

The Role of the IGF Axis in Epithelial-to-Mesenchymal Transition during the Progression of Prostate Cancer

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Abstract: Prostate cancer is the second most common lethal cancer in men worldwide. Despite the fact that the prognosis for patients with localized disease is good, many patients succumb to metastatic disease with the development of resistance to hormone treatments. This is normally termed castration-resistant prostate cancer (CRPC). The development of metastatic, castration-resistant prostate cancer has been associated with epithelial-to-mesenchymal transition (EMT), a process where cancer cells acquire a more mesenchymal phenotype with enhanced migratory potential, invasiveness and elevated resistance to apoptosis. The main event in EMT is the repression of epithelial markers such as E-cadherin and upregulation of mesenchymal markers such as N-cadherin, vimentin and fibronectin. The insulin-like growth factor (IGF) signalling axis is essential for normal development and maintenance of tissues, including that of the prostate, and dysregulation of this pathway contributes to prostate cancer progression and malignant transformation. It is becoming increasingly clear that one of the ways in which the IGF axis impacts upon cancer progression is through promoting EMT. This review will explore the role of EMT in prostate cancer progression with a specific focus on the involvement of the IGF axis and its downstream signalling pathways in regulating EMT in prostate cancer.

Keywords: Epithelial-to-mesenchymal transition, insulin-like growth factor family, prostate cancer progression, lifestyle factors.

PROSTATE CANCER

Prostate cancer is the second most common lethal cancer diagnosed in men. According to The American Cancer Society, it is estimated that more than 220 000 new cases of prostate cancer will occur in the United States during 2015 accounting for about 26% of all cancers in men with a mortality rate of 9%: the second highest cause of cancer deaths in men after lung cancer [1].

Age is the biggest risk factor for PCa with the incidence of prostate cancer being higher in older men particularly in men aged 65 and above [2]. The incidence of, and mortality from, prostate cancer varies hugely within different racial and ethnic groups. Much higher incidence and death rates are reported in black men compared to other races [3,4]: the reasons underlying these observations are still unclear but both genetic and/or environmental influences may be involved. A number of epidemiology studies have also reported that family history is a risk factor for PCa- with approximately 5-10% of cases being attributable to inherited genetic factors or PCa susceptibility genes [5,6].

The prognosis for patients with localized disease is good, however the morbidity and mortality for prostate cancer patients who develop metastatic disease is high [7]. Androgen deprivation therapy (ADT) which involves surgical or chemical castration plays an important role in the treatment and management of prostate cancer and is also utilised as an adjuvant or neo-adjuvant treatment for high-risk disease [8]. The response to the treatments is however temporary and the patient often undergoes disease recurrence and metastasis and inevitability progresses to castration-resistance prostate cancer (CRPC) [9]. An epidemiology study conducted on 2494 non-metastatic prostate cancer patients undergoing ADT showed that within five years, 80% of the patients developed CRPC and nearly 25% developed metastases, with the majority having bone metastases [10]. Metastatic prostate cancer particularly bone metastases have been regarded as a lethal form of prostate cancer and remains an incurable disease at present since current therapy can only delay the progression of the cancer [11,12].

The extensive use of prostate-specific antigen (PSA) testing has dramatically changed the prostate cancer landscape, enhancing prostate cancer detection at an earlier stage for which immediate treatment can be given [7]: screening has led to a large increase in the number of detected prostate cancers. The major issue in the clinical management of prostate cancer is

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that there is no way to accurately determine at the time of diagnosis which cancers will develop into a lethal disease from those cancers that will not impact on a man's life. Consequently, almost all men with PSA-detected cancer, which has increased due to screening, opt for treatment, even though for the majority it is probably not required.

This has led to many researchers focusing on other contributory factors that could be important in determining whether a cancer progresses to a life-threatening disease including environmental exposures, endogenous hormones and the complex interactions between these influences [4,13]. Epidemiology studies have contributed greatly to our understanding of the impact of the environment in the pathogenesis of prostate cancer as well as other epithelial cancers. This has been convincingly demonstrated by migrant studies: migrants from Asia who move to Western countries develop a higher prevalence of prostate cancer compared with the native Asian populations and adopt the cancer risk of the host population within a couple of generations [14]. Such evidence clearly indicates that the development of clinically aggressive cancers in migrants cannot be due to genetic mutations over this short period of time but must be heavily influenced by an altered lifestyle and different environmental exposures. A western lifestyle or western diet is characterised by physical inactivity accompanied with excessive intake of processed foods, red meat, dietary fat, refined carbohydrates, or excess calories [15]. As prostate cancer is detected in older men they often also present with co-morbidities including obesity, hyperinsulinemia, and insulin resistance. Recent studies have shown an association between metabolic syndrome and an increased risk of prostate cancer progression to advanced disease and prostate cancer-specific mortality [16,17]. A meta-analysis on the association between diabetes and PCa showed that diabetic patients had a reduced risk of developing PCa [18]. However, despite the risk of developing PCa being reduced by diabetes, [19] men having both prostate cancer and diabetes, suffered more aggressive tumours, a higher risk of cancer recurrence and worse overall survival [20] which suggests metabolic exposures affect progression but not initiation, consistent with the epidemiology observations of geographical variations.

