

Differential Roles of Androgen Receptor in Prostate Development and Cancer Progression

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Abstract The androgen depletion therapy (ADT) has become the major treatment for the cancer patients through the use of chemical castration and/or antiandrogens, yet the therapy eventually fails and cancers progress to more advanced stages. The mutation, amplification, overexpression of AR, and cross-talk between AR, AR co-regulators, and other growth factor pathways have provided potential explanations for the failure of androgen ablation therapies in some cases. However, whether the differential AR roles in different types of prostate cells could contribute to the failure of ADT remains unclear and will be the focus of this review.

AR expresses in both stromal and epithelial compartments of prostate. It has been shown that there are three basic types of prostatic epithelial cells: (i) cytokeratin 8 (CK8)-positive, CK5-negative luminal cells, (ii) CK5/CK8-double positive intermediate cells, and (iii) CK8-negative, CK5-positive basal cells. In addition to prostatic stromal cells, AR expression could be detected in some basal cells, some intermediate cells, and all luminal cells in prostate. By Cre-LoxP strategy, the prostate epithelium-specific AR knockout (pes-ARKO) and inducible-cre ARKO mice were recently established and have allowed the field to address the differential and distinct AR roles in different types of prostatic cells. These ARKO mice were bred with TRAMP prostate cancer model, and results from these models suggest that (i) prostatic epithelial AR plays dual roles as a suppressor of basal cell proliferation and as a survival factor for luminal cells, and (ii) the stromal AR plays a proliferator role to support the epithelial cell survival and proliferation. Using microarray analysis of primary tumor cells isolated from the prostate tumors of pes-ARKO-TRAMP mice, it was found that a series of metastatic genes were altered and responsible for the higher invasiveness and metastatic rates.

These recent ARKO animal studies not only advance our understanding of the differential roles of AR in different type of prostatic cells, but also closely reflect the pathological changes for the patients undergoing the ADT. Together, these findings provide new evidences to support the potential beneficial effects of

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intermittent ADT therapy, and they also urge the development of cell type and stage selective anti-AR therapies for the prostate cancer patients.

1 Introduction

Prostate cancer remains a major factor of cancer morbidity and mortality in man (Jemal et al. 2008). Nearly 200,000 new prostate cancer cases would be diagnosed in the USA and close to 30,000 men would die of the disease yearly. Androgen and androgen receptor (AR) signals are required for prostate development, differentiation, and normal functioning as well as cancer initiation and progression (Huggins and Hodges 1972; Niu et al. 2008a, b). Androgen exerts its biological effects through binding to the AR and inducing AR transcriptional activity. The androgen-induced AR transactivation is modulated by the interaction of the AR with various coregulators in response to various growth factors (Buchanan et al. 2001b; Heinlein and Chang 2002, 2004; Heemers and Tindall 2007). Initial stages of prostate cancer growth could be suppressed by reducing the availability of androgens to cancer cells via surgical or chemical androgen deprivation therapy (ADT). However, ADT ultimately fails, and prostate cancer progresses to a castration-recurrent (androgen-independent) state that is frequently metastatic and almost always fatal. The AR is expressed throughout prostate cancer progression as well as in the majority of patients with castration-recurrent disease (Chodak et al. 1992; Hobisch et al. 1996; Mohler et al. 1996; Sadi et al. 1991; van der Kwast et al. 1991). The reasons for failure of ADT are not well understood and require further investigation. This chapter discusses findings that the AR can function to stimulate tumor growth or suppress tumor metastasis depending on its expression in different prostatic cells. These differential functions of the AR in different types of prostatic cells could lead to emerging new concepts to treat prostate cancer.

2 The Classic Concept for an AR Role in Stimulating the Growth and Differentiation of Epithelial Cells During Prostate Development

The prostate develops from the endodermal urogenital sinus (UGS) (Cunha et al. 2004). The UGS consists of an outer layer of embryonic connective tissue called the urogenital sinus mesenchyme (UGM), and an inner layer called the urogenital sinus epithelium (UGE). UGM expresses the AR, which could mediate the effect of androgens in the initial step of prostatic development. In response to androgen secreted from fetal testis, epithelial buds emerge and grow into the surrounding UGM. In rodents, solid prostate buds start to elongate and undergo branching morphogenesis in the prenatal period, and the process continues until maturation

at the end of puberty (Cunha et al. 2004; Kinbara and Cunha 1996; Sugimura et al. 1986a). Beginning at the neonatal period, the epithelial cords undergo ductal canalization during which the epithelial cells differentiate into the luminal and basal cells (Cunha et al. 2004; Prins and Putz 2008). Prostatic epithelial differentiation is accompanied by differentiation of the mesenchyme into fibroblasts and smooth muscle cells (Hayward et al. 1996a, 1997).

