming event that helps in creating a permissive environment for outgrowth and continued morphogenesis of prostatic ducts at different stages of the development [87]. This again tells us how PCa cells hijack this evolutionary conserved process to decrease the expression of E-cadherin. Another well-known histone

methyltransferase i, e EZH2, a component of polycomb repressive complex 2 (PRC2), has been implicated in EMT and is known to repress the E-cadherin expression. EZH2 is also known to suppress disabled homolog 2-interacting protein (DAB2IP) [88]. Forced suppression of DAB2IP was found to activate EMT [89] and its expression was also found to be lost in PCa samples generally through an epigenetic mechanism [90]. Furthermore, BMI, a component of PRC1 is known to modulate EMT and found to be upregulated in PCa [91, 92]. However, it remains to be known whether BMI is able to induce EMT like state in PCa.

4.5. microRNAs

MicroRNAs (miRNAs) are non-coding RNA molecules that consist of 21–23 nucleotides and can bind selectively to mRNAs. Generally, miRNAs are involved in regulation of gene expression mainly through participating in post-transcriptional silencing of target genes. They express differentially in site, tissue and developmental context. miRNAs are known to regulate various important physiological and pathological processes such as differentiation, proliferation, migration, invasion, survival and EMT. Like most cellular genes; the expression of miRNAs is influenced by transcription factor binding, chromosomal rearrangements, genetic and epigenetic alterations [93].

Several miRNAs have been shown to directly target families of EMT transcription factors and are known to facilitate EMT like state in PCa. Based on their possible targets, miRNAs can act both as EMT inducers or inhibitors. For example, the most studied miRNA-200 family which consists of miR-200a, miR-200b, miR-200c, miR-141 and miR-449, are significantly down-regulated during PCa progression and act as tumor suppressors in PCa mainly by inhibiting EMT [94, 95]. Kong *et al* showed that miR-200 can inhibit the platelet-derived growth factor-D (PDGF-D)-induced EMT in PC3 cells via targeting both ZEB1 and ZEB2 [96]. In benign prostate hyperplasia (BPH) miR-200 can reverse the TGFb-induced EMT phenotype [44]. Similarly, Liu *et al* evaluated miRNA expression associated with tumorigenesis and EMT in a model of Pten- and TP53-null prostate adenocarcinoma and found that both miRNA-200 in association with miRNA-1 was reduced with progression of prostate adenocarcinoma, and identified Slug as one of the phylogenetically conserved targets of these miRNAs [97]. Forced expression of miRNA-200 inhibited both EMT and tumorigenesis in human and mouse model systems [98]. Other well-known tumor suppressor miRNAs like miRNA-203 and 205 were also found to inhibit EMT in PCa. Both miRNA-203 and 205 inhibit PCa progression via their ability to restore epithelial phenotype of PCa cell lines [99].

In addition, miR-143/-145 cluster that consists of miR-143 and miR-145 are known to inhibit EMT and down-regulated in metastatic PCa. Forced expression of both miRNAs in PC3 cells represses fibronectin and enhances E-cadherin expression and both can reverse EMT [100]. Similarly, miRNA-29b expression was also found to be lower in PCa cells (PC3 and LNCaP) with regard to immortalized prostate epithelial cells [101]. Here, N-cadherin, Twist and Snail expression was down-regulated in PC3 cells expressing miR-29b [102]. Additionally, loss of well-known miR-100 enhances migration, invasion, EMT and stemness properties in PCa cells through targeting Argonaute 2 [103]. Very recently, Gandelini *et al*

demonstrated that miRNA-205 is down-regulated in PCa cells upon cancer associated fibroblasts (CAF) stimulation, mainly via direct transcriptional repression by HIF-1. In this case, forced expression of miR-205 in PCa cells reversed CAF-induced EMT [104]. These studies clearly highlight the enigmatic roles of miR-NAs in EMT. miRNAs can regulate different components of EMT like transcription, post transcription and signaling that are important for EMT induction in epithelial subtypes. These qualities make miRNAs an important prognostic marker and therapeutic tool in EMT-associated PCa progression.

