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Review

Stroma–epithelium crosstalk in prostate cancer

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Abstract

The critical role played by stroma–epithelium crosstalk in carcinogenesis and progression of prostate cancer has been increasingly recognized. These interactions are mediated by a variety of paracrine factors secreted by cancer cells and/or stromal cells. In human prostate cancer, reactive stroma is characterized by an increase in myofibroblasts and a corresponding amplification of extracellular matrix production and angiogenesis. Permanent genetic mutations have been reported in stromal cells as well as in tumour cells. Transforming growth factor-β, vascular endothelial growth factor, platelet-derived growth factor and fibroblast growth factor signalling pathways are involved in the process of angiogenesis, whereas hepatocyte growth factor, insulin-like growth factor-1, epidermal growth factor, CXC12 and Interleukin-6 play active roles in the progression, androgen-independent conversion and distal metastasis of prostate cancer. Some soluble factors have reciprocal interactions with androgens and the androgen receptor (AR), and can even activate AR in the absence of the androgen ligand. In this article, we review the complex interactions between cancer cells and the surrounding microenvironment, and discuss the potential therapeutic targets in the stromal compartment of prostate cancer.

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1 Introduction

Carcinomas that originate from the epithelia are the most common type of human cancer. In cancer tissue, following epithelial changes, the surrounding stroma is inevitably modified by cancer cell-derived cytokines, such as transforming growth factor-β (TGF-β). These modifications drive the emergence of the characteristic reactive stroma, which promotes the invasive and metastatic properties of cancer. Carcinoma-reactive stromal tissues include non-epithelial cells such as fibroblasts, inflammatory cells (lymphocytes, macrophages and mast cells) and vasculature-related cells (endothelial cells, pericytes and smooth muscle cells). These modified stromal cells secrete extracellular matrix (ECM) proteins and soluble factors, which in turn play important roles in carcinoma development, as they not only permit but also induce the initiation and progression of certain carcinomas [1–4].

Prostate cancer is the most common malignancy in men in the USA and the second leading cause of cancer-related deaths. In 2007, the estimated number of new cases in the USA was 218 890, accounting for 29% of all new cancer cases in men. The estimated number of deaths in the same period was 27 050, representing 9% of all cancer-related deaths in men [5]. Clinically undetectable, though histologically evident, prostate cancers are thought to be even more common, so that the advancement of prostate cancers from undetectable to clinically positive states, as well as from androgen-sensitive to androgen-independent conditions is of clear importance. The mechanisms

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underlying these activities are not fully understood. Several research groups have recently focused on the role of reactive stroma in the progression and metastasis of prostate cancer, and more and more data show that a dynamic interaction between stroma and epithelia plays a critical role in this procession [6–9].

The prostate is a tubulo-alveolar gland composed of epithelial tissues embedded in stromal components. The main cell types in the prostate stroma include fibroblasts, myofibroblasts and smooth muscle cells. These stromal cells secrete growth factors, produce ECM, and express androgen receptor (AR), estrogen receptor, adrenergic receptor and 5-α reductase. Fibroblasts express vimentin and laminin, whereas smooth muscle cells express desmin, α-actin, calponin, caldesmon, myosin, smoothelin and dystrophin, and myofibroblasts characteristically express procollagen-1 [10].

Reactive stroma in prostate cancer tissue is characterized by an increase in myofibroblasts and fibroblasts, with a significant decrease or loss of smooth muscle cells as prostate cancer progresses. Loss of normal tissue architecture, angiogenesis, nuclear atypia and genetic alterations also occur throughout this process. This altered stroma microenvironment may permit or initiate mechanisms that would allow a histologic cancer to escape from immunosurveillance or a hormone-sensitive cancer to escape from hormonal control, leading to an androgen-independent cancer and disease progression [11, 12].

In this review, we will discuss the reciprocal interactions between stroma cells and epithelial cells in the initiation and progression of prostate cancer.

2 Stroma–epithelia interactions in prostate cancer

In 2003, De Wever and Mareel [11] proposed two closely interactive pathways in their model of the crosstalk between cancer cells and stromal tissue, namely the efferent and afferent pathways. In the efferent pathway, cancer cells trigger a reactive response in the stroma by releasing soluble factors such as TGF-β and platelet-derived growth factor (PDGF). These factors can directly or indirectly transdifferentiate fibroblasts into myofibroblasts or induce epithelial–mesenchymal transition (EMT) in the surrounding cancer-associated stroma, resulting in cells that exhibit increased expression of vimentin, unchanged levels of smooth muscle α-actin and decreased expression of calponin, which together constitute the characteristic myofibroblast phenotype [13]. Cancer cells are also capable of inducing neoplastic transformation in the stromal cells of the host organ. This efferent pathway seems necessary and may serve as an early event in prostate cancer progression. In the afferent pathway, cancer cells respond to modified stromal cells in the surrounding microenvironment. Reactive stroma exerts multiple effects on the behaviour of cancer cells. Reactive stromal cells release soluble factors, secrete solid matrix components, repress cell apoptosis, increase motility and invasion, and guide progression and distal spread. It has been reported that when prostate cancer cells were co-inoculated with embryonic prostate fibroblasts *in vivo*, their malignant progression was inhibited [14]. In contrast, recombination of carcinoma-associated fibroblasts (CAF) or tumorigenic rat prostate mesenchymal cells with non-tumorigenic epithelial cells stimulated progression of tumorigenesis *in vivo* [15–17]. From these results, we can infer that in the efferent pathway stromal cells are activated by cancer tissue, whereas in the afferent pathway reactive stroma directs the carcinogenesis of prostate epithelia and cancer progression.

