Influence of stromal–epithelial interactions on androgen action

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Abstract
Androgen receptor (AR) signaling is vital to the development and function of the prostate and is a key pathway in prostate cancer. AR is differentially expressed in the stroma and epithelium, with both paracrine and autocrine control throughout the prostate. Stromal–epithelial interactions within the prostate are commonly dependent on AR signaling and expression. Alterations in these pathways can promote tumorigenesis. AR is also expressed in normal and malignant mammary tissues. Emerging data indicate a role for AR in certain subtypes of breast cancer that has the potential to be exploited therapeutically. The aim of this review is to highlight the importance of these interactions in normal development and tumorigenesis, with a focus on the prostate and breast.

Introduction
The role of androgens in the maintenance of prostate structure, development, and growth and in the stimulation of prostate cancer cells has been studied since the pioneering work of Huggins in the 1940s (Huggins et al. 1939, Huggins & Clark 1940, Huggins & Sommer 1953). The androgen receptor (AR) is a central mediator of androgen action in all tissues that are androgen-responsive. The role of AR can be paradoxical, with both stimulatory and anti-proliferative actions, depending on the microenvironment and hormone levels. Reciprocal AR responses in the epithelium and stroma are involved in tissue development and homeostasis and aberrant responses can lead to the disruption of these processes, resulting in tumorigenesis. Understanding these processes could lead to strategies designed to halt or prevent the development of malignant disease. AR is also expressed in normal mammary tissue and has a demonstrated hormonal role in breast development. AR may regulate tumorigenic properties and contribute to epithelial–mesenchymal signaling in the breast, though the information is limited. There are many other well-characterized AR target tissues throughout the body, but specific information on the role of AR in epithelial–mesenchymal interactions is minimal or non-existent (Chang et al. 2013). This review focuses on the role of AR in the prostate and, to a lesser extent, in the breast, specifically in the context of interactions between the epithelium and the stroma.

Androgens in development
Prostatic development
The human prostate develops from the urogenital sinus (UGS). 5α-Reductase is present in the fetal UGS and is the main enzyme involved in the conversion of testosterone into the more potent dihydrotestosterone (DHT; Shimazaki et al. 1965). In Fig. 1, this mechanism is shown in relation to the AR. Androgens produced by Leydig cells in males stimulate the UGS to undergo epithelial bud
formation and branching. This process occurs during weeks 8–10 in human gestation and E13.5–E15.5 in murine gestation (Meeks & Schaeffer 2011). The expression of AR in the developing prostate indicates that there is a functional role for androgens prior to maturation. The development of the Ar-knockout (ARKO) mouse has significantly strengthened our understanding of the tissue-specific functions of AR in vivo (Matsumoto et al. 2003, Yeh et al. 2003, De Gendt et al. 2004, Holdcraft & Braun 2004, Notini et al. 2005). The double-stroma and smooth-muscle ARKO (dARKO) mouse exhibits decreased prostatic bud formation and branching patterns (Lai et al. 2012). Evidence of this is also described in the context of the external genitalia (Weiss et al. 2012). Further differentiation of the prostate occurs post-natally. Fetal prostatic epithelium and surrounding stroma exhibit similar levels of AR mRNA and protein expression, but the epithelial AR lacks ligand-binding ability until post-parturition (Takeda & Chang 1991). Complete differentiation of the prostate is delayed until puberty, when a surge in androgen levels stimulates differentiation (Van Wagenen 1947).

Cunha et al. have rigorously shown that AR-positive urogenital mesenchyme (UGM) is the driving force behind the differentiation of the AR-negative fetal urogenital epithelium through tissue recombination experiments. In tissue recombination, fetal rat UGM is recombined with the external genitalia (Weiss et al. 2012). Further differentiation of the prostate occurs post-natally. Fetal prostatic epithelium and surrounding stroma exhibit similar levels of AR mRNA and protein expression, but the epithelial AR lacks ligand-binding ability until post-parturition (Takeda & Chang 1991). Complete differentiation of the prostate is delayed until puberty, when a surge in androgen levels stimulates differentiation (Van Wagenen 1947).