The crucial underlying molecular mechanism that promotes the progression of localised prostate cancer to become metastatic and castration resistant remains largely elusive. Contributing mechanisms have been

suggested for development of metastatic androgen-independent prostate cancer including androgen receptor (AR) over-expression, AR splice variants such as AR3/AR-V7 and AR4/AR-V1, and mutations in the ligand binding domain of the AR as well as cross-talk with other growth factor signalling pathways particularly the insulin-like growth factor (IGF) axis [21]. But still, targeted therapy to the AR signalling axis for treatment of castration resistant prostate cancer has yet to be fully effective.

INSULIN-LIKE GROWTH FACTOR (IGF) AXIS

The IGF system comprises two ligands (IGF-I and IGF-II); three receptors: IGF-I receptor (IGF-IR), insulin receptor (IR) and the IGF-II/mannose-6-phosphate receptor (IGF-IIR) and a family of six high-affinity IGF binding proteins (IGFBP-1 to -6) (see Figure 1). Together they are involved in multiple signalling pathways that promote cell proliferation, differentiation, migration and survival. Both IGF-I and -II are multifunctional regulatory peptides and have similar structural homology with proinsulin [22, 23]. The synthesis of IGF-I, IGF-II, and insulin is regulated by diverse mechanisms with the major regulators being nutrient intake and growth hormone [24]. Unlike insulin and other peptide hormones, IGFs are not synthesized and stored within specialized cells in a given tissue. Instead, they are ubiquitously produced and released by virtually every tissue [25]. IGFs exert their effect by predominantly binding to the IGF-IR that exhibits structural homology to the IR [26] especially in the intracellular tyrosine kinase domain. IGF-IR and IGF-IIR have different structures and functions. The IGF-IR is composed of two identical α -subunit and two identical β -subunit linked by disulfide bonds [27] whereas the IGF-IIR is a single, transmembrane structure that is thought to act as a clearance receptor for IGF-II [28]. Differential splicing of the IR gene produces two isoforms of IR, IR-A and IR-B. IGFs, particularly IGF-II has high affinity for IR-A compared to its affinity for IR-B. Insulin on the other hand binds with similar affinity to both isoforms. IR-B is thought to mediate the metabolic response of insulin such as glucose uptake [29] whereas IR-A is considered to play more of a role in cell growth, proliferation and survival [30]. IR-A is the predominant isoform expressed in many cancer cells consistent with the increased risk of developing cancer in patients with hyperinsulinemia/hyperglycaemia, such as those with concomitant obesity and type 2 diabetes [31].

The interaction between the IGFs and their receptor is modulated by IGF-binding proteins (IGFBPs). The