5. OTHER FACTORS

Apart from the some conventional well known factors recent studies had highlighted role of other factors that are involved in EMT-associated with PCa. Wu *et al* showed monoamine oxidase –A (MAOA) induces EMT in PCa epithelial cells [105]. Knockdown and overexpression studies for MAOA in PCa cell lines showed that MAOA induces EMT mainly via activation of VEGF and its co-receptor neuropilin-1. Authors found MAOA-dependent stimulation of neuropilin-1 expression promoted AKT/FOXO1/TWIST1 signaling, permitting FOXO1 binding at the *TWIST1* promoter. Authors also found MAOA/VEGF-A/TWIST1 pathway was activated in high-grade PCa specimens. Other studies had also established the role of VEGF in EMT associated with PCa [106, 107].

6. TRANSCRIPTION FACTORS DRIVING EMT IN PCA

EMT inducers discussed above relay the necessary signals for epithelial switching to the downstream molecules, known as executioners (or transcription factors) that finally accomplish EMT (Fig. 2). Some of the well-established transcription factors (TFs) include Twist1, Snail, Slug, Zeb1, Zeb2 etc. The role of a specific transcription factor in EMT depends on the site specific microenvironment, cell type and functionality of other signaling pathways within the cell [108]. The main function of these EMT related transcription factors is to repress the expression of epithelial associated genes and induction of mesenchymal genes [109]. The information related to the functions and molecular targets of the established TFs in PCa is well known and has been reviewed extensively [43, 110, 111]. Here, we focus more on some of the newly identified TFs like YB-1, p63, OVOL family and their role during EMT in PCa.

6.1. Y Box Protein-1

YB-1 is encoded by the *YBX1* gene; a member of DNA- and RNA-binding proteins which contains a highly conserved nucleic-acid-binding motif known as the cold shock domain (CSD) that binds to both types of nucleic acids. Initially, YB-1 was recognized as a repressor factor that binds to an inverted CCAAT box of MHC class II promoters, however using DNA probes it was later found that YB-1 also binds to enhancers of some genes. The YB-1 protein is remarkably multifunctional, and is associated with a variety of oncogenic features like cell proliferation, migration, invasion, EMT, cell cycle progression, DNA damage repair, angiogenesis, and genomic instability in different type of cancers. These features strongly suggest that YB-1 should be considered an oncogene [112, 113]. YB-1 is linked with EMT related factors like Twist1 and clusterin clearly suggesting some

involvement of YB-1 in EMT during PCa progression [114, 115]. However, no direct evidence is available for this presumption.

Recently, we observed that the forced expression of YB-1 in prostate epithelial cells induced a mesenchymal morphology that was associated with down-regulation of epithelial markers. Moreover, silencing of YB-1 in different PCa cells was shown to reverse the mesenchymal phenotype to an epithelial morphology, reducing cell migration, invasion and proliferation [1]. Also, a significant inverse correlation between phospho-YB-1^{Ser102} and E-cadherin in these samples was observed [1]. Overall, it may be concluded that YB-1 has the potential to induce EMT in prostate epithelial cells and has an inverse association with E-cadherin expression.

6.2. p63

The p63 is a part of a well-known family of transcription factors that also include p53 and p73. Due to the presence of two promoters, p63 encodes two proteins; one that contains TAp63 and another ΔNp63 which lacks an N-terminal transactivating (TA) domain homologous to that present in p53. However, the C terminus generates three isoforms (α, β, and γ) of both TAp63 and ΔNp63 due to alternative splicing. Interestingly, ΔNp63α via a second C-terminal TA domain can transactivate a spectrum of genes distinct from that recognized by the N-terminal TA domain [116, 117]. Mice lacking p63 fail to develop prostate. Moreover, p63 is normally expressed in basal cells of the adult prostate but it is nearly lost in differentiated luminal cells. Similarly, PCa cells also barely express p63. Both type of p63 isoforms i.e. TAp63 and ΔNp63 have been shown to regulate the expression of EMT related genes such as ZEB1 and vimentin in PCa. It was found that the effects of p63 on EMT markers are mediated mainly through miR-205 and p53 mutations [118], because mutant p53 can suppress the actions of p63. It has been reported that p63 is highly down-regulated when epithelial EP156T cells underwent EMT to become EPT1 cells [119] and over-expression of ΔNp63 in mesenchymal type cells led to gain of several epithelial characteristics [120, 121].