Figure 1
Overview of AR-regulated stromal–epithelial signaling pathways in prostate cancer. DHT, dihydrotestosterone; HSP90, heat-shock protein 90; AR, androgen receptor; ARE, androgen response element; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; IGF1, insulin-like growth factor 1; TGFβ, transforming growth factor beta; PTEN, phosphatase and tensin homolog; PI3K, phosphatidylinositol-4,5-biphosphate 3-kinase; AKT, protein kinase B.
epithelial cells and implanted under the murine renal capsule. The graft is allowed to grow for \( \geq 8 \) weeks. The result is a graft of fully differentiated prostatic tissue. The UGM is the driving inductive force behind prostatic differentiation; provided the epithelium is of endodermal origin (i.e. prostate, bladder, and salivary gland), its source is not as critical. Importantly, neither UGM nor epithelium alone can produce a graft with a prostatic phenotype; androgens and stromal–epithelial interactions are required for proliferation and differentiation (Cunha 1972, 1984, Cunha & Chung 1981, Cunha et al. 1983, 1987).

Donjacour & Cunha elaborated on the temporal relationship between differentiation and AR with testicular feminized mice (XTfm) mice, which have a defect in the Ar locus. When prostatic, bladder, or urethral epithelium was recombined with normal UGM, fully differentiated prostatic structures developed, complete with mouse dorsolateral secretory protein (mDLP) staining. The prostate, bladder, and urethra are endodermal tissues, so UGM drives prostatic differentiation, and germline differences are not important (Donjacour & Cunha 1995). When XTfm UGM is recombined with WT bladder/urethral epithelium, a vaginal-like phenotype is observed, indicating that the expression of AR exclusively in the epithelium is not enough to drive prostatic differentiation. Embryonic AR in the UGM is required to form a prostate-like graft. When WT UGM was recombined with XTfm bladder/urethral epithelium, a well-organized prostatic graft developed (Cunha & Lung 1978, Cunha & Chung 1981, Shannon & Cunha 1984, Sugimura et al. 1986). However, when histologically analyzed, mDLP staining was negative. This demonstrates that the complete exocrine differentiation of luminal cells is dependent on post-embryonic AR in the epithelium (Donjacour & Cunha 1993). UGM recombined with epithelium from XTfm mice does not produce a graft when implanted in a male host. A summary of these experiments is given Table 1.

Prostatic development in the prostate epithelia-specific ARKO (pes-ARKO) mouse also showed an important role for the epithelial AR. Niu et al. (2008) determined that pes-ARKO mice exhibit increased apoptosis of luminal cells and increased proliferation of basal cells. Reciprocally, maintenance of the prostatic epithelium is dependent on the stromal AR. Cunha et al. used tissue recombination experiments to show that loss of Ar in the stroma affects the apoptotic index of the epithelium. The authors suggest that the stromal AR, rather than the epithelial AR, is the primary determinant of the hormone-dependent apoptotic index (Kurita et al. 2001). In the context of the dARKO mouse, loss of Ar in the stroma results in a decrease in epithelial cell proliferation and an increase in apoptosis. This effect is thought to occur due to a lack of stromal factors such as collagen matrix, altered gene expression, and reduction of growth factors (Lai et al. 2012, Yu et al. 2012). Altogether, this demonstrates the powerful cues of both the epithelium and the stroma, as well as the role of AR. A summary of ARKO mouse models used to study the role of AR in the prostate is given in Table 2.

Breast development

During embryonic development, both male and female mice initially develop a mammary bud at day 12 of gestation, but the mammary bud of male mice regresses during late gestation. The mammary epithelium signals the surrounding mesenchyme to express AR. The AR-positive mesenchyme encloses the mammary epithelium and induces involution.

**Table 1** Summary of the recombinant experiments reviewed

<table>
<thead>
<tr>
<th>Tissue recombinants</th>
<th>Graft phenotype</th>
<th>References</th>
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<tbody>
<tr>
<td></td>
<td>AR-negative basal layer</td>
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<tr>
<td></td>
<td>AR-positive luminal layer</td>
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<tr>
<td></td>
<td>mDLP-positive</td>
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<td></td>
<td>AR-negative basal layer</td>
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<tr>
<td></td>
<td>AR-negative luminal layer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mDLP-negative</td>
<td></td>
</tr>
<tr>
<td>WT UE + Tfm UGM</td>
<td>Vaginal</td>
<td>Cunha &amp; Lung (1978)</td>
</tr>
<tr>
<td>Tfm UE + Tfm UGM</td>
<td>Undifferentiated growth in male host</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vaginal in female host</td>
<td></td>
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UE, urothelium; UGM, urogenital mesenchyme; Tfm, testicular feminized; AR, androgen receptor; mDLP, mouse dorsolateral secretory product B.
of the mammary bud. Androgens directly act on the surrounding stroma to control subsequent epithelial changes (Kratochwil 1969, Kratochwil & Schwartz 1976, Durnberger & Kratochwil 1980, Heuberger et al. 1982, Wasner et al. 1983). The induction of mesenchymal AR expression by the adjacent mammary epithelium appears to be mammary epithelium-specific. When mammary mesenchyme was recombined with epithelium from epidermis or pancreas, no expression of AR was observed in the mammary mesenchyme. Salivary epithelium was found to elicit a mild response (Heuberger et al. 1982).