In a recent study, Hill *et al.* [18] proposed a selection theory, which predicts that in the mouse model of prostate cancer, inhibition of retinoblastoma gene function in tumour cells induces upregulation of p53 in stromal fibroblasts through a paracrine mechanism. This process creates a selective pressure that promotes the expansion of a subpopulation of fibroblasts that lack p53. These p53-null stromal fibroblasts contribute to tumour progression. The authors thus found evidence for the selection of a highly proliferative p53-null subpopulation of CAF, rather than induction of widespread p53 mutations in tumour-associated fibroblasts [18, 19].

The stroma is usually genetically stable, but permanent genetic changes have been found in carcinoma-associated stromal cells. Transformed murine stromal cells with altered chromosomal constitutions were reported in human prostate cancer xenografts [20]. In microdissected human prostate stromal tissues genetic lesions on chromosome 8p have been detected, and a significant incidence (33%) of loss of heterozygosity (LOH) was found in carcinoma-associated stromal samples [21]. In recent studies, promoter methylation of glutathione S-transferase 1 (GSTP1) and retinoic acid receptor β2 (RARβ2) was documented in four of five cancer-reactive stroma samples and adjacent normal areas [22, 23]. These observations of genetic changes in the stromal cells of the prostate cancer microenvironment may advance our understanding of carcinogenesis and progression of prostate cancer.

AR-mediated transcriptional activity in normal stromal cells was enhanced by co-culture with epithelial cells or epithelial cell-conditioned media. However, AR-mediated transcriptional activity in prostate cancer stromal cells was consistently repressed upon co-culture with cancerous epithelial cells. Epithelial cells can upregulate AR-mediated transcriptional activity by increasing recruitment of coactivators to the AR transcriptional complex on androgen-responsive genes, but in the prostate cancer

stromal microenvironment this ability seems compromised [24]. Concurrent overexpression of AR in the malignant epithelia and loss of AR immunoreactivity in the surrounding stroma was associated with higher clinical stage, higher pre-treatment PSA level, and earlier and higher relapse rates after radical prostatectomy [25, 26]. These results suggest the need to further investigate the mechanistic basis for the loss of AR expression in the malignant stroma and its potential role in the progression of prostate cancer. In a related study, nuclear AR staining in epithelial and stromal cells initially decreased but reappeared some months after castration [27]. Local tumour relapse and conversion to an androgen-independent state are probably associated with reappearance of nuclear AR, not only in tumour epithelial cells but also in the tumour stroma.

2.1 ECM and cell adhesion molecule (CAM)

Interactions among ECM, CAM and other cellular components can promote the ability of cells to adhere, as well as cancer cell proliferation, survival, migration and metastasis. De-arrangement of ECM barriers is noticed at the invasion front of tumour cells [28, 29]. Most important enzymes related to ECM degradation are produced by stroma constituents, including myofibroblasts, inflammatory and endothelial cells. By binding to ECM proteins or immunoglobulin superfamily molecules, integrins play key roles in regulating tumour growth and metastasis as well as tumour angiogenesis. Moreover, integrins may have a more complex role in cancer metastasis as they cooperate with serine proteases and metalloproteases to promote tumour cell invasion, migration and extravasation [30, 31].

Besides the loss of E-cadherin function in the epithelia, upregulation of N-cadherin and cadherin-11 in the stroma may serve important functions in prostate cancer progression [32]. N-cadherin is expressed in stromal cells, such as myofibroblasts, neurons, smooth muscle cells and endothelial cells, as well as in tumour cells. N-cadherin transduces the invasion signal across its extracellular, juxtamembrane and β-catenin-binding domains. These N-cadherin-dependent heterotypic contacts may promote matrix invasion, perineural invasion, muscular invasion and transendothelial migration [11]. Cadherin-11 is expressed in the stroma of all prostatic cancers and in the membrane of high-grade cancer cells. In a number of metastatic lesions, N-cadherin and cadherin-11 are homogeneously expressed [33]. Cadherin expression changes probably have significant impacts in prostate cancer metastasis.

Fibroblast activation protein-α (FAP) is a membrane-bound glycoprotein with serine protease activity. FAP is expressed neither by fibroblasts or other cell types in normal tissues, nor by cancer epithelial cells, but is overexpressed on the surface of reactive stromal fibroblasts in the stromal compartment of most human epithelial tumours. Thus, the proteolytic activity of FAP represents a potential novel pan-tumour therapeutic target [34].

2.2 Soluble factors in carcinoma-associated stroma

Interactions between stroma and epithelia in the normal and malignant prostatic microenvironment involve a number of soluble factors and their receptors. Soluble factors act in paracrine fashion by binding to their respective receptors. Soluble factors can work in coordination with other signalling molecules, such as the ECM and integrins mentioned above, as well as other intracellular signalling factors, such as steroid hormones and their corresponding receptors. These synergistic interactions could trigger and facilitate carcinogenesis, aggressive local cancer growth and distal metastasis [35].