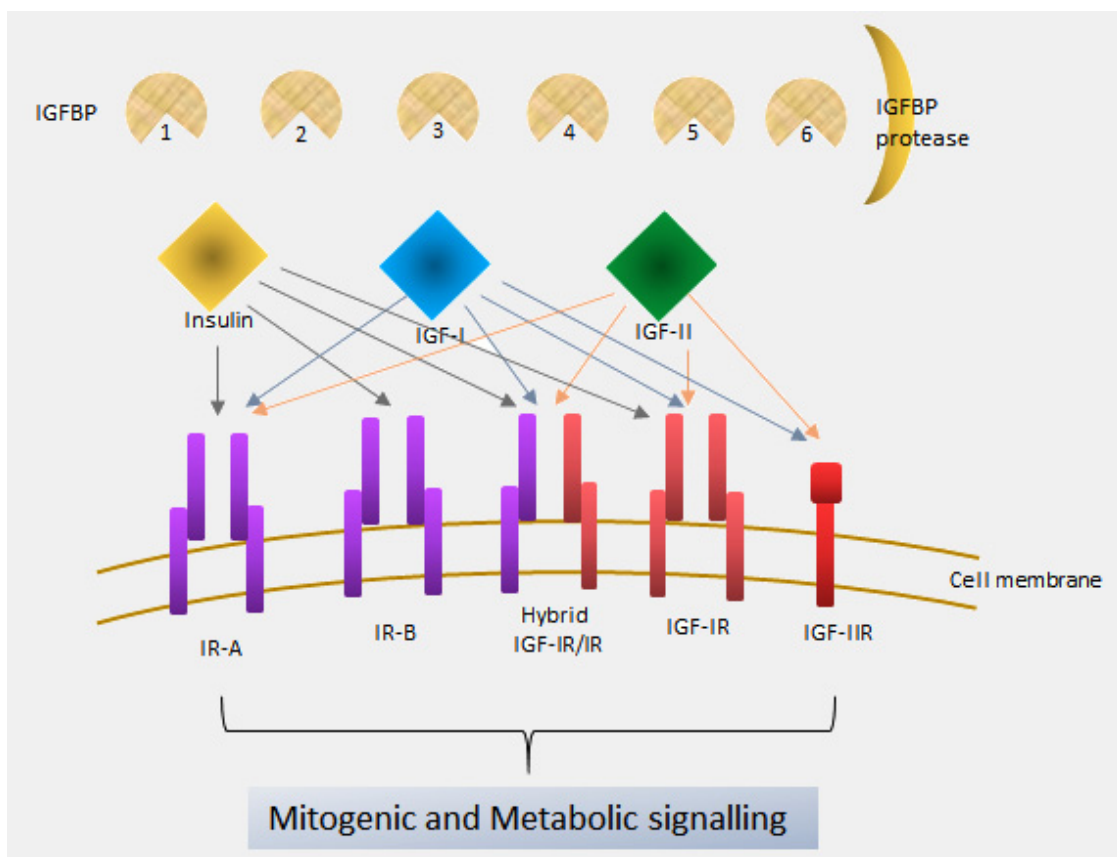


Figure 1: The Insulin-like growth factor (IGF) system.

The IGF system consists of the receptors (IR, IGF-IR, IGF-IR/IR hybrids and IGF-IIR), the ligands (IGF-I, IGF-II and insulin), six high affinity IGFBPs (-1 to -6) and IGFBP proteases. IGF ligands bind to IGFBPs in the circulation. Binding of an IGF ligand to its receptor activates downstream mitogenic and metabolic signalling that regulates cell survival, growth, differentiation and metabolism.

Abbreviations: IGF: insulin-like growth factor; IGFBP: insulin-like growth factor-binding protein; IR: insulin receptor.

IGFBP family consists of six circulating proteins that bind with high affinity to the IGFs. They have multiple and complex functions in which they exert their action dependent or independent of IGF binding. IGFBP association with IGFs may either enhance or inhibit their actions which results in different outcomes on cellular functions. For example, binding of IGFBPs with IGFs can hinder the interaction between IGFs and IGF-IR as the IGFBPs have a higher affinity for the IGFs than the receptors which results in suppression of IGF actions [32]. Circulating IGFs are almost entirely bound to the IGFBPs. IGF binding to its binding protein protects the IGF from proteolytic degradation resulting in increased bioavailability of circulating IGFs. In addition to their role in regulating the bioavailability of circulating IGF, it has become clear that IGFBPs can also exert effects on numerous cell functions, including proliferation, survival, gene transcription and the initiation of apoptosis [33–36](see review Baxter, R 2014) [37].

EPITHELIAL TO MESENCHYMAL TRANSITION

Epithelial-to-mesenchymal transition (EMT) is a coordinated and organized process that is important during development. During this process epithelial cells lose their epithelial properties and acquire mesenchymal, fibroblast-like characteristics [38]. EMT begins when E-cadherin, a calcium dependent cell-cell adhesion molecule that mediates epithelial connections to neighbouring cells and the basement membrane, is down-regulated [39,40]. Apart from loss of E-cadherin another critical molecular feature of EMT is repression of other cell adhesion proteins including occludin and plakoglobin and decreased expression of keratin intermediate filaments such as cytokeratin; as well as decreased in desmosomes such as desmoplakin resulting in dismantling of adherens junctions, reorganization of cytoskeletal and subsequent loss in cell polarity. The loss in these molecules is accompanied by increases in mesenchymal markers comprising N-cadherin, vimentin as well as

extracellular matrix molecules such as fibronectin. The acquisition of a mesenchymal phenotype leads to increases in migratory capacity and invasiveness of the cells undergoing EMT [38]. The reduced expression of E-cadherin and increased expression of N-cadherin has been termed as the “cadherin switch” and is proposed as a crucial process in cancer progression [39,41]. Another epithelial marker β -catenin acts to link the cytoplasmic domain of E-cadherin to α -catenin and connects the adhesion complex to the actin skeleton [42]. In addition, β -catenin also participates in signal transduction through the Wnt signalling pathway. Contrary to the non-invasive cells where β -catenin is localised at the membrane, for invasive cells that have undergone EMT loss of E-cadherin releases β -catenin into the cytoplasm and the nucleus [43]. Furthermore activation of the Wnt pathway also mediates β -catenin nuclear translocation where it associates with transcription factors from the T-Cell Factor (TCF)/lymphoid enhancing factor (LEF) family and drives transcription of Wnt/ β catenin target genes such as MYC, inhibitor of differentiation 2 (ID2), achaete-scute like 2 (ASCL2), matrix metalloproteinase 7 and 14 (MMP7 and MMP14) that are known to regulate different cellular processes including proliferation, differentiation, migration, invasion, survival and angiogenesis [44]. During EMT, epithelial cells also become resistant to both stress-induced cell death and that provoked by pro-apoptotic signals that activate the death receptor pathway such as tumour necrosis factor α (TNF- α) [45]. This increase in cell survival is triggered by the Snail zinc-finger transcription factor by suppressing apoptosis and provides a selective advantage to invasive cells to migrate and colonize distant sites [45].