6.3. OVOLs

Ovo gene family is evolutionarily conserved zinc-finger's that encodes transcription factors which regulates gene expression in various developmental processes [122]. In Drosophila, the ovo functions for epidermal denticle formation and oogenesis. However, in mammals, three ovo homologues are found, known as OVOL 1, OVOL *2*, and OVOL 3. In mice, OVOL 1 is tightly regulated by wnt signaling and is required for hair follicle differentiation, kidney and male germ cell development [123, 124].

In humans, OVOL1 is highly responsive to TGF-β1/BMP7 stimulation through Smad4-dependent pathway. All the members of this gene family act downstream of signaling pathways required for diverse processes during both early and late stages of embryonic development [125]. Recently OVOLs were found to be positively correlated with the E-cadherin expression in primary tumors of PCa. Further analysis by using Oncomine database demonstrated negative correlation of OVOLs with the protein expression of ZEB1 and Vimentin. This transcriptional repression of ZEB1 was mainly regulated by OVOL2 via

direct interaction of OVOL2 with in the promoter region of ZEB1. However, OVOL1 fails to show the same effect on ZEB1 expression [126]. Overall these results clearly suggest that the mesenchymal or epithelial state of prostate cells is controlled, in part, by a regulatory feedback loop between the OVOLs and ZEB1 [127].

7. SOME IMPORTANT QUESTIONS

Even though a role for EMT has been observed in PCa development and progression the evidence is not compelling enough for specific targeting of the components involved in EMT. Based on this fact we discuss some issues that need to be addressed. ***Can EMT be used as a biomarker for staging PCa?*** Much of the current literature suggests that EMT related events are correlated with high grade and metastatic PCa. The same was also found to be true during the progression of hormone independent PCa. However, the clinical relevance of EMT is frequently questioned due to the lack of confirmatory evidences of full EMT in different grades of human PCa samples. Also right now there is no direct scientific evidence or report that confirms the role of EMT specifically in bone metastasis cases in PCa. All these observations create a limitation in using EMT as a biomarker for high grade and metastatic PCa. ***Can EMT be developed as a therapeutic target for PCa management?*** Classical cytotoxic chemotherapy or androgen ablation has proved less beneficial in PCa because of the reappearance of the tumor acquired through drug resistance along with some signs of EMT and stemness. Newer strategies are being used to not only inhibit proliferation but also EMT and stem cell-like properties to prevent the metastatic spread. A similar strategy could be employed against PCa to target cancer stem cells and EMT. In this direction, large numbers of studies have tried design inhibitors of EMT in PCa but with limited success. The reasons for this could be: (a) EMT may not be appropriate therapeutic target, since metastasis also contains evidences of epithelial subtype, (b) The role of EMT in metastatic dissemination is still debatable (c) Failure of desirable effects of EMT inhibition in reversing current aspects of therapy resistance. Though EMT can be induced by various signaling pathways and regulatory networks; however, it is mainly executed by TFs and their targeting remains a challenge due to their intracellular localization. ***Does PCa microenvironment interact with EMT?*** Since, tumor microenvironment plays an influential role in determining EMT. In this context, many of the tumor microenvironment components like tumor associated macrophages (TAMs), myeloid-derived suppressor cells, regulatory T cells (MDSCs) and cancer-associated fibroblasts (CAFs) have been observed to be associated with EMT in a variety of epithelial cancers. However, the role of PCa microenvironment components in EMT related events or vice versa remains unclear. It would be interesting to understand if such unique paracrine crosstalk occurs in Pca or other such cancers.