2.2.1 Transforming growth factor β

The TGF-β family includes three isoforms [1–3], which act through transmembrane type I and type II receptors (TβRI and TβRII). TGF-β proteins are involved in multiple processes, including regulation of cell proliferation, functional differentiation, ECM production, cell motility and apoptosis. TGF-β maintains homeostasis in benign prostatic epithelia by eliciting differentiation, inhibiting proliferation and inducing apoptosis. Conditional inactivation of the TβRII gene in mouse fibroblasts resulted in intraepithelial neoplasia in prostate tissue in association with an increased number of stromal cells. Thus, TGF-β signalling in fibroblasts modulates the growth and oncogenic potential of adjacent epithelia [36]. TGF-β enhances carcinogenesis by inducing EMT or fibroblast–myofibroblast transdifferentiation, and also promotes local invasion and distal metastasis by stimulating angiogenesis, inhibiting immune surveillance or promoting the degradation of ECM. The signalling factors that mediate these activities include Smad, phosphatidylinositol 3-kinase (PI3K)/AKT and/or mitogen-activated protein kinase (MAPK) pathways [37, 38].

TGF-β expression is enhanced in both stroma and epithelia of prostate cancer, unlike in PIN or benign prostate hyperplasia (BPH). As the prostate cancer progresses, cancer cells become insensitive to the growth inhibition of TGF-β1 [38, 39]. Blocking TGF-β signalling by loss of TβRII promotes prostate cancer metastasis [40]. Prostate cancer cells can also become refractory to TGF-β1 due to transcriptional silencing of TβRII through promoter CpG methylation. This expression is restored after treatment with epigenetic silencing modifiers 5-aza-2′-deoxycytidine (5-aza) and trichostatin A, which in turn restores cellular sensitivity to TGF-β1 [41, 42]. Promoter CpG methylation may have extensive effects on the invasive and metastatic properties of prostate cancer. High expression of TGF-β1

and loss of TβRII are poor prognostic factors related to cancer progression.

Interactions between androgen and TGF-β1 have been observed in prostate stromal cells. TGF-β1 is a stimulator of stromal cell myodifferentiation and androgens regulate prostate stromal cells. During cellular differentiation, AR was transiently induced to translocate from the nucleus to the cytoplasm by TGF-β1, followed by a resumption of nuclear localization in myodifferentiated cells [43]. These data indicate that androgen and TGF-β1 crosstalk may cooperatively regulate myodifferentiation of stromal cells in the reactive stroma in prostate cancer. Recent investigation showed that 5α-dihydrotestosterone (DHT) can downregulate the expression of TβRII by a transcriptional mechanism [44]. In addition, inhibition of TGF-β signalling suppressed the progression of androgen-independent human prostate cancer in nude mice [45]. These results provide fresh insight into the mechanism of growth control by androgens and the progression of prostate cancer to androgen independence. TGF-β can also upregulate basic fibroblast growth factor (bFGF) messenger RNA (mRNA) and protein expressions by post-transcriptional mechanisms in cultured human prostate stromal cells [46]. TGF-β2 was reported to promote the survival of cancer cells and resistance to apoptosis by stimulating the activation of nuclear factor-kappaB (NF-κB) in PC3 cells [47]. These findings identify TGF-β2 as a potential therapeutic target that could inhibit the growth of tumour cells that depend on constitutively active NF-κB.

Prostate-derived factor (PDF) is another member of the TGF-β superfamily that is significantly upregulated in human prostate tumours. PDF was shown to promote AR-positive prostate tumour progression by upregulating cell proliferation via the ERK1/2 and p90RSK signalling pathways [48].

2.2.2 Vascular endothelial growth factor (VEGF)

VEGF is required for blood vessel and lymphatic vessel formation. The function of VEGF in vessel formation is complemented by additional factors such as bFGF, TGF-β, PDGF and the angiopoietins. VEGF expression is confined to the stroma in BPH, but is found in both the stroma and epithelia of prostate cancer, where it plays a central role in tumour angiogenesis. DHT induced a twofold increase in VEGF mRNA and protein levels in human fetal fibroblasts [49]. VEGF is secreted by stromal cells in prostate cancer and bone marrow, and initiates paracrine activation of bone marrow endothelial cells, thereby supporting tumour neovascularization [50]. Increased expression of VEGF-C and VEGFR-3 is associated with prostate cancer progression and metastasis to regional lymph nodes [51]. Finasteride can decrease VEGF expression as well as microvessel density in specimens of benign prostatic hyperplasia. In prostate cancer, androgen deprivation has been shown to decrease VEGF expression and may be a mechanism of castration-mediated apoptosis [52]. VEGF was found to be a significant prognostic factor for disease-specific survival in locally invasive prostate cancer after radiotherapy [53].

2.2.3 Platelet-derived growth factor (PDGF) and receptor (PDGFR)

PDGF family proteins signal through transmembrane tyrosine kinase receptors and stimulate various cellular functions, including growth, proliferation and differentiation. Fibroblasts, neurons, endothelial cells and epithelial cells all express PDGFs. PDGF can stimulate TGF-β release and promote myofibroblast differentiation. PDGF can also stimulate secretion of insulin-like growth factor (IGF-1), hepatocyte growth factor (HGF), FGF2 and endothelin-3. PDGF is a potent mitogen and a chemoattractant for mesenchymal cells [54]. PDGF signalling has been shown to contribute to EMT, regulate cancer cell invasion and angiogenesis. High levels of PDGF-D resulted in the significant induction of EMT in PC3 cells. Activation of the mammalian target of rapamycin and NF-κB, and Bcl-2 overexpression were observed in PDGF-D-transfected PC3 cells, which were associated with enhanced adhesive and invasive behaviours [55]. In a rat prostate cancer model, stromal PDGF-Rβ-targeted therapy enhanced castration effects, resulting in a decreased vascular density, increased tumour cell apoptosis and decreased tumour growth compared with castration therapy alone [56].