EMT AND PROSTATE CANCER PROGRESSION

Despite the importance of the EMT program during embryogenic development, such as during formation of the mesoderm and neural crest and throughout life during wound healing, emerging evidence suggests that the atypical activation of the EMT program, attributed to a response to microenvironmental alterations and aberrant stimuli, may contribute to various pathologic conditions including tissue fibrosis and cancer progression [46]. The activation of this program in epithelial cancers provides the tumour cells with increased migratory and invasive capacity and the ability to metastasise. Unwarranted increases in epithelial cell proliferation and angiogenesis have been regarded as an important step occurring during the initiation and early growth of primary epithelial cancers

followed by the invasion-metastasis cascade, where the epithelial cancer cells switch from a collective invasion form towards a detached and disseminated cell migration mechanism [47]. These changes occur through the loss of the cell-cell adhesion complex attributed to mutations or loss in cadherin or catenins, or from the increase in cadherin cleavage resulting in the cancer cells losing their polarity and detaching from the basement membrane [47–49]. The acquisition of this malignant phenotype by epithelial cancer cells, facilitated by the EMT program [48], allows the tumour cells to leave the primary tumour site and invade through the basement membrane and surrounding tissues. Intra-vasation of the cells into a lymphatic or blood vessel then occurs, thereby entering the systemic circulation. The cells are then transported in the vasculature and invade the vascular basement membrane and extracellular matrix at secondary sites in the process of extravasation. Ultimately these cells will attach at the distant organ forming micro-metastases with the potential to proliferate into fully malignant, secondary tumours. The final step in metastasis called colonization requires the reversion of EMT and/or activation of the mesenchymal to epithelial transition (MET) program [48,50] (see Figure 2).

As mentioned previously, loss of E-cadherin is the defining feature of EMT and is of crucial importance in cancer invasion and metastasis. The loss of this adhesion molecule is accompanied by increases in other mesenchymal markers such as N-cadherin, vimentin and fibronectin [51]. An increasing number of studies demonstrate that in many tumours including prostate, loss of E-cadherin increases tumour cell motility and invasiveness and is associated with poor prognosis in cancer patients [39,52–54]. Similarly, examination of prostate cancer tissue samples using immunohistochemistry also revealed a reduction in the expression of cellular membrane E-cadherin that was concomitant with increasing Gleason score, clinical stage and a decline in survival [55]. In the clinical setting, plasma concentrations of E-cadherin are low in the later stage of prostate cancer patients and correlate with raised levels of PSA. In keeping with this, the use of plasma E-cadherin concentrations as a biomarker of the metastatic potential and to predict prognosis of prostate cancer has been proposed in clinical practice along with the use of PSA, particularly when aberrant modification of E-cadherin is likely to be involved in cancer invasion [53]. In addition to this, *in vivo* and *in vitro* studies have found that inhibition of N-cadherin using antibodies is able to delay the progression of

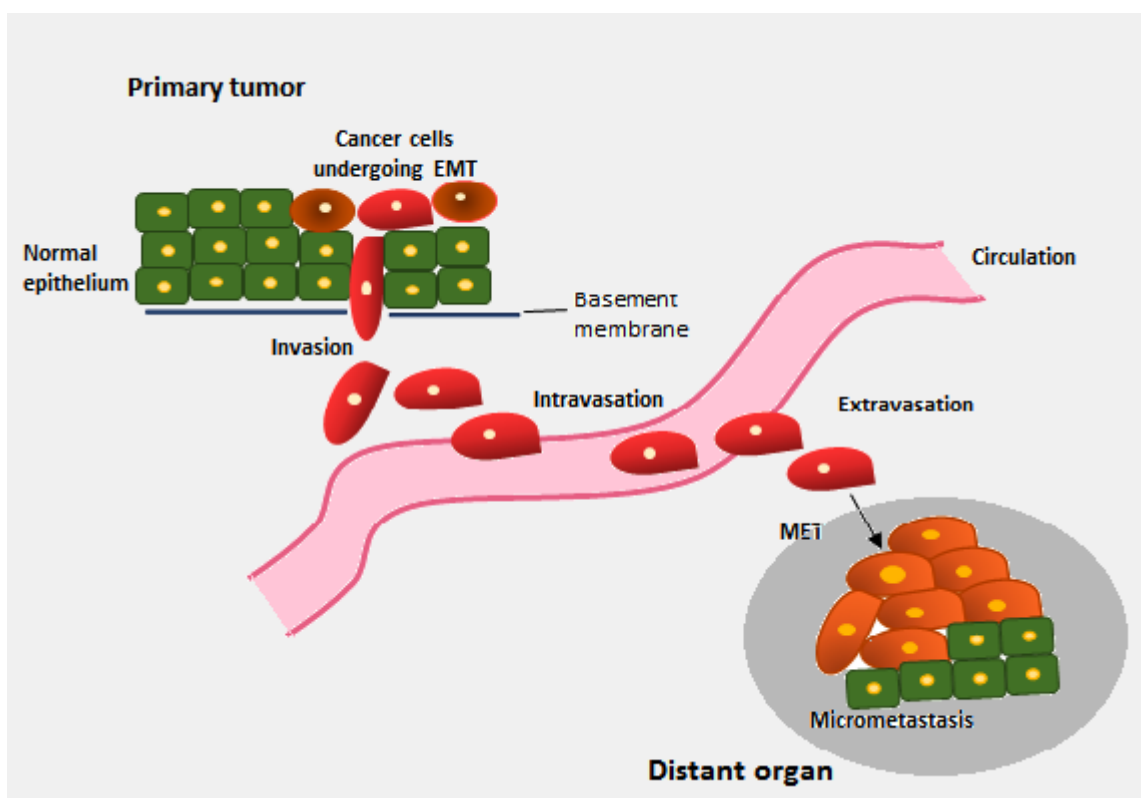


Figure 2: Epithelial-to-mesenchymal transition (EMT) during prostate cancer progression.