Female murine and human mammary tissues express AR (Boutin & Cunha 1997), and androgens play a role in counteracting the proliferative effects of estrogens. This is especially important during puberty when the AR level in the mammary fat pad doubles (Peters et al. 2011). In Ar-null female mice, mammary gland development and branching are inhibited (Yeh et al. 2003). Overall, the available data indicate that normal mammary tissue expresses AR, which plays an anti-proliferative role.

### Androgens post-puberty

#### The normal prostate

The completely differentiated prostate is composed of an epithelial bilayer surrounded by stroma. The stroma consists of AR-positive smooth-muscle cells, fibroblasts, myofibroblasts, extracellular matrix (laminin and collagen-rich), immune cells, innervation, and vasculature. The basal lamina surrounds the epithelium and forms a barrier between the epithelium and the stroma. The epithelial compartment is composed of an AR-negative basal cell layer with rare AR-negative neuroendocrine cells interspersed. The epithelial compartment also contains an AR-positive luminal cell layer, which produces prostatic secretory protein. Along with these fundamental prostatic epithelial cells, there are also prostatic stem cells, progenitor cells, and transit-amplifying cells (Barron & Rowley 2012, Frank & Miranti 2013). Though the AR-positive stroma produces strong inductive cues, these are not enough to form a completely differentiated prostate (see the discussion above). Furthermore, without a constant supply of androgens, the prostate gland will begin to regress, especially the AR-positive luminal cells (Hayward et al. 1996).

### The normal breast

AR is found throughout human and murine mammary tissues, specifically in luminal, myoepithelial, and stromal cells within the acini. AR is also found in the adipose tissue (Hickey et al. 2012). The overall AR protein level in female mice is about one-third that in male mice. In normal mammary tissue, AR serves as a tumor suppressor, inhibiting the stimulatory effects of estrogen receptor alpha (ERα; Peters et al. 2009). The levels of AR remain relatively consistent throughout a menstrual cycle, but drop during pregnancy and lactation. This is probably to allow for higher ERα-regulated proliferation, but the mechanism is still unclear (Hickey et al. 2012).

Overall, studies in both mammary and prostatic tissues define an essential role of AR in the development and maintenance of normal glandular organization and integrity.

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### Table 2 Mouse models used to study the role of AR expression in the prostate

<table>
<thead>
<tr>
<th>ARKO model</th>
<th>Prostatic phenotype</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>FSP-ARKO</td>
<td>↑ Apoptosis, ↓ Epithelial proliferation, ↓ Collagen matrix</td>
<td>Yu et al. (2012)</td>
</tr>
<tr>
<td>dARKO</td>
<td>↑ Epithelial apoptosis, ↑ Anterior lobe size, ↓ Branching morphogenesis, ↓ Epithelial proliferation</td>
<td>Lai et al. (2012)</td>
</tr>
<tr>
<td>pes-ARKO</td>
<td>↑ Luminal proliferation, ↓ CK5+/CK8+-intermediate progenitor, ↓ Epithelial proliferation, ↓ Differentiation, Hyperproliferative</td>
<td>Wu et al. (2006), Niu et al. (2008, 2011) and Lee et al. (2012)</td>
</tr>
<tr>
<td>Total ARKO</td>
<td>No prostate formation, Undifferentiation</td>
<td>Matsumoto et al. (2003), Yeh et al. (2003), De Gendt et al. (2004), Holdcraft &amp; Braun (2004) and Notini et al. (2005)</td>
</tr>
</tbody>
</table>

ARKO, androgen receptor knockout; FSP-ARKO, prostatic stromal fibroblast ARKO; dARKO, double-stroma and smooth-muscle ARKO; pes-ARKO, prostate epithelia-specific ARKO.
Cancer