2.2.4 Insulin-like growth factor-1 (IGF-1)

IGF-1 is synthesized mainly in prostatic stroma, whereas its receptor (IGF-1R) is expressed by epithelial cells. Stromally expressed c-Jun may promote prostatic epithelial proliferation through the paracrine action of IGF-1, which in turn promotes prostate epithelial proliferation [57]. IGF-1 overexpression has been shown to drive neoplastic transformation of murine prostate epithelium, whereas the antisense RNA for IGF-1R inhibited prostate cancer proliferation and invasion. IGF-1 significantly increased the invasive capacity of DU145 cells *in vitro*; this increase was inhibited by blocking the IGF-1R. Specific inhibitors for the MAPK and PI3-K pathways also decreased IGF-1-mediated invasion [58].

Many interactions between IGF signalling and AR have been detected in the context of prostate cancer. IGF-1 can stimulate androgen response genes in epithelial prostate cancer cells, and inhibition of IGF-1R signalling can result in cytoplasmic AR retention and significant changes in androgen-regulated gene expression [59, 60]. Prostate cancer epithelia are sensitive to the surrounding IGF-1 levels regardless of their androgen sensitivity status.

Castration reduced stromal IGF-1 mRNA levels in normal tissues but not in carcinoma-associated stroma, because of the low AR expression. In prostate cancer epithelium, IGF-1 expression was also shown to be increased and androgen could induce synthesis of IGF-1. Reduction of IGF-1 mRNA levels in the tumour stroma and/or epithelium was associated with epithelial apoptosis [61]. Gonadotropin-releasing hormone agonists can also exert significant inhibitory effects on the migratory and invasive behaviours of androgen-independent prostate cancer cells by interfering with the pro-metastatic activities of IGF-1 [62]. Activation of IGF-1R can block the apoptosis induced by TGF-β1 in prostatic epithelial cells, indicative of an interaction between IGF-1 and TGF-β1 [38]. Interactions among IGF-1, TGF-β1 and androgen may therefore provide prostate cancer cells with the microenvironment for invasion, metastasis and androgen-independent conversion in the absence of the androgen ligand.

2.2.5 HGF/scatter factor (SF) and c-met

HGF/SF activates its transmembrane tyrosine kinase receptor, c-met, in normal and prostate cancer epithelial cells through a paracrine mechanism. HGF/SF is expressed throughout the prostate stroma, which is consistent with a paracrine effect on the adjacent epithelium, where the c-met receptor is expressed. HGF/SF plays important roles in the regulation of cell growth, motility, morphogenesis and angiogenesis. The c-met was found to be expressed in approximately 50% of localized prostate cancers and in almost all prostate cancer metastatic lesions [63]. The aberrant expression of HGF/SF or c-met, was shown to regulate invasion and growth of carcinoma cells in a variety of tumors, including prostate cancer [64]. HGF can upregulate bone morphogenetic protein-7 (BMP-7) and BMP-7 receptor in the prostate cancer both *in vitro* and *in vivo*, particularly in bone metastasis, which can be completely blocked by the HGF antagonist NK4 [65]. Several approaches have been used to inhibit the HGF/SF and c-Met pathway, such as HGF/SF antagonists, monoclonal antibodies against HGF/SF or c-Met, agents that reduce expression of c-Met, c-Met kinase inhibitors, and inhibitors of c-Met induced signaling pathways [63, 66].

Androgen signalling can repress the expression of c-met in prostate cancer cells by inhibiting Sp1-induced transcription in a ligand-dependent manner [64]. Both androgen withdrawal and AR-specific small interfering RNA can activate c-met expression, although expression of androgen-activated genes is repressed. Knockdown of both AR and c-met expression markedly inhibited cancer cell growth. These results indicate that activation of c-met signalling may contribute to androgen-independent progression in prostate cancer [67]. Membrane-localized nucleolin functions as a novel HGF receptor and appears to be upregulated during progression of androgen-independent prostate cancer. Antibodies against nucleolin can ameliorate the stimulatory effects of HGF on c-met-negative prostate cancer cells [68]. Therapeutic strategies that inhibit the activation of the HGF/c-Met or nucleolin pathways may thus be beneficial when combined with the current androgen ablation treatment.

2.2.6 Fibroblast growth factor (FGF)

The FGF family contains many members, including acidic FGF (FGF1), basic FGF (FGF2), FGF6, FGF8 and KGF/FGF7, which are expressed at increased levels by stromal cells in prostate cancer tissue and function as paracrine and/or autocrine soluble factors. FGF2, FGF8 and KGF/FGF7 were also found to interact with androgen or AR. All the four types of FGF receptors (FGFR1–4) are expressed in prostate cancer epithelia, although FGFR-1 and FGFR-4 are most closely associated with prostate cancer progression. FGF receptor signalling plays a role in prostate cancer progression by activating several signal transduction pathways, including phospholipase Cγ, PI3K/AKT and the MAPK pathway. Enhanced FGF receptor signalling can have myriad effects, including a promotion of proliferation, resistance to cell death, increased motility and invasiveness, increased angiogenesis, enhanced metastasis, resistance to chemotherapy and radiation, and progression to androgen independence. These data suggest that the FGF signalling pathway is an important potential therapeutic target in both tumour cells and cancer angiogenesis [69].

2.2.7 Other soluble factors

Other soluble factors involved in stroma–epithelia crosstalk in prostate cancer include epidermal growth factor (EGF), CXCL12/SDF-1 (stromal cell-derived factor-1), caveolin 1, hypoxia inducible factor and interleukin-6 (IL-6).