During cancer progression, cancer cells undergo EMT to acquire a mesenchymal phenotype which is characterised with loss of cell polarity and increased migratory capacity and invasiveness. The acquisition of this mesenchymal phenotype facilitates the cancer cells to invade through the basement membrane and enter the circulatory system *via* intravasation into blood or lymph vessels. After arrival at the distant site, the cells facilitate extravasation and form micrometastases with the potential to proliferate into fully malignant, secondary tumours. Ultimately they undergo mesenchymal-to-epithelial transition (MET) to colonize distant organs thereby generating the final, life-threatening stage of cancer progression.

prostate cancer to castration resistance and also to inhibit the invasion and metastasis of castration-resistant tumour growth suggesting the fundamental role of N-cadherin in promoting castration resistance and metastasis [56].

During cancer development, various extracellular signals could trigger the activation of EMT [57]. However, the full spectrum of critical mediators contributing to the activation of EMT remains uncertain. A few molecules capable of inducing EMT in many cell types have been identified including transforming growth factor- β (TGF- β), hepatocyte growth factor, platelet-derived growth factor, fibroblast growth factor, Wnt and Notch ligands, Hedgehog, epidermal growth factor (EGF) as well as the IGF family [57]. The action of these growth factors is thought to be cell type- or tissue type-specific and probably involves crosstalk between multiple pathways. Therefore combination of extracellular signals in the tumour microenvironment is needed to induce EMT [50]. The growth factors released by the cancer-associated stromal cells appear

to trigger the functional activation of EMT-inducing transcription factors particularly zinc finger E-box binding homeobox 1 (ZEB1), Slug, Snail, FOXC2, Gooseoid and Twist [48,57–62].

Among all of the EMT-inducing growth factors, TGF- β is undoubtedly a multifunctional cytokine that not only appears to be a primary inducer of EMT but also mediates critical cellular processes including apoptosis and immune responses [63]. During development, TGF- β is crucial for heart development [64], palatogenesis [65] as well as for testis development [66]: all are by virtue of its effect in regulating EMT. TGF- β signal *via* activation of Smad and non-smad signalling pathways including p38 MAP kinase (p38 MAPK). Apart from TGF- β , p38 MAPK can also be activated by Ras and Wnt pathways, which cooperate with TGF- β to induce EMT. Many studies have shown that activation of the TGF- β pathway induces EMT and single cell migration in cancer cells. Its activation is also required for intravasation, extravasation and blood borne metastasis [67,68]. In prostate cancer, during the

early stage of tumorigenesis, TGF- β acts as tumour suppressor by inhibiting cell proliferation and inducing apoptosis [69]. However, when the tumour progresses to clinically relevant disease, it becomes pro-oncogenic and switches its role to a promoter of EMT and metastasis, accelerating the pathologic malignant changes in the prostate [70]. TGF-beta also behaves in this manner during breast cancer progression and we have shown that both its pro- and anti-tumourigenic roles were at least partly mediated by IGFBP-3 [71]. The dual functions of TGF- β signalling on tumour initiation and progression are thought to be due to changes in the cell environment and consequent alterations in intracellular signalling. The tumour suppressive function of TGF-beta is mediated by a Smad-dependent pathway and inactivation of this pathway coupled with functional loss of TGF- β receptors is believed to activate its pro-oncogenic role. It has been suggested that TGF- β induces cancer cell invasive properties and dissemination from the primary tumour. However, when the cancer cells form micrometastasis at the distant organ, loss of TGF- β may stimulate an epithelial phenotype and colonization [21]. Intriguingly, it has also been shown that together with EGF, TGF- β may also promote prostate cancer progression by inducing immune suppression and promoting prostate cancer cell survival through the regulation of human leukocyte antigen class I antigens (HLA-I). TGF- β induces activation of Snail which leads to reduction in the transcription of nuclear factor kappa β (NF- κ B) and subsequent loss in HLA-I protein levels. This in turn results in the attenuation of cytotoxic T cell-mediated lysis of and provides a survival effect in prostate cancer cells [72]. In addition, TGF- β can also promote bone metastasis in prostate cancer through regulating microRNA-96 (miR-96) in a Smad-dependent transcription manner. Induced expression of miR-96 in response to TGF- β reduces its downstream target, AKT1S1. As the AKT1S1 protein is a negative regulator of mammalian target of rapamycin (mTOR), reduction in its expression enhances mTOR function thereby promoting an aggressive prostate cancer phenotype [73].

