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Androgen receptor signalling in prostate: Effects of stromal factors on normal and cancer stem cells

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ABSTRACT

The prostate gland is the most common site for cancer in males within the developed world. Androgens play a vital role in prostate development, maintenance of tissue function and pathogenesis of prostate disease. The androgen receptor signalling pathway facilitates that role in both the epithelial compartment and in the underlying stroma. Stroma is a key mediator of androgenic effects upon the epithelium and can regulate both the fate of the epithelial stem cell and potentially the initiation and progression of prostate cancer. Different groups of growth factors are expressed by stroma, which control proliferation, and differentiation of prostate epithelium demonstrating a critical role for stroma in epithelial growth and homeostasis. Paracrine stromal proteins may offer the possibility to control tumour stem cell growth and could permit prostate specific targeting of both therapies and of androgen responsive proteins. The effect of 5α-dihydrotestosterone, the more potent metabolite of testosterone, on expression of androgen-regulated genes in stroma from benign prostatic hyperplasia is a key mediator of epithelial cell fate. Global gene expression arrays have recently identified new candidate genes in androgen responsive stroma, some of which have androgen receptor binding sites in their promoter regions. Some of these genes have direct androgen receptor binding ability.

1. The prostate gland and pathology

The prostate gland is the most common site of neoplastic disorders in men, which mainly develop after the age of 50. By this time, half of men will have a degree of benign prostate hyperplasia (BPH). At 80 years of age, 90% of men will have BPH symptoms (Rizzo et al., 2005) and 23% of men will develop prostate cancer (CaP). CaP is now the most common cancer in men. Within the UK, 32,000 men were diagnosed with CaP in 2004, representing 12% of cancer incidence (Cancer Research UK). Increasingly, amongst the elderly male population prostate disease is having a significant effect on morbidity, mortality and health care resources.

The pathogenesis of BPH and CaP is still poorly understood (Bonkhoff and Remberger, 1996). In BPH there is an androgen-mediated proliferation of the anterior prostate lobe possibly in response to decreasing levels of androgen, and changes in the balance between epithelial cell division and differentiation (Rizzo et al., 2005). This leads to symptoms of bladder outflow obstruction and abdominal pain, which are the most common referral reasons. A recent review even proposes mechanical urethral angulation to be a causative factor (Cho et al., 2007). BPH is not a precursor for CaP, other precursor lesions are recognised and are discussed below. Current therapeutic options for CaP remain inadequate (Chung et al., 2003). While tumours initially respond well to androgen reduction, the duration of response is short, typically 12–33 months. At this time a population of cells resistant to androgen deprivation therapy emerges (Rizzo et al., 2005).

Prostatic intraepithelial neoplasia (PIN) represents a precursor lesion for invasive CaP development. This lesion is characterised by expression of bcl2, c-Met and nuclear phosphoprotein pp32 (Bui and Reiter, 1999). A low grade PIN lesion is often a well differentiated early invasive tumour with a high percentage of basal cells. The high grade lesions form poorly differentiated tumours with a secretory luminal cell population (Bonkhoff and Remberger, 1996).

Abbreviations: AR, androgen recepetor; ARE, androgen response element(s); BPH, benign prostatic hyperplasia; CaP, prostate cancer; CD, cluster differentiation marker; CK, cytokeratin; DHT, 5alpha-dihydrotestosterone; EGF/EGFR/erb1/erbb2, epidermal growth factor and receptors; ERα/ERβ, oestrogen receptor; FAP, fibroblast activation protein; FGF, fibroblast growth factors; GSTP1, glutathione *S*-transferase 1; HGF, hepatocyte growth factor; HIF1α, hypoxia induced factor α; IGF1, insulin like growth factor 1; IL6, interleukin 6; LBD, ligand binding domain; LOH, loss of heterozygocity; PAP, prostatic acid phosphatase; PDF, prostate derived factor; PDGF, platelet derived growth factor; PIN, prostate intraepithelial neoplasia; PSA, prostate specific antigen; PSCA, prostate stem cell antigen; RARβ, retinoic acid receptor β; TGFα/TGFβ, transforming growth factor α/β; TP/A, transiently proliferating/amplifying cells; VEGF, vascular endothelial growth factor.

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2. Prostate architecture

The normal prostate is a walnut sized gland composed of different cell types that can be distinguished by their cytokeratin (CK) and cluster differentiation (CD) expression patterns. Three main cell types are discernible within normal, mature prostatic epithelium-basal, secretory luminal and neuroendocrine. The luminal or glandular cells constitute the exocrine compartment of the prostate, secreting prostate specific antigen (PSA) and prostatic acid phosphatase (PAP) into the glandular lumina. They are terminally differentiated, and represent the major cell type in normal and hyperplastic epithelium. They express high levels of the androgen receptor (AR), and depend on androgens for their survival (Collins and Maitland, 2006). They also express CK-8 and CK-18.