EGF is well known for its involvement in prostate cancer progression and works by binding receptors such as EGFR/erbB1 and erbB2/Her-2. Stromal expression of EGF has been reported, especially in osteogenic stroma [70]. It has also been shown that in androgen-independent prostate cancer cells, many cell cycle-regulated genes are constitutively activated by EGF and androgen. Of those activated genes, 75% are co-regulated by androgen and EGF, which indicates that EGF can induce androgen-regulated genes even in the absence of androgen [71]. Interactions between EGFR and AR at the membrane level in AR-transfected PC3 and LNCaP cells have been shown: ectopic AR expression in PC3 cells conferred a less malignant phenotype by interfering with EGFR signalling through a mechanism involving an interaction between AR and EGFR. This interaction may also be important for invasion and metastasis of prostate cancer [72].

Within the tumour microenvironment, mesenchymal or marrow-derived stromal cells constitutively secrete the

chemokine CXCL12/SDF-1. CXCL12 acts by binding its cognate receptor CXCR4 on the membrane of cancer cells, which activates multiple signalling pathways such as PI3K/AKT, MAPK and NF-κB. CXCR4 expression is a poor prognostic marker in prostate cancer as CXCR4 protein expression is significantly elevated in localized and metastastic cancers. CXCR4 signalling promotes tumour progression and metastasis by several mechanisms. First, CXCL12 itself can promote survival and growth of cancer cells in a paracrine manner. Second, CXCL12 can promote tumour angiogenesis by attracting endothelial cells to the neoplastic microenvironment. When CXCR4-knockout cells were co-grafted with vascular endothelial cells into severe combined immunodeficiency mice, significantly fewer human blood vessels and parallel inhibition of the invasive behaviours of prostate cancer cells were observed [73]. Expression of CXCR4 in tumour cells is essential for distal metastasis to organs where CXCL12 is expressed, such as the bone marrow. Neutralizing antibodies targeted to CXCL12 or CXCR4 decreased the proliferation of bone metastatic lesions [74]. Finally, the CXCR4 pathway was shown to interact with TGF-β1 in the process of CAF-induced carcinogenesis [75]. Current observations suggest that CXCL12–CXCR4 pathway antagonists may be potential agents for the treatment of prostate cancer [76].

IL-6 is involved in regulation of immune reaction, cell growth and differentiation. IL-6 is expressed mainly by prostate stromal cells, although both stroma and epithelium express the IL-6 receptor. Levels of both IL-6 and its receptor increase during the process of carcinogenesis in prostate tissue. Activation of the JAK/STAT (Janus kinase/signal transducer and activator of transcription), MAPK and PI3K/AKT signalling pathways has been reported in various prostate cancer cell lines in response to IL-6 [77]. IL-6 promotes growth of most prostate cancer cells, with the exception of the LNCaP cell line, in which IL-6 causes growth arrest and induces cell differentiation. However, a recent study showed that short-term treatment of IL-6 inhibited LNCaP cell growth by a paracrine mechanism associated with neuroendocrine differentiation, whereas long-term IL-6 treatment promoted LNCaP cell growth by an autocrine mechanism accompanied by activation of AR signalling [78]. The transition of IL-6 from a paracrine growth inhibitor to an autocrine growth stimulator, as well as its ability to activate AR in the absence of androgen suggest a role for IL-6 during prostate cancer progression and possibly androgen-independent progression. IL-6 is also involved in regulation of VEGF expression as well as neuroendocrine differentiation in prostate tissue. Studies using an IL-6 antibody have reported induction of apoptosis, inhibition of tumour proliferation and elimination of the progression to androgen-independent status in a prostate cancer xenograft model [79].

3 Conclusions

In this review, we emphasise the importance of cross-talk between stroma and epithelia in carcinogenesis and progression of prostate cancer. Reactive stroma is induced after epithelial changes. In human prostate cancer, reactive stroma is characterized by increase of myofibroblasts, and corresponding amplification of ECM and angiogenesis. The stromal cells in cancer tissue subsequently influence epithelial transformation by producing a variety of paracrine factors that affect normal epithelia, carcinoma cells and the stromal cells. In addition, recent studies have shown that permanent genetic mutations arising in stromal cells can precede tumour development. TGF-β, VEGF, PDGF and FGF signalling were shown to be involved in the process of angiogenesis, whereas HGF, IGF-1, EGF, CXC12 and IL-6 play active roles in cancer progression, androgen-independent conversion and distal metastasis. Furthermore, several of these soluble factors have reciprocal regulatory relationships with androgen and AR, and some can even activate AR in the absence of the androgen ligand. Integrin and N-cadherin also play active roles in this process. Further study is required to fully understand the complex interactions between cancer cells and the tumour microenvironment. Targeted therapy to the stromal compartment as well as to the epithelia is expected to be clinically promising, and further elucidation of the molecular mechanisms underlying tumour–stroma interactions may yield novel therapeutic targets for prostate cancer, especially for androgen-independent and metastatic diseases.