Inductive effects of stroma in prostate cancer

The role of the tumor microenvironment in prostate tumorigenesis has gained increasing attention since the seminal experiments of Cunha & Chung (1981) have demonstrated that prostate cancer-associated fibroblasts (CAFs) stimulate tumorigenesis in the benign prostatic hyperplasia (BPH)-1 cell line. BPH1 is an SV40 T-antigen-transformed human prostatic cell line that originated from BPH cells. Olumi et al. (1999) were the first to show that prostate CAFs promote BPH1 tumorigenesis. When grafted alone, or with normal peripheral zone-associated fibroblasts (NPFs), BPH1 cells are not tumorigenic. However, when BPH1 cells are combined with CAFs and grafted under the renal capsules of nude mice, they form tumors. Signaling by transforming growth factor beta (TGFβ) and stromal cell-derived factor 1 (SDF1) has been proposed as a mechanism by which CAFs can induce adjacent epithelial cells to proliferate (Ao et al. 2007). Subsequent studies carried out by several groups have confirmed these findings and extended them. BPH epithelial cells can also be stimulated to form aggressive, metastatic cancer cells via hormone stimulation alone. Wang et al. showed that when BPH1 cells were recombined with rat UGM, a well-organized, benign graft formed under the renal capsule. In the presence of testosterone or 17β-estradiol (E2), BPH1 cells with UGM formed aggressive cancer cells that maintained this phenotype through several in vivo passages. Epithelial apoptosis occurred when these animals were castrated (Wang et al. 2001). The 5α-reductase inhibitor finasteride decreases the expression of AR exclusively in the epithelium (Bauman et al. 2014). Similar findings associated with CAF induction and hormone-stimulated growth have also been reported (Hayward et al. 2001). In addition, Thalmann et al. (2010) have demonstrated the ability of CAFs to promote castration resistance in an LNCaP model, indicating that the CAFs contribute to lethal disease. We demonstrated that distinct populations of stromal cells reside in the prostate tumor microenvironment relative to stromal cells from normal prostate peripheral zone tissue and, more importantly, relative to stromal cells in BPH tissue (Barclay et al. 2005).

McNeal (1990) has suggested that BPH is a disease of the prostatic stroma. His observations have led to the hypothesis that BPH occurs as a result of the reawakening of the embryonic inductive abilities of the prostatic stroma. Recent work has also reported BPH to be associated with inflammation via activating protein 1 (Lin-Tsai et al. 2014). BPH tissue has significantly greater AR expression in both the epithelium and stroma compared with normal glandular tissue. Interestingly, the expression of ERs (ESR1) dramatically increases in the epithelium and is much lower than normal levels in the stroma (Nicholson et al. 2013). The estrogen antagonist raloxifene has been shown to inhibit BPH epithelial growth and may be a major mediator of epithelial-to-mesenchymal transition (EMT) in BPH tissue (Yang et al. 2010, Shao et al. 2014). The inductive abilities of the BPH stroma are thought to be embryonic signals that induce normal epithelial structures. The overexpression of cyclin D1 in CAFs has been suggested as a possible mechanism for their inductive capabilities. Normal prostate fibroblasts exhibiting cyclin D1 overexpression have been shown to induce a malignant transformation of the BPH1 epithelial cell line in vivo (He et al. 2007). Cathepsin D is required for the contribution of cyclin D1 to NPF malignancy, and overexpression of cathepsin D in the stroma promotes tumor growth (Pruitt et al. 2013). This is only one of the many examples of genetic alterations promoting tumorigenesis in CAFs. Love et al. (2009) identified numerous AR-regulated genes in human BPH xenografts grown in mice. CAFs and prostate cancer epithelium are also able to reciprocally regulate metabolism. Fiaschi et al. (2012) have shown that CAFs are stimulated to favor Warburg metabolism after contact with prostate cancer epithelium and that prostate cancer cells favor aerobic metabolism after contact with CAFs.

The zone of the prostate is an important prognostic factor in prostate cancer. BPH commonly occurs in the transition zone, while prostate cancer is mostly found in the peripheral zone (McNeal et al. 1988, Grignon & Sakr 1994, Brossmer et al. 2003, Pavelic et al. 2003). These zones are markedly different. The transition zone has greater cell density and ER activity, while the peripheral zone has greater AR activity (Feneley et al. 1995). It was shown that, under elevated levels of DHT and E2, AR is more highly expressed in the peripheral zone than in the transition zone. Under these conditions, the peripheral zone also has a greater capacity to induce tumor growth via androgen-regulated growth factors such as TGFβ1 and insulin-like growth factor 1 (IGF1; Jiang et al. 2011). In Fig. 1, a schematic of androgen-regulated signaling pathways in prostate cancer is shown. CAFs from both the peripheral and transitional zones can induce a metastatic phenotype in the normally non-metastatic LNCaP cell line in vivo, but the mechanisms are unclear (Thalmann et al. 2010). Reactive stroma is also a major...
problem in prostate cancer progression. The stroma develops a ‘wound that will not heal’ phenotype (Dvorak 1986), with a constant immune response that leaves the microenvironment unstable. The role of AR in reactive stroma is still undefined (Barron & Rowley 2012).