As discussed earlier, EMT-inducing transcription factors particularly Snail, Slug, ZEB1 and Twist are activated during EMT by various growth factors particularly TGF- β [48,57-62]. A well-known EMT inducer, Snail transcription factor has been extensively studied in many cancers as well as in prostate cancer. This zinc finger transcription factor induced by TGF- β mediates the activation of EMT through repression of adhesion molecules particularly E-cadherin by binding

to E-boxes in the proximal promoter region [61]. A number of studies have shown a strong correlation between Snail-mediated EMT with cell invasiveness and metastatic capacity. It has been shown that high expression of Snail is inversely correlated with E-cadherin expression in tumour tissues and is associated with poor prognosis of patients suffering from cancer. Loss of E-cadherin is concomitant with activation of Wnt signalling hence increasing cell invasion and survival [74]. In prostate cancer, immunohistochemical studies using tissue microarray assessing the gene expression profiles of Snail, have discovered that the expression of Snail is positively correlated with Gleason grade, with the highest expression seen in metastatic prostate cancer [75,76]. This is also supported by a recent *in vitro* study showing higher Snail expression in PC3 cells which exhibit advanced cancer properties compared to androgen-dependent LNCaP cells which exhibit more epithelial properties [77]. The importance of Snail in EMT-associated cell migration has further been revealed by overexpression of Snail in LNCaP cells which resulted in relocalization of E-cadherin from the cell membrane to the cytosol and increases in vimentin [78]. Additionally, inhibition of Snail was also found to inhibit EMT in human androgen-independent prostate cells (AIPC) and inhibit cancer metastasis in mouse models [79]. Apart from inducing EMT-mediated migration, Snail is also found to promote prostate tumour proliferation by inducing a neuroendocrine phenotype in LNCaP cells [80], suggesting that multiple pathways are involved in inducing prostate cancer aggressiveness.

Twist1, a basic Helix-Loop-Helix (bHLH) transcription factor, is initially found to regulate the expression of various target genes such as heparan sulfate 6-O-sulfotransferase 2 (HS6ST2), collagen, type I, alpha 1 (COL1A1) and coagulation factor II (thrombin) receptor (F2RL1) that are involved in cell differentiation, migration and survival. It is regarded as a proto-oncogene and its role in inducing EMT has been widely studied in many *in vitro* and *in vivo* models. Overexpression of TWIST1 induces EMT in breast, pancreatic and gastric cancers[81-83]. In prostate cancer, TWIST1 has been shown to induce EMT by increasing N-cadherin and fibronectin expression, promoting a more mesenchymal phenotype associated with increased prostate cancer cell migration and invasion [84,85].

In addition to promoting invasion and metastasis, EMT also induces resistance to therapy. EMT has been

associated with resistance to chemotherapy in many cancers including epithelial ovarian [86], lung [87], breast [88] as well as prostate cancer [89,90]. In prostate cancer, Snail and ZEB1 transcription factors which are known to induce EMT, mediate resistance to the chemotherapeutic drugs cisplatin and docetaxel respectively [90,91].

THE ROLE OF IGF SYSTEM IN PROSTATE CANCER

The insulin-like growth factor (IGF) signalling axis is essential for normal development and maintenance of tissues including that of the prostate, and dysregulation of this pathway contributes to cancer progression and malignant transformation.

Circulating plasma IGF-I is consistently found to be highly elevated in many cancer patients including breast [92], colorectal [93] and cervical cancer [94]. With prostate cancer, case control and prospective studies have reported associations of IGF-I with the risk and development of prostate cancer [95–98]. It has also been reported that higher levels of serum IGF-I with lower concentrations of IGFBP-3 are associated with prostate cancer mortality in men with advanced as opposed to localized prostate cancer, suggesting that IGF-I and IGFBP-3 may be potential biomarkers predicting death in men with advanced disease [99]. *In vitro* and *in vivo* studies have also contributed to our understanding of the IGF axis in the development of prostate cancer demonstrating that members of the IGF family are involved promoting a number of different processes involved in cancer development including growth, survival and migration (see review Biernacka 2012) [100]. In this review we will focus on the roles of members of the IGF axis in promoting EMT.

Alterations in insulin and IGFs, related to nutritional lifestyle and metabolic syndrome have been postulated to be important factors mediating the increased risk of prostate cancer [101]. Studies using dietary restriction (DR) in mice which is a classic experimental model to inhibit cancer development showed that DR was associated with a marked reduction in circulating IGF-I levels. The growth of chemically-induced liver tumours in mice was also inhibited by diet restriction and this was accompanied by a decrease in IGF-I concentrations [102]. Dietary restriction in mice has also been shown to increase apoptotic rates and reduce progression of bladder cancers, that was similarly associated with reduced levels of circulating IGF-I and in this study the positive impact of dietary

restriction was inhibited when IGF-I levels were restored indicating a key role for IGF-I in mediating the effects of nutrition on tumour progression [103]. The increased expression of IGF-I by prostate cancer cells that have metastasized to bone suggests a role of IGF-I in specifically promoting osteoblastic metastases in prostate cancer patients [75].