In 1978, Cunha (Cunha and Lung 1978) reported that when the UGM compartment of UGS from wild-type mouse embryo was combined with epithelium from the neonatal bladder of wild-type or testicular feminized mouse (Tfm, a mouse model lacking functional AR) and transplanted into the kidney capsules of recipient mice, the tissues developed into glandular structures histologically resembling prostatic glands. In contrast, when the UGM of embryonic UGS from Tfm mice was combined with epithelium from bladder of wild-type or Tfm mice, the combined tissues developed into structures histologically resembling vaginal epithelium. These results suggested that (a) the AR in the UGM regulates signals for ductal morphogenesis for the initiation of prostatic development through a paracrine mechanism, and (b) Tfm bladder epithelium is capable of participating in an androgenic response from wild-type UGM cells.

In spite of the importance of stromal AR signals, the postnatal development and maturation of the prostate are believed to depend on a reciprocal interaction between the stromal (mesenchymal) and epithelial compartments (Hayward et al. 1998). Indeed, experimental evidence showed that the epithelial AR is required for epithelial cell differentiation, expression of some prostatic secretory proteins (Cunha and Young 1991; Donjacour and Cunha 1993), and differentiation of stromal fibroblasts into the smooth muscle cells (Cunha et al. 1992; Donjacour and Cunha 1988; Hayward et al. 1996b). Other experiments indicated that castration of the adult mice resulted in prostate involution, which was attributed to epithelial cell apoptosis (Isaacs 1984), and androgen supplementation in castrated animals resulted in restoration of prostate size and ductal morphology (Kyprianou and Isaacs 1988; Sugimura et al. 1986b). These experiments indicated that the growth of adult prostate epithelial cells is dependent on AR signaling. Further reported data indicated that apoptosis of prostate tissues recombined from rat UGM + wild-type epithelium or rat UGM + Tfm epithelium was similar in castrated hosts, and androgen supplementation inhibited apoptosis of these recombinants in a similar manner (Kurita et al. 2001). Together, those observations suggested that the regeneration of prostate epithelial cells is dependent on stromal, but not epithelial, AR signaling.

Our recent study of transgenic mice, in which the AR was specifically knocked out from stromal smooth muscle cells or fibroblasts, indicated that reducing AR signaling in the prostatic stromal cells resulted in the development of smaller prostate glands due to a moderate decrease in proliferation and substantial increase in apoptosis in the epithelium (Yeh and Chang et al. unpublished observations). These observations strongly support the view that prostatic stromal AR is an important modulator for the epithelial cell survival, proliferation, and differentiation.

3 Prostatic AR as a Suppressor of Basal Cells and a Survival Factor for Luminal Cells

Although results from the embryonic tissue recombination studies suggested that stromal AR plays a dominant role over the epithelial AR, the studies using embryonic tissues would allow only 4–6 weeks of observation. Thus, tissue recombination studies were not adequate to assess the relative contributions of epithelium and stroma in the adult prostate. To study AR functions in adult prostate, a temporal and tissue-specific strategy to control AR gene expression was necessary, and the Cre-loxP recombination system (Lakso et al. 1992; Nagy 2000; Yeh et al. 2002) is very useful for generating transgenic animal models with conditional AR gene knockout.