Effects of stromal androgens

The mRNA levels of AR are very similar in the stroma and the epithelium, but transcription is differentially regulated in each. This allows for more active co-regulator recruitment by the epithelium. In prostate cancer, this co-regulator recruitment is altered within the microenvironment, especially within the androgen-responsive genes. Co-culture between stromal and epithelial cells was used to assess co-regulator recruitment to an exogenous MMTV-driven promoter in the stroma. CAFs were found to have impaired recruitment relative to NFPS. Some of the major co-regulators found in this system were SRC1, NCoR, and SMRT (Cano et al. 2007). AR may also regulate the stroma by inducing the differentiation of myofibroblasts via TGFβ, altering the tumor microenvironment according to androgen action (Gerdes et al. 2004). These findings demonstrate that stromal–epithelial interactions influence AR signaling as well as tumorigenesis.

The previously described BPH1 cell line was recombined with UGM from X<sup>Tim</sup>/Y mice, and it was shown that BPH1 cells did not develop tumors without the stromal AR. However, when human BPH1 cells were recombined with WT rat UGM, grafts formed consistently (Ricke et al. 2012). It is important to note that ERβ (ESR2), expressed exclusively in the epithelium, has also been reported to be involved in anti-proliferative effects in the prostate. These effects are dependent on functional aromatase in the stroma. Furthermore, conditioned media from the stroma have been shown to stimulate growth in human prostatic cell lines, possibly through the AR-mediated ERK pathway (Shigemura et al. 2009). Studies carried out in the dARKO mouse, in the context of hypothymic loss of phosphataze and tensin homolog (Pten), have demonstrated that loss of stromal Ar could inhibit prostatic intraepithelial neoplasia (PIN) (Lai et al. 2012). PTEN function is commonly lost in prostate cancer cells and is associated with increased cell proliferation via Akt and PI3K activities (Fig. 1; Simpson & Parsons 2001). There is evidence that a transition from a paracrine to an autocrine mechanism of growth stimulated by the stroma occurs within the prostatic epithelial cells, which are also stimulated by androgens (Gao et al. 2001).

There is conflicting evidence concerning the AR status of the stroma. Ricke et al. (2012) showed that prostate cancer is dependent on stromal androgens and not on epithelial androgens. Previous studies have demonstrated that low stromal AR levels are indicative of a poor outcome after castration. This may be dependent on the high levels of AR in the epithelia, but further study is required (Henshall et al. 2001, Ricciardelli et al. 2005, Wikstrom et al. 2009). Recent work has shown that the AR in myofibroblasts may be an indicator of a better outcome. Hic5 collaborates with AR in the stroma to inhibit migration and invasion (Leach et al. 2014). In addition, there is evidence that typically androgen-independent growth factors such as fibroblast growth factor 7 (FGF7 or KGF) may be activated under androgen deprivation therapy (ADT) conditions (Ishii et al. 2009). Specific targeting of the stromal AR may yield different results. Yu et al. (2013) have suggested that the expression of AR in CAFs regulates epithelial proliferation via various growth factors such as IGFl, FGFl, FGF10, SDF1, HGF, and TGFβ2. This would indicate that preferential targeting of AR in the stroma might be a therapeutic option. The AR status of the stroma plays an important role in outcome, but primary cells tend to lose AR expression in culture. For this reason, an AR-positive stromal cell line was developed via lentiviral overexpression in the WPMY cell line (Tanner et al. 2011). It is unclear exactly what role the stromal AR plays in cancer progression, but co-targeting both the stroma and the epithelium may be a promising therapeutic strategy (Hsieh et al. 2004, Niu et al. 2008).