SUMMARY

The ongoing research suggests several important functions of EMT that have been correlated with therapy resistance, stemness, tumor recurrence in PCa. Therefore, it is not surprising that the research on EMT has got attention and is on forefront of PCa research. Many established (Snail, Twist and Zeb families) along with some newly identified TFs (YB-1, p63 and OVOLs) are being linked with EMT. However, a difficulty in

therapeutically targeting these TFs remains a challenge for the development of an effective EMT inhibitor. Also the dependency of EMT on different cell type and initiating signalling pathways poses another major challenge in finding new druggable EMT targets. Furthermore, the biggest challenge still is the EMT characterization in PCa samples, as it is heavily affected by the transient and reversible nature of the process. Beside this, current focus of PCa research on tumor microenvironment had another dimension on EMT perspective, as it is clear now, signals arising from microenvironment play a key role in governing EMT and thereby may have a direct impact on either tumor progression or possible clinical outcomes in PCa patients. Future efforts shall focus to delineate the mysteries of EMT will form a basis for developing novel anti-metastatic therapeutic approaches, as well as prognostic or diagnostic markers for PCa management.

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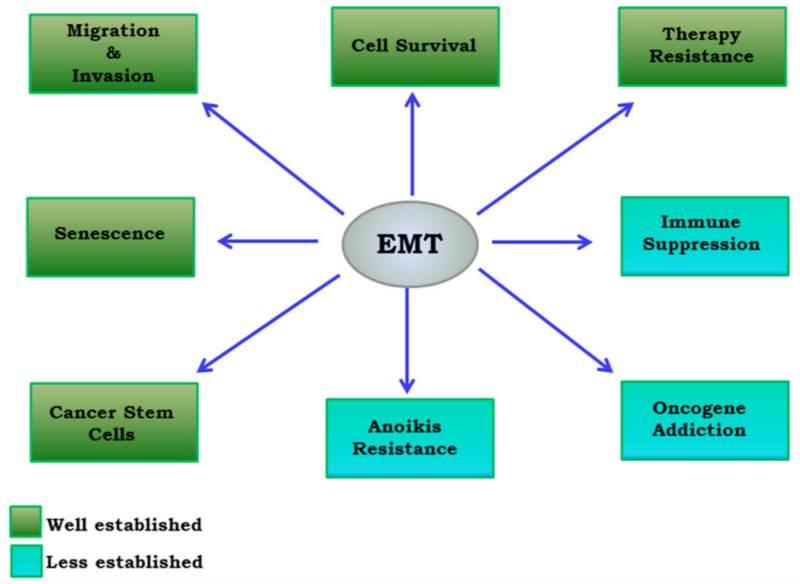

Fig. 1. Important role of EMT in PCa progression

The role of EMT in migration, invasion and cell survival is well established. However the impact of EMT on anoikis resistance and immune suppression is still quite not established and debatable.

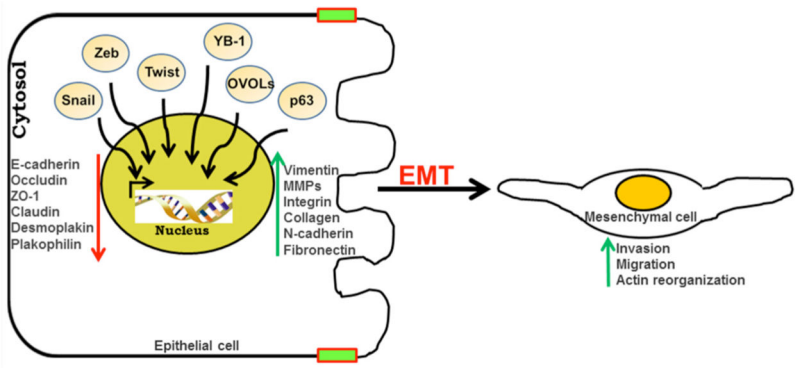

Fig. 2. Schematic representation of the induction of EMT by different transcription factors

In the presence of a stimulus the transcription factor moves into the nucleus, interacts at different gene loci and further modulates the expression of genes related to migration, invasion and EMT.