Prostatic epithelial cells develop from stem cells through proliferation stepwise into basal and intermediate cells that finally differentiate into epithelial luminal cells (Litvinov et al. 2003). Earlier studies have shown that there are three basic types of prostatic epithelial cells: (a) cytokeratin 8 (CK8)-positive, CK5-negative luminal cells, (b) CK5/CK8-double positive intermediate cells (van Leenders and Schalken 2003), and (c) CK8-negative, CK5-positive basal cells (van Leenders et al. 2001). AR expression is detected in some epithelial basal cells, some intermediate cells, and all luminal cells (Mirosevich et al. 1999). By crossing floxed AR mice (Yeh et al. 2002) with probasin-Cre mice (Wu et al. 2001), we have generated prostate epithelium-specific AR knockout (pes-ARKO) mice to study the role of the epithelial AR in prostatic tissue homeostasis (Wu et al. 2007). Results suggest that prostatic epithelial AR plays dual roles as a suppressor of basal cell proliferation and a survival factor for luminal cells. Within the epithelium, the probasin promoter transgene is expressed increasingly from 2 to 7 weeks and expression is sustained throughout life (Wu et al. 2001). Consistent with increasing probasin-Cre expression, our data showed that the epithelial AR expression gradually decreased in the prostate of pes-ARKO mice beginning at 6 weeks of age and was undetectable at 24 weeks of age (Wu et al. 2007). Histological analysis of the ventral prostate in pes-ARKO mice suggested a progressive decrease in epithelial height, loss of glandular infolding, and increase in luminal epithelial cell apoptosis at 6–32 weeks of age. As the pes-ARKO animals matured, the p63-positive epithelial basal cell population increased during puberty and remained elevated, while the CK8/CK18-positive epithelial luminal cell population declined. The loss of epithelial luminal cells was accompanied by decreased expression of the differentiation markers, probasin, PSP-94, and Nkx3.1. At 24 weeks of age, the glands of pes-ARKO mice exhibited one layer of undifferentiated epithelial cells with increased incorporation of 5-bromodeoxyuridine (BrdU) suggesting an increase in epithelial cell proliferation. Those BrdU-positive cells primarily colocalized with CK5-positive basal cells.

To rescue the phenotype, we then generated the AR(T857A)-pes-ARKO double transgenic mice by crossing pes-ARKO mice with AR(T857A) transgenic mice carrying an androgen-responsive AR mutant (Han et al. 2005). These mice exhibited normal prostatic morphology and glandular histology. These results suggested that the increase in epithelial basal proliferation and the loss of epithelial luminal

cells in pes-ARKO mice are attributable to the loss of epithelial AR signaling. These observations not only support the view that the AR in prostatic epithelial cells is required for epithelial cell differentiation (Donjacour and Cunha 1993), but also suggest that the epithelial AR functions as a suppressor for basal cell proliferation and a survival regulator for differentiated epithelial luminal cells.

4 Differential Roles of AR in Prostate Cancer Progression: The Stromal AR as a Tumor Proliferation Stimulator and Epithelial AR as a Tumor Suppressor Through In Vitro and In Vivo Evidences

The success in generating cell-type selective ARKO mice using the cre-loxP strategy (Yeh et al. 2002) permitted further investigation of the role of epithelial AR in prostate cancer progression by creating a pes-ARKO in a mouse TRAMP prostate cancer model. The TRAMP mouse develops spontaneous prostate tumors starting at 10–12 weeks of age (Gingrich et al. 1997).

There are three basic types of prostate epithelial cells: luminal, intermediate, and basal cells. We have bred TRAMP mice with the floxed AR transgenic and probasin-Cre mice to generate pes-ARKO-TRAMP mice (Niu et al. 2008a). The knockdown of the AR from the epithelial cells in pes-ARKO-TRAMP mice proceeded gradually with age in a time course very similar to that of pes-ARKO mice (Wu et al. 2007). The prostate of pes-ARKO-TRAMP mice exhibited elevated epithelial cell apoptosis compared to the prostates of wild-type AR (WtAR) TRAMP mice at 16 weeks of age. Double immunostaining indicated that the apoptotic cells were CK8-positive epithelial luminal cells. In contrast, there was an increasing number of proliferating cells in the CK5-positive epithelial basal compartment as indicated by the expression of Ki 67 (a proliferation marker) and BrdU incorporation in pes-ARKO-TRAMP mice compared with that in WtAR TRAMP mice (Niu et al. 2008a). Increased apoptosis in the epithelial luminal cells and increased proliferation in the epithelial basal cells resulted in an increase in a population of cells that were CK5/CK8-double positive intermediate cells. Interestingly and unexpectedly, we observed that pes-ARKO-TRAMP mice developed larger and less differentiated primary tumors in the ventral prostate than TRAMP mice at 16 weeks of age (Niu et al. 2008a).