Many studies have shown the link between metabolic syndrome and clinical cancers and growing evidence has shown the involvement of the IGF pathway in the mechanism that promotes tumour development in patients with metabolic syndrome [104]. It has been known for many years that the IGF pathway plays a central role in mediating the effects of nutrition on cell growth and metabolism [105] and so could be important in mediating cancer growth and survival in patients with a disturbed metabolism. It was suggested that chronic hyperinsulinaemia (commonly seen in metabolic syndrome) increases circulating IGF-I by reducing gene expression and protein production of IGFBP-1 and IGFBP-2 in the liver, leading to activation of the IGF pathway which results in abnormal cellular proliferation and inhibition of apoptosis [23,104,106]. *In vitro*, it has also been reported that exposure of prostate cancer cells to 7mM glucose concentrations (and above), often seen in poorly controlled patients with type 2 diabetic induces chemoresistance which was associated with increased IGFBP-2 production [107]. Taken together, these reports suggest the involvement of the IGF axis in mediating the effect of altered metabolism on prostate cancer pathogenesis and metastatic progression.

THE ROLE OF IGF SYSTEM IN MEDIATING EMT

Aberrant stimulation of the IGF axis in prostate cancer cells is believed to contribute to the induction of EMT and subsequent progression of localised prostate cancer to metastatic disease. It has been widely known that IGF-I regulates the proliferation of prostate epithelial cells during growth and development. However, abnormal stimulation of IGF-IR by IGF-I has been found to induce spontaneous formation of neoplasia in prostate epithelium in BK5.IGF-I transgenic mice [108]. In addition, increased IGF-IR expression coupled with increase in IGF-I responsiveness has been implicated with the development of androgen independent progression with increased protection from apoptosis and enhanced mitotic activity [109].

The two main signalling pathways downstream of the IGF-IR (PTEN/PI3K/Akt pathway and RAS/MAPK

pathway) are both involved in the EMT program by regulating the transcription factors twist, snail and ZEB1 [110]. One of the key features during EMT is loss of epithelial markers; ie E-cadherin and β -catenin and increases in mesenchymal markers such as N-cadherin and fibronectin. IGF-I has been shown to promote epithelial prostate cancer cells to attain a more mesenchymal phenotype by regulating ZEB1 expression [111]. ZEB1, a transcriptional repressor known to regulate EMT, mediates its action by down regulating E-cadherin expression through its binding with CANNTG sequence in the promoter region of E-cadherin. Addition of exogenous IGF-I to human prostate cancer cell lines (ARCaP) induces up-regulation of ZEB1 transcription and protein level in a MEK/ERK dependent manner [111]. Increased ZEB1 induces EMT, manifested by reduction in E-cadherin and β -catenin and induction of mesenchymal markers N-cadherin, vimentin and fibronectin, thereby enhancing the ability of the cancer cells to migrate. Consistently, when ZEB1 is down-regulated through the use of siRNA, the epithelial phenotype of the cancer cells is restored as demonstrated by epithelial-like changes in cell morphology and E-cadherin and β -catenin re-expression [111]. This finding is also consistent with other studies that showed a similar effect of IGF-I in inducing EMT-mediated invasion through regulating the ZEB1 expression in colorectal and breast cancer [112,113].

The ability of IGF-I to induce EMT in many cancer cells including prostate is also dependent in its ability to increase the cellular level of β -catenin, another epithelial marker in which its accumulation could lead to neoplastic transformation [114]. Apart from its role in cadherin-based cell adhesion, β -catenin also participates in signal transduction through the Wnt signalling pathway, another important driver of EMT. As described earlier, activation of Wnt stabilises β -catenin and induces its translocation from the cytoplasm to the nucleus where it associates with transcription factors from the TCF/LEF family and promotes transcription of Wnt/ β catenin target genes that promote cell survival, proliferation and invasion [44]. Increases in cellular levels of β -catenin in androgen dependent and independent prostate cancer cells in response to IGF-I, either by increases in its phosphorylation or as a consequence of E-cadherin loss in the cell membrane could also lead to β -catenin nuclear translocation and subsequent activation of TCF/LEF transcription factor family members [115–117]. IGF-I also increases cyclin D1, one of the target genes of the β -catenin/TCF

complex, and stimulates cell cycle transition leading to cancer cell proliferation [116]. Supporting these data is a study conducted using NBTII bladder carcinoma cells which showed that activation of the IGF pathway by IGF-II binding to the IGF-IR promoted EMT through the induction of intracellular sequestration and degradation of E-cadherin along with translocation of β -catenin from the plasma membrane to the nucleus [118]. It has also been shown that IGF-I enhances the stability of β -catenin through glycogen synthase kinase-3 β (GSK3 β) inhibition [114]. GSK3 β , an enzyme involved in the regulation of glycogen synthesis in response to insulin, is known to induce proteosomal targeting and degradation of β -catenin by inducing its phosphorylation. It also acts as a potent suppressor for EMT-inducer transcription factors, Snail and Slug [119,120]. Inhibition of GSK3 β by IGF-I in prostate cancer can therefore regulate EMT and cancer metastasis by directly upregulating Snail and Slug expression. Taken together, these studies suggest an interplay between activation of IGF/IGF-IR and cell adhesion signalling as a crucial regulator in promoting EMT in prostate cancer cells by inducing atypical cell proliferation and tumour cell invasion.