References

1 Bhowmick NA, Moses HL. Tumor-stroma interactions. Curr Opin Genet Dev 2005; 15: 97–101.
2 Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. Nature 2004; 432: 332–7.
3 Hu M, Polyak K. Microenvironmental regulation of cancer development. Curr Opin Genet Dev 2008; 18: 27–34.
4 West RB, van de Rijn M. Experimental approaches to the study of cancer-stroma interactions: recent findings suggest a pivotal role for stroma in carcinogenesis. Lab Invest 2007; 87: 967–70.
5 Jemal A, Siegel R, Ward E, Murray T, Xu J, *et al*. Cancer statistics, 2007. CA Cancer J Clin 2007; 57: 43–66.
6 Condon MS. The role of the stromal microenvironment in prostate cancer. Semin Cancer Biol 2005; 15: 132–7.
7 Yang F, Tuxhorn JA, Ressler SJ, McAlhany SJ, Dang TD, *et al*. Stromal expression of connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis. Cancer Res 2005; 65: 8887–95.
8 Arnold JT, Gray NE, Jacobowitz K, Viswanathan L, Cheung PW, *et al*. Human prostate stromal cells stimulate increased PSA production in DHEA-treated prostate cancer epithelial cells. J Steroid Biochem Mol Biol 2008; 111: 240–6.
9 Morrissey C, Vessella RL. The role of tumor microenvironment in prostate cancer bone metastasis. J Cell Biochem 2007; 101:

873–86.
10 Berry PA, Maitland NJ, Collins AT. Androgen receptor signalling in prostate: effects of stromal factors on normal and cancer stem cells. Mol Cell Endocrinol 2008; 288: 30–7.
11 De Wever O, Mareel M. Role of tissue stroma in cancer cell invasion. J Pathol 2003; 200: 429–47.
12 Zhang Y, Nojima S, Nakayama H, Jin Y, Enza H. Characteristics of normal stromal components and their correlation with cancer occurrence in human prostate. Oncol Rep 2003; 10: 207–11.
13 Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, *et al*. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. Clin Cancer Res 2002; 8: 2912–23.
14 Yan G, Fukabori Y, McBride G, Nikolaropolous S, McKeehan WL. Exon switching and activation of stromal and embryonic fibroblast growth factor (FGF)-FGF receptor genes in prostate epithelial cells accompany stromal independence and malignancy. Mol Cell Biol 1993; 13: 4513–22.
15 Chung LW, Chang SM, Bell C, Zhau HE, Ro JY, *et al*. Co-inoculation of tumorigenic rat prostate mesenchymal cells with non-tumorigenic epithelial cells results in the development of carcinosarcoma in syngeneic and athymic animals. Int J Cancer 1989; 43: 1179–87.
16 Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, *et al*. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res 1999; 59: 5002–11.
17 Hayward SW, Wang Y, Cao M, Hom YK, Zhang B, *et al*. Malignant transformation in a nontumorigenic human prostatic epithelial cell line. Cancer Res 2001; 61: 8135–42.
18 Hill R, Song Y, Cardiff RD, Van Dyke T. Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. Cell 2005; 123: 1001–11.
19 Bierie B, Moses HL. Under pressure: stromal fibroblasts change their ways. Cell 2005; 123: 985–7.
20 Pathak S, Nemeth MA, Multani AS, Thalmann GN, von Eschenbach AC, *et al*. Can cancer cells transform normal host cells into malignant cells? Br J Cancer 1997; 76: 1134–8.
21 Macintosh CA, Stower M, Reid N, Maitland NJ. Precise microdissection of human prostate cancers reveals genotypic heterogeneity. Cancer Res 1998; 58: 23–8.
22 Hanson JA, Gillespie JW, Grover A, Tangrea MA, Chuaqui RF, *et al*. Gene promoter methylation in prostate tumor-associated stromal cells. J Natl Cancer Inst 2006; 98: 255–61.
23 Rodriguez-Canales J, Hanson JC, Tangrea MA, Erickson HS, Albert PS, *et al*. Identification of a unique epigenetic sub-microenvironment in prostate cancer. J Pathol 2007; 211: 410–9.
24 Cano P, Godoy A, Escamilla R, Dhir R, Onate SA. Stromal-epithelial cell interactions and androgen receptor-coregulator recruitment is altered in the tissue microenvironment of prostate cancer. Cancer Res 2007; 67: 511–9.
25 Ricciardelli C, Choong CS, Buchanan G, Vivekanandan S, Neufing P, *et al*. Androgen receptor levels in prostate cancer epithelial and peritumoral stromal cells identify non-organ confined disease. Prostate 2005; 63: 19–28.