Castration-resistant prostate cancer

Castration-resistant prostate cancer (CRPC) is an aggressive, late stage of prostate cancer that develops after ADT. It is not known whether there is an enclave of androgen-insensitive cells within the primary tumor or whether the lack of androgens during ADT drives the tumor to acquire mutations and amplifications in the AR, allowing ligand-independent signaling. Recent studies indicate that there may be a population of androgen-insensitive cells within primary prostate tumors using the xenografts of clinical tumors in mice (Lawrence et al. 2013, Toivanen et al. 2013). The AR itself adapts to the sudden lack of androgens by increasing the number of receptors in the cell so that any available androgens are readily bound or by becoming hypersensitive to ligands that may be scarce (Chen et al. 2004, Taplin 2008). Mutations in the AR gene are much more frequent in metastatic tumors than in primary tumors, indicating that mutations may be adaptive (Taplin et al. 1995, 1999). Deep sequencing of tumor samples from patients with CRPC revealed that cells may acquire novel
mechanisms of AR synthesis and signaling, which will undoubtedly have significant effects on stromal–epithelial interactions (Grasso et al. 2012).

**AR-positive breast cancer**

AR status in breast cancer has only more recently been examined in relevance to treatment, although studies have shown that AR is the most common nuclear steroid receptor in breast tumors. Some data indicate that AR is more abundant in malignant mammary tissue than ERα (Lea et al. 1989, Isola 1993, Schippinger et al. 2006, Hanley et al. 2008, Park et al. 2010). Androgen treatment has yielded conflicting proliferation results in classic breast cancer cell lines, which indicates that the mechanistic role of AR in breast tumors is more complex (Birrell et al. 1995). In ER-positive breast tumors, AR levels dramatically decrease when treated with an aromatase inhibitor, when compared with those observed on treatment with the inhibitor in addition to tamoxifen (Harvell et al. 2008). Also, when excluding basal tumors, ER-negative breast cancer cells nearly always express AR, giving a possible therapeutic treatment target (Farmer et al. 2005). Enzalutamide, an AR antagonist used clinically in the treatment of prostate cancer patients, has been reported to be a possible endocrine therapy option. Treatment response has been linked to the ratio of AR:ER in the tumor (Cochrane et al. 2014). For these reasons, some have adopted a new breast cancer classification based solely on the ER and AR status of the tumor. Basal breast cancer is ER-negative/AR-negative, luminal breast cancer is ER-positive/AR-positive, and apocrine breast cancer is ER-negative/AR-positive. These apocrine tumors share AR-associated transcriptional characteristics of prostate cancer (Farmer et al. 2005).

The expression of AR in triple-negative breast cancer (TNBC) cells has been reported to be associated with a decrease in the expression of E-cadherin (Tang et al. 2012). The expression of AR and E-cadherin may be a strong indicator of chemotherapeutic response (Koo et al. 2009). Further study has shown that AR can directly repress E-cadherin in breast cancer, indicating that it contributes to EMT and metastasis. In the context of TNBC, Graham et al. (2010) demonstrated that zinc-finger binding protein 1 (ZEB1) and AR cross-talk to regulate EMT. The expression of ZEB1 and AR is increased in TNBC patients. Further data are available regarding the expression of AR in metastases as well as PSA levels correlating with tumor grade (Garay & Park 2012, Hickey et al. 2012), but there are very few data regarding the role of stromal–epithelial interactions.

These studies highlight the importance of AR in the regulation of tumorigenesis in both breast and prostate cancers via epithelial–mesenchymal signaling. They also highlight potential therapeutic targets in these tumors. Presumably, a similar role for AR signaling and epithelial–mesenchymal interactions in tumorigenesis of other glandular derived carcinomas, such as salivary gland cancer, will be discovered with appropriate inquiry.

**Signaling pathways**

There are many androgen-regulated signaling networks in prostate cancer or signaling pathways that impinge on AR signaling; some of the major pathways are described below. We describe some specific examples below and show their relationships in a simple schematic form in Fig. 1.

**Fibroblast growth factor**

Members of the FGF family are found in the adult human prostate stroma, specifically FGF2, FGF7, and FGF9, in relatively high quantities (Giri et al. 1999). FGF10 is found in smaller quantities in the adult stroma, but plays a major role in development (Ropiquet et al. 2000). The ventral-mesenchymal pad cells have been suggested as a model to examine FGF10 signaling in vitro. This model retains AR expression and has shown that FGF10 is regulated by TGFβ (Tomlinson et al. 2004). The expression of AR in the epithelium was shown to increase with higher FGF10 activity, and overexpression in mice results in prostate cancer (Memarzadeh et al. 2007). The expression of AR in the epithelium is required for stromal FGF10-induced PIN in vivo (Memarzadeh et al. 2011).