As mentioned previously IGF-BPs are the main regulatory molecules of the IGFs: they bind with high affinity to IGF-I and IGF-II ligands in the circulation and modulate their availability and activity [121]. The IGF-dependent and independent roles of IGF-BPs in the development of cancers are well established and the *in vitro* and *in vivo* data have been extensively reviewed previously [37]. We will focus on the data indicating a role for IGF-BPs in cancer progression and EMT: reduced levels of IGF-BP-3 have been associated with cancer metastasis and increased survival in many cancers [122–125]. Similarly, prostate cancer patients with bone metastases have been reported to have significantly lower plasma levels of IGF-BP-3 compared with clinically localised-prostate cancer patients, suggesting low levels of circulating IGF-BP-3 are associated with reduced metastasis [126,127]. Low plasma IGF-BP-3 coupled with increases in IGF-I have also been associated with all-cause prostate cancer mortality in men with clinically advanced disease as discussed previously [99]. An important role for the IGF-IR and IGF-BP-3 in the homeostasis of prostate carcinoma cells has also been reported: inhibition of IGF-IR gene expression was shown to up-regulate IGF-BP-3 which led to the inhibition of cell proliferation and invasion [128]. The anti-proliferative properties of IGF-BP-3 in prostate cancer are also supported by a

study from Liu *et al.* that showed IGFBP-3 suppressed tumor growth by inhibiting angiogenesis in an IGF-independent way in CaP xenograft models [129]. In contrast to pro-apoptotic and anti-metastatic functions of IGFBP-3, there are a number of reports indicating the involvement of IGFBP-3 in promoting tumour progression. For example, immunohistochemistry studies have shown an increased level of IGFBP-3 in prostate adenocarcinoma compared with levels in benign tissue [130]. Evidence suggests that the response of a cancer cell to IGFBP3 is dependent on the cell environment, which may explain why both positive and negative effects of IGFBP-3 have been described for IGFBP-3 on prostate cancer progression. TGF- β is undoubtedly one of the most important inducers of EMT as we discussed earlier. TGF β can alter a cells environment in many ways, for example it enhances the production of extracellular matrix proteins, such as fibronectin [131] as part of its role in inducing EMT. TGF-beta is also one of the main regulators of IGFBP-3: activation of TGF- β has been found to up-regulate IGFBP-3 in cancer cells including those of the prostate [132] and a number of studies have shown functional interactions between the two (see review Huang SS 2005) [133] :it is likely therefore that IGFBP-3 will mediate some of the effects of TGF-beta on EMT in prostate cancer.

IGFBP-2, the second most abundant IGFBP in serum after IGFBP-3, also plays an important role in prostate cancer progression by stimulating prostate cancer cell proliferation in an IGF-dependent and independent manner. We have shown that IGFBP-2 could promote prostate tumour progression by acting as a growth promoter and as a survival factor against docetaxel-induced cell death [134]. It has also been shown that IGFBP-2 plays an important role in promoting the metastatic ability of many cells including human dermal fibroblasts, breast cancer and glioma cells [33–35] through the interaction with β -1 integrin receptors *via* the RGD sequence of IGFBP-2 [135]. This interaction leads to inactivation of phosphatase and tensin homolog (PTEN), a tumour suppressor gene and the phosphatase that normally restrains the PI3K/Akt pathway [136]. Inactivation of PTEN is associated with increases in the incidence and progression of prostate cancer in mouse models; a reduction in PTEN massively accelerated tumour growth, ultimately resulting in high-grade prostatic intraepithelial neoplasia and locally invasive carcinoma [137]. Immunohistochemistry studies have also reported higher levels of IGFBP-2 in malignant

compared with benign prostatic epithelial cells [138,139]. A report describing LNCaP xenografts shows a significant increase in IGFBP-2 levels after androgen withdrawal as a result of an adaptive response to an androgen depleted environment that promotes IGF-I mediated cell survival and cancer progression [140].

FUTURE PERSPECTIVES

There is overwhelming evidence showing that a poor lifestyle is associated with progression of a number of different cancers including the prostate: currently obesity and associated type 2 diabetes represent one of today's major health problems. Together this suggests that progression to metastatic disease will undoubtedly become more prevalent. The IGF axis clearly plays a number of important roles in effecting EMT and as IGFs are the major mediators of nutrition and metabolism, it will become even more important to gain a better understanding of these so that more effective interventions can be identified for prostate cancer patients that develop metastatic disease. A number of agents targeting the IGF-IR have already been developed and taken forward into clinical trials [141–143]. Despite promising preliminary data, the advanced clinical trials failed to show clinical benefit, which resulted in most drug companies ending current programs targeting the IGF-IR. These trials were undertaken on unselected patients: success of the drugs will be dependent upon the ability to identify which sub-set of patients will respond and this will require a greater understanding of the context; including an understanding of the effect of both the stage of progression of the cancer and the metabolic status of the host.

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