In contrast, basal cells are relatively undifferentiated and lack secretory activity. As their name suggests, basal cells rest on the basement membrane and morphologically range from small flattened to cuboidal cells. They express low/undetectable levels of AR (Bonkhoff and Remberger, 1996) and are independent of androgens for their survival (Collins and Maitland, 2006). Basal cells focally express the oestrogen receptor and can proliferate under oestrogen therapy (Collins and Maitland, 2006). Basal cells express CK-5, CK-14, CD44, p63, a homologue of the tumour-suppressor gene *p53*, and the anti-apoptotic protein bcl-2. Basal cells do not express androgen receptor, although there may be focal expression of nuclear oestrogen, progesterone, and glucocorticoid receptors (Bonkhoff and Remberger, 1996), and hence they are androgen-independent. Within this basal compartment are the epithelial stem cells and transiently proliferating/amplifying (TP/A) cells expressing CK-5, CK-18, CK-17, CK-19 and bcl-2 (Rizzo et al., 2005). A further subpopulation of partially differentiated basal cells expressing CK-5, CK-14, and weak CK-18 is also documented (Rizzo et al., 2005). Expression patterns change as cells differentiate, resulting in an increase in CK-18 expression. The epithelial stem cells can also express CD133, a human analogue of prominin which is localised at membrane protrusions (Richardson et al., 2004). This stem cell population adheres rapidly to collagen as the cells also express high levels of $\alpha_2\beta_1$ integrin. The CD133+ population was shown to have high proliferative output, and high colony-forming efficiency, expressing CK-18, PSA, PAP, and AR negative (Richardson et al., 2004; Collins et al., 2005). A further cell surface antigen, prostate stem cell antigen (PSCA) has also been found to be expressed on basal cells (Bui and Reiter, 1999), although this population is mainly localised to the TP/A basal cell compartment rather than the stem cell compartment, and PSCA mRNA is also expressed in high grade PIN (Bui and Reiter, 1999). The breast cancer resistance protein (Huss et al., 2005) is a further putative stem cell marker. This too is predominantly expressed by TP/A basal cells and in BPH.

Significant populations of neuroendocrine cells, sometimes referred to as endocrine-paracrine cells also reside among the more abundant secretory epithelium in the normal prostate gland. These cells are found in the epithelium of the acini and in ducts of all parts of the gland. The major type of neuroendocrine cells contain serotonin, thyroid-stimulating hormone and chromogranin A. Neuroendocrine cells are terminally differentiated, post-mitotic cell types that are androgen-insensitive (Bui and Reiter, 1999) and can also express neuropeptide Y. Immunoreactivity to neuropeptide Y was demonstrated in up to 75% of CaP, suggesting a role in growth and progression of CaP (Ruscica et al., 2007). These differentiated cell populations can become more prominent in CaP with changes in number, histology and function (Nelson et al., 2007; Schalken and van Leenders, 2003) suggesting a regulatory role and demonstrating the plasticity of the differentiation program even in CaP. Mitogenic and oncogenic activities have also been demonstrated, for example expression of vascular endothelial growth factor (VEGF) leading to angiogenesis.

Some neuroendocrine cells can also express somatostatin and even PSA (Nelson et al., 2007).

2.1. Stromal–epithelial interactions in the prostate

The prostate is a complex tubulo-alveolar gland composed of an epithelial parenchyma embedded within a stromal tissue matrix. The stromal cells are responsible for direction of epithelial cell development, maintenance and differentiation. Stromal cells supply nutrients and growth factors, express adrenergic receptors, steroid receptors including AR, oestrogen receptor (ERα, ERβ) and 5-α-reductase (required for conversion of testosterone to 5α-dihydrotestosterone; DHT). The three major cell types within stroma are: myofibroblasts, fibroblasts and smooth muscle cells within a connective tissue matrix. Myofibroblasts express procollagen 1, and are the main cell type within CaP reactive stroma (Ayala et al., 2003). Desmin, α-actin, calponin, caldesmon, myosin, smoothelin and dystrophin are expressed by smooth muscle cells (Antonioli et al., 2007). Fibroblasts express vimentin and laminin (Micke and Östman, 2004; De Wever and Mareel, 2003). Expression of these markers has been associated with CaP grade and survival (Ayala et al., 2003). Reactive CaP stroma is characterised by an increase in myofibroblasts and fibroblasts, with a significant decrease or complete loss of smooth muscle cells (Ayala et al., 2003), see Section 2.4.

2.2. Paracrine growth factors

Stromal cells produce several growth factors under the influence of androgens, some of these are discussed below. Androgens play a central role in the maintenance of prostate tissue and can regulate growth and differentiation of the different epithelial cell types, either directly or indirectly via the stroma. In the absence of ligand some of these growth factor pathways can still activate AR (Fig. 1).

2.2.1. Transforming growth factor β (TGFβ)

TGFβ exists in three different isoforms (1–3); all are potent inhibitors of epithelial cell growth and migration, but stimulate mesenchymal (stromal) cell proliferation and migration (Micke and Östman, 2004). Other actions include transdifferentiation of fibroblasts to myofibroblasts, and induction of extracellular-matrix

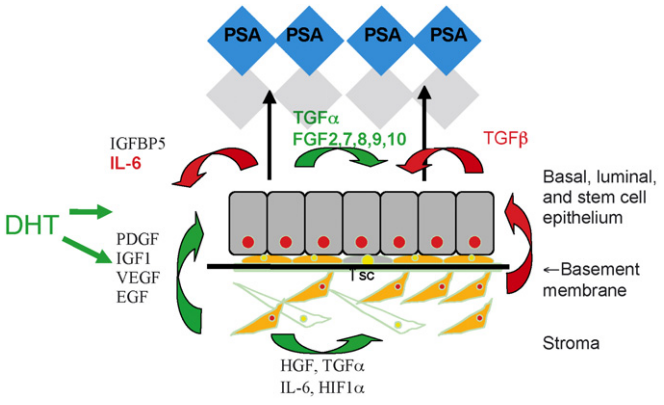

Fig. 1. Stromal–epithelial interactions in the prostate. The basal cells are in direct contact with the basement membrane. The cancer stem cell (↑sc) is also shown. The stromal cells secrete multiple classes of growth factors important for maintenance of the prostate. Growth factors can be inhibitory (shown in red) or stimulatory (shown in green), some of these are androgen (DHT) regulated. Both cells within the stroma and the luminal epithelial cells within the basal cell compartment express AR. Luminal epithelial cells secrete PSA in response to DHT.