Findings regarding FGF7 have been particularly controversial; there have been conflicting studies showing that it may have either a direct or an indirect role in androgen signaling. For example, it was suggested that AR could mediate the upregulation of FGF7 (Yan et al. 1992, Nemeth et al. 1998, Planz et al. 1998, Giri & Ittmann 2000). This would be important in the context of prostate cancer because embryology studies have shown that FGF7 is a potent stimulator of epithelial growth and may assist androgens in early prostate growth (Hom et al. 1998). FGF7 can bypass AR-dependent growth, indicating a possible role in CRPC (Ishii et al. 2009). In the context of the stromal fibroblast ARKO mouse, FGF7 and FGF10 levels are reduced. This is associated with decreased epithelial proliferation and has been suggested as a possible therapeutic target.
The secretion of FGF7 is substantially higher in the cancer-associated peripheral zone than in the BPH-associated transitional zone (Jiang et al. 2011). Though further investigation is necessary to understand the exact role that FGF7 plays in prostate cancer cells, it is clear that the levels of FGF receptors are elevated and linked to androgen-dependent transcription, potentially through the MAPK pathway (Culig et al. 1994, Rowan et al. 2000, Debes et al. 2003).

Hedgehog

The Hedgehog (Hh) pathway has been reported to be involved in prostatic development, specifically in ductal branching. Mutations in the downstream target, sonic Hh (Shh), result in embryos without prostates. However, this is rescued when the pregnant females are treated with androgens, implicating Hh to be a major mediator of differentiation through stromal–epithelial interactions and the AR (Culig et al. 1994, Rowan et al. 2000, Debes et al. 2003). EMT is involved in prostate cancer progression and Hh plays a major role in this process (Huber et al. 2005). SHH has been suggested as a potential therapeutic target to prevent androgen-insensitive tumor progression, especially bone metastases, in prostate cancer (Shigemura et al. 2011). Additionally, members of the Hh pathway have been found preferentially in undifferentiated basal cells, which indicates that Hh is involved in stem/progenitor cell maintenance (Chen et al. 2007). Hh is active in both normal human prostate and prostate tumor stroma, and its downstream targets are upregulated in human prostatic fibroblasts. Smoothened (Smo), a downstream target of Hh, is particularly necessary for the survival of CAFs (Wilkinson et al. 2013).

Insulin-like growth factor 1

IGF1 is produced in the prostatic stroma, stimulated by androgens. The increase in IGF1 levels has a pro-proliferative effect on the surrounding epithelia (Moschos & Mantzoros 2002, Bogdanos et al. 2003, Garrison & Kyprianou 2004). Reciprocally, androgens enhance the levels of IGF receptor and IGF1 promotes the production of steroid receptors (Culig et al. 1994). The inhibition of IGF1 receptor causes the nuclear AR to relocate to the cytoplasm, with subsequent transcriptional consequences for AR-mediated gene activity (York et al. 2005, Wu et al. 2006). Stromal ARKO mice exhibit reduced IGF1 expression. Lowered IGF1 expression in the stroma has been reported to be associated with a reduction in epithelial proliferation and maintenance (Yu et al. 2013). IGF1 has been implicated in CRPC, but IGF1-mediated growth may also affect androgens by promoting ligand-independent androgen action (Mantzoros et al. 1997). Circulating IGF1 is associated with a higher risk of prostate cancer as well as other types of cancers (Sachdev & Yee 2007). Growth hormone-releasing hormone antagonists in conjunction with ADT have yielded promising therapeutic results by reducing IGF1 levels (Sciarrà et al. 2008).