26 Henshall SM, Quinn DI, Lee CS, Head DR, Golovsky D, *et al*. Altered expression of androgen receptor in the malignant epithelium and adjacent stroma is associated with early relapse in prostate cancer. Cancer Res 2001; 61: 423–7.
27 Wikström P, Ohlson N, Stattin P, Bergh A. Nuclear androgen receptors recur in the epithelial and stromal compartments of malignant and non-malignant human prostate tissue several months after castration therapy. Prostate 2007; 67: 1277–84.
28 Ingber DE. Cancer as a disease of epithelial-mesenchymal interactions and extracellular matrix regulation. Differentiation 2002; 70: 547–60.
29 Ghersi G. Roles of molecules involved in epithelial/mesenchymal transition during angiogenesis. Front Biosci 2008; 13: 2335–55.
30 Jin H, Varner J. Integrins: roles in cancer development and as treatment targets. Br J Cancer 2004; 90: 561–5.
31 Guo W, Giancotti FG. Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 2004; 5: 816–26.
32 Bussemakers MJ, Van Bokhoven A, Tomita K, Jansen CF, Schalken JA. Complex cadherin expression in human prostate cancer cells. Int J Cancer 2000; 85: 446–50.
33 Tomita K, van Bokhoven A, van Leenders GJ, Ruijter ET, Jansen CF, *et al*. Cadherin switching in human prostate cancer progression. Cancer Res 2000; 60: 3650–4.
34 Aggarwal S, Brennen WN, Kole TP, Schneider E, Topaloglu O, *et al*. Fibroblast activation protein peptide substrates identified from human collagen I derived gelatin cleavage sites. Biochemistry 2008; 47: 1076–86.
35 Chung LW, Baseman A, Assikis V, Zhau HE. Molecular insights into prostate cancer progression: the missing link of tumor microenvironment. J Urol 2005; 173: 10–20.
36 Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, *et al*. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science 2004; 303: 848–51.
37 Ao M, Williams K, Bhowmick NA, Hayward SW. Transforming growth factor-beta promotes invasion in tumorigenic but not in nontumorigenic human prostatic epithelial cells. Cancer Res 2006; 66: 8007–16.
38 Danielpour D. Functions and regulation of transforming growth factor-beta (TGF-beta) in the prostate. Eur J Cancer 2005; 41: 846–57.
39 Bello-DeOcampo D, Tindall DJ. TGF-betal/Smad signaling in prostate cancer. Curr Drug Targets 2003; 4: 197–207.
40 Tu WH, Thomas TZ, Masumori N, Bhowmick NA, Gorska AE, *et al*. The loss of TGF-beta signaling promotes prostate cancer metastasis. Neoplasia 2003; 5: 267–77.
41 Zhao H, Shiina H, Greene KL, Li LC, Tanaka Y, *et al*. CpG methylation at promoter site -140 inactivates TGFbeta2 receptor gene in prostate cancer. Cancer 2005; 104: 44–52.
42 Pulukuri SM, Rao JS. CpG island promoter methylation and silencing of 14–3–3sigma gene expression in LNCaP and Tramp-C1 prostate cancer cell lines is associated with methyl-CpG-binding protein MBD2. Oncogene 2006; 25: 4559–72.
43 Gerdes MJ, Larsen M, Dang TD, Ressler SJ, Tuxhorn JA, *et al*. Regulation of rat prostate stromal cell myodifferentiation by androgen and TGF-beta1. Prostate 2004; 58: 299–307.
44 Song K, Wang H, Krebs TL, Kim SJ, Danielpour D. Androgenic control of transforming growth factor-beta signaling in prostate epithelial cells through transcriptional suppression of transforming growth factor-beta receptor II. Cancer Res 2008; 68: 8173–82.
45 Zhang F, Lee J, Lu S, Pettaway CA, Dong Z. Blockade of transforming growth factor-beta signaling suppresses progression of androgen-independent human prostate cancer in nude mice. Clin Cancer Res 2005; 11: 4512–20.
46 Story MT, Hopp KA, Meier DA. Regulation of basic fibroblast growth factor expression by transforming growth factor beta in cultured human prostate stromal cells. Prostate 1996; 28: 219–26.
47 Lu T, Burdelya LG, Swiatkowski SM, Boiko AD, Howe PH, *et al*. Secreted transforming growth factor beta2 activates NF-kappaB, blocks apoptosis, and is essential for the survival of some tumor cells. Proc Natl Acad Sci USA 2004; 101: 7112–7.

48 Chen SJ, Karan D, Johansson SL, Lin FF, Zeckser J, *et al*. Prostate derived factor as a paracrine and autocrine factor for the proliferation of androgen receptor-positive human prostate cancer cells. Prostate 2007; 67: 557–71.
49 Levine AC, Liu XH, Greenberg PD, Eliashvili M, Schiff JD, *et al*. Androgens induce the expression of vascular endothelial growth factor in human fetal prostatic fibroblasts. Endocrinology 1998; 139: 4672–8.
50 Muir C, Chung LW, Carson DD, Farach-Carson MC. Hypoxia increases VEGF-A production by prostate cancer and bone marrow stromal cells and initiates paracrine activation of bone marrow endothelial cells. Clin Exp Metastasis 2006; 23: 75–86.
51 Jennbacken K, Vallbo C, Wang W, Damber JE. Expression of vascular endothelial growth factor C (VEGF-C) and VEGF receptor-3 in human prostate cancer is associated with regional lymph node metastasis. Prostate 2005; 65: 110–6.
52 Cheng L, Zhang S, Sweeney CJ, Kao C, Gardner TA, *et al*. Androgen withdrawal inhibits tumor growth and is associated with decrease in angiogenesis and VEGF expression in androgen-independent CWR22Rv1 human prostate cancer model. Anticancer Res 2004; 24: 2135–40.
53 Green MM, Hiley CT, Shanks JH, Bottomley IC, West CM, *et al*. Expression of vascular endothelial growth factor (VEGF) in locally invasive prostate cancer is prognostic for radiotherapy outcome. Int J Radiat Oncol Biol Phys 2007; 67: 84–90.
54 Heldin CH, Eriksson U, Ostman A. New members of the platelet-derived growth factor family of mitogens. Arch Biochem Biophys 2002; 398: 284–90.
55 Kong D, Wang Z, Sarkar SH, Li Y, Banerjee S, *et al*. Platelet-derived growth factor-D overexpression contributes to epithelial-mesenchymal transition of PC3 prostate cancer cells. Stem Cells 2008; 26: 1425–35.
56 Johansson A, Jones J, Pietras K, Kilter S, Skytt A, *et al*. A stroma targeted therapy enhances castration effects in a transplantable rat prostate cancer model. Prostate 2007; 67: 1664–76.
57 Li W, Wu CL, Febbo PG, Olumi AF. Stromally expressed c-Jun regulates proliferation of prostate epithelial cells. Am J Pathol 2007; 171: 1189–98.
58 Saikali Z, Setya H, Singh G, Persad S. Role of IGF-1/IGF-1R in regulation of invasion in DU145 prostate cancer cells. Cancer Cell Int 2008; 8: 10.
59 York TP, Plymate SR, Nelson PS, Eaves LJ, Webb HD, *et al*. cDNA microarray analysis identifies genes induced in common by peptide growth factors and androgen in human prostate epithelial cells. Mol Carcinog 2005; 44: 242–51.
60 Wu JD, Haugk K, Woodke L, Nelson P, Coleman I, *et al*. Interaction of IGF signaling and the androgen receptor in prostate cancer progression. J Cell Biochem 2006; 99: 392–401.
61 Ohlson N, Bergh A, Stattin P, Wikström P. Castration-induced epithelial cell death in human prostate tissue is related to locally reduced IGF-1 levels. Prostate 2007; 67: 32–40.
62 Montagnani Marelli M, Moretti RM, Mai S, Procacci P, Limonta P. Gonadotropin releasing hormone agonists reduce the migratory and the invasive behavior of androgen-independent prostate cancer cells by interfering with the activity of IGF-I. Int J Oncol 2007; 30: 261–71.
63 Knudsen BS, Edlund M. Prostate cancer and the met hepatocyte growth factor receptor. Adv Cancer Res 2004; 91: 31–67.
64 Verras M, Lee J, Xue H, Li TH, Wang Y, *et al*. The androgen receptor negatively regulates the expression of c-Met: implications for a novel mechanism of prostate cancer progression. Cancer Res 2007; 67: 967–75.
65 Ye L, Lewis-Russell JM, Sanders AJ, Kynaston H, Jiang WG. HGF/SF up-regulates the expression of bone morphogenetic protein 7 in prostate cancer cells. Urol Oncol 2008; 26: 190–7.
66 Liu X, Yao W, Newton RC, Scherle PA. Targeting the c-MET signaling pathway for cancer therapy. Expert Opin Investig Drugs 2008; 17: 997–1011.
67 Maeda A, Nakashiro K, Hara S, Sasaki T, Miwa Y, *et al*. Inactivation of AR activates HGF/c-Met system in human prostatic carcinoma cells. Biochem Biophys Res Commun 2006; 347: 1158–65.
68 Tate A, Isotani S, Bradley MJ, Sikes RA, Davis R, *et al*. Met-independent hepatocyte growth factor-mediated regulation of cell adhesion in human prostate cancer cells. BMC Cancer 2006; 6: 197.
69 Kwabi-Addo B, Ozen M, Ittmann M. The role of fibroblast growth factors and their receptors in prostate cancer. Endocr Relat Cancer 2004; 11: 709–24.
70 Festuccia C, Angelucci A, Gravina GL, Biordi L, Millimaggi D, *et al*. Epidermal growth factor modulates prostate cancer cell invasiveness regulating urokinase-type plasminogen activator activity. EGF-receptor inhibition may prevent tumor cell dissemination. Thromb Haemost 2005; 93: 964–75.
71 Oosterhoff JK, Grootegoed JA, Blok LJ. Expression profiling of androgen-dependent and -independent LNCaP cells: EGF versus androgen signalling. Endocr Relat Cancer 2005; 12: 135–48.
72 Bonaccorsi L, Carloni V, Muratori M, Formigli L, Zecchi S, *et al*. EGF receptor (EGFR) signaling promoting invasion is disrupted in androgen sensitive prostate cancer cells by an interaction between EGFR and androgen receptor (AR). Int J Cancer 2004; 112: 78–86.
73 Wang J, Wang J, Sun Y, Song W, Nor JE, *et al*. Diverse signaling pathways through the SDF-1/CXCR4 chemokine axis in prostate cancer cell lines leads to altered patterns of cytokine secretion and angiogenesis. Cell Signal 2005; 17: 1578–92.
74 Sun YX, Schneider A, Jung Y, Wang J, Dai J, *et al*. Skeletal localization and neutralization of the SDF-1 (CXCL12)/CXCR4 axis blocks prostate cancer metastasis and growth in osseous sites *in vivo*. J Bone Miner Res 2005; 20: 318–29.
75 Ao M, Franco OE, Park D, Raman D, Williams K, *et al*. Cross-talk between paracrine-acting cytokine and chemokine pathways promotes malignancy in benign human prostatic epithelium. Cancer Res 2007; 67: 4244–53.
76 Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood 2006; 107: 1761–7.
77 Culig Z, Steiner H, Bartsch G, Hobisch A. Interleukin-6 regulation of prostate cancer cell growth. J Cell Biochem 2005; 95: 497–505.
78 Lee SO, Chun JY, Nadiminty N, Lou W, Gao AC. Interleukin-6 undergoes transition from growth inhibitor associated with neuroendocrine differentiation to stimulator accompanied by androgen receptor activation during LNCaP prostate cancer cell progression. Prostate 2007; 67: 764–73.
79 Wallner L, Dai J, Escara-Wilke J, Zhang J, Yao Z, *et al*. Inhibition of interleukin-6 with CNTO328, an anti-interleukin-6 monoclonal antibody, inhibits conversion of androgen-dependent prostate cancer to an androgen-independent phenotype in orchiectomized mice. Cancer Res 2006; 66: 3087–95.