Transforming growth factor beta

TGFβ is produced by the stroma and is normally involved in tumor-suppressive activity by inducing epithelial apoptosis and controlling growth (Massague et al. 1992). As has been stated previously, TGFβ promotes stromal myodifferentiation via the AR, which may involve the translocation of AR to the nucleus. Myodifferentiation is of particular interest in stromal–epithelial interactions because an increase in the number of myofibroblasts can facilitate angiogenesis (Gerdes et al. 2004). The formation of myofibroblasts is associated with an androgen-mediated Hic5 nuclear translocation. Hic5 then alters the expression of AR target genes to make the microenvironment less favorable for migration and invasion. As has been described previously, the presence of the stromal AR in this context would be favorable (Leach et al. 2014). After ADT, TGFβ-mediated apoptosis is responsible for a dramatic reduction in PSA levels as well as tumorigenicity (Guo & Kyprianou 1999, Wikstrom et al. 1999). For example, the expression of TGFβ in CAFs is elevated and is associated with a greater capacity for colony formation in vitro (San Francisco et al. 2004). Furthermore, the TGFβ accessory receptor, endoglin, has been shown to promote the tumorigenicity of CAFs (Kim et al. 2013). In the context of the pes-ARKO mouse, TGFβ1 has been suggested as a mediator of EMT (Niu et al. 2008). TGFβ indirectly enhances the transcription of AR via Smad3 and Smad4, but the exact mechanism is not well understood (Massague & Chen 2000).

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF (VEGFA)) is a critical mediator of angiogenesis. Its expression is indirectly upregulated in prostate cancer stroma by the AR, thus facilitating angiogenesis. Normally, VEGF is only found in fetal stroma when angiogenesis is necessary for...
normal development. VEGF is not found in BPH stroma, and its expression is upregulated, at both the mRNA and protein levels, by DHT treatment of cancer stroma in vitro (Levine et al. 1998). Therefore, it is not surprising that ADT downregulates the expression of stromal VEGF (Cheng et al. 2004). This improvement is not long lasting, however, and the expression of VEGF is upregulated again in CRPC cells (Pan et al. 2013). IGF and TGFβ upregulate the expression of VEGF transcriptionally (Eisermann et al. 2013, Li et al. 2013). There are currently multiple VEGF inhibitors in clinical use, as well as dual hepatocyte growth factor (MET) (an important signaling pathway in bone) and VEGF inhibitors currently being explored (Lee & Smith 2013).

**WNT**

Wnt signaling through β-catenin is necessary for lineage-specific growth in the normal prostate (Memarzadeh et al. 2007). In the mouse embryo, Wnt/β-catenin signaling is required for the normal development of the genital tubercle (Miyagawa et al. 2009). The Wnt pathway has been implicated in the self-renewal of prostate cancer cells, and its action combined with AR may be a driving force behind rapid renewal and differentiation (Bisson & Prowse 2009). Furthermore, Wnt signaling inhibits E-cadherin and is involved in EMT (Liebner et al. 2004). Wnt signaling also seems to have an important role in androgen deprivation, wherein it is upregulated during ADT, but downregulated if androgens are added back. This, along with the self-renewal properties described, indicates that Wnt signaling may be vital for the treatment of CRPC patients (Prins & Putz 2008). WNT11 protein is expressed in prostate cancer cells, and there is evidence that this protein is a mediator of invasion and migration as well as more aggressive neuroendocrine differentiation (Uysal-Onganer et al. 2010).

AR mediates Wnt signaling in breast cancer cells. Ni et al. (2011) showed that AR transcriptionally activates the Wnt and Her2 pathways in a ligand-dependent manner. This indicates a possible role for AR therapeutic targeting in Her2-positive breast cancer.

Clearly, numerous ligands and their receptors are important for mediating the effects of AR signaling on epithelial–mesenchymal interactions in the normal development and tumorigenesis of both breast and prostate cancer cells. These are prime targets for intervention to disrupt feed-forward signaling pathways that drive tumorigenesis.

**Conclusions**

Based on the information reported herein, it is clear that stromal–epithelial interactions have major effects on androgen action in both prostate cancer and breast cancer. AR signaling is both temporally and spatially regulated throughout development. Normal prostate and breast organization continue to be dependent on the expression of AR throughout adult life. There is evidence that the expression of AR is linked to prostate cancer progression, but the role of AR in breast cancer progression is not known. The inductive action of prostatic stroma is undeniable, and it is a major void in our understanding of prostate cancer. The genomic effect of CAFs is still not known. There is a pressing need for therapeutic targets for preventing CRPC or treating CRPC patients, and stromal–epithelial interactions are a likely candidate. Our knowledge of AR expression in breast cancer cells is growing, but the role of stromal–epithelial interactions is unclear. AR-dependent signaling pathways have been well established in prostate cancer patients, but are only marginally understood in breast cancer patients. Discoveries reported herein could lead to new endocrine therapeutic targets in both sets of cancer patients. Future work should also be focused on the role of AR in epithelial–mesenchymal reciprocal signaling in other tissues. These studies may lead to a more comprehensive understanding of the roles of AR signaling in normal development and its misregulation in diseases such as cancer.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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