CD44, an early progenitor cell marker, expresses in basal and intermediate prostatic cells (Liu et al. 2004). In addition, it has been found that CD44-positive and AR-negative prostate cancer cells purified from human prostate cancer xenografts are enriched in tumorigenic and metastatic progenitor cells (Patrawala et al. 2006). The primary tumors of pes-ARKO-TRAMP exhibited a higher population of CK5/CK8- and CD44-positive cells than WtAR TRAMP mice. These results suggest that knockout of AR in the prostatic epithelium of TRAMP mice resulted in cell population changes with expansion of intermediate-like tumor cells and

decrease of secretory epithelial luminal cells in the prostate of pes-ARKO-TRAMP mice (Niu et al. 2008a). This is in agreement with a prior report that CK5-positive intermediate cells increased from 29% to 75% when prostate cancer patients received ADT (van Leenders et al. 2001). Moreover, these results confirm the observations in pes-ARKO mice that epithelial AR functions both as a proliferation suppressor of basal intermediate cells and as a survival factor for epithelial luminal cells. Since prostate cancer arises from epithelial cells in TRAMP mice, the dual opposing functions of the epithelial AR might also influence prostate cancer development by favoring the survival of differentiated luminal cells and suppress the expansion of CK5/CK8-positive basal intermediate epithelial cancer cells. Using animal cancer models, it was also found the AR signaling might influence metastasis of prostate cancer. The size of metastatic tumors in pelvic lymph nodes (PLN) in pes-ARKO-TRAMP mice was increased compared with that in their WtAR TRAMP littermates at 24 weeks of age. We observed that primary cultured PLN tumor cells from pes-ARKO-TRAMP mice were more invasive than those from WtAR TRAMP mice in the matrigel invasion assay. Furthermore, restoring the functional AR by transfection of human AR cDNA reduced the invasiveness of PLN tumor cells from pes-ARKO-TRAMP mice (Niu et al. 2008a).

It was further examined whether the AR status affected the invasiveness of several human prostate cancer cell lines, which include PC-3 and CWR22rv1 cells. The AR-negative PC-3 cells were isolated from a bone metastasis and the AR-positive CWR22rv1 cells were isolated from a prostate tumor growing despite ADT (Nagabhushan et al. 1996). Stable AR-positive transfectants of PC-3 cells (PC-3-AR9 cells) also exhibited less invasiveness than control vector-transfected PC-3 (PC-3-v) cells. AR was knocked down in CWR22rv1 cells using a homologous AR gene recombination strategy (Yeh et al. 2003) to obtain CWR22rv1 cells with knockdown (KD) of AR expression (CWR22rv1-AR^{KD} cells). CWR22rv1-AR^{KD} cells express much less AR and are more invasive than parental cells in the Boyden chamber invasion assay. Since PC-3 cells were isolated from a bone metastasis, these cells were injected into the tibia of athymic nude mice according to Corey's method (Corey et al. 2002). We found that PC-3-v tumors grew more aggressively and more invasively than PC-3-AR9 tumors as determined by X-ray analysis and measurement of tumor weights in the mouse tibia. These data from knockin of the functional human AR show that absence of AR signaling in prostate cancer cells promotes invasion both in vitro and in vivo. PC-3-v or PC-3-AR9 cells were also orthotopically inoculated into the anterior prostate of nude mice. Results showed that mice inoculated with PC-3-v cells developed bigger primary tumors that were less differentiated and larger PLN metastatic tumors than mice inoculated with PC-3-AR9 cells (Niu et al. 2008a). Nelius et al. transfected PC-3 cells with an inducible AR-expressing transgene and observed that induction of AR expression resulted in decreased invasion in vitro and decreased tumorigenicity due to decreased microvesicular density and increased tumor cell apoptosis (Nelius et al. 2007). These results showed that loss of prostatic epithelial AR expression leads to the development of more invasive and metastatic prostate cancers and restoration of

a functional AR reverses these characteristics. Together, these results suggest that the epithelial AR is defined as a suppressor of prostate tumor growth and metastasis.

Tumor microenvironment and stromal–epithelial interaction remain important for tumor initiation, progression, and metastasis of prostate cancer (Bhowmick and Moses 2005; Condon 2005). Our recent data also indicated that the AR in prostatic stroma could function as a stimulator of prostate cancer progression and metastasis (Niu et al. 2008b). Furthermore, human prostatic stromal WPMY1 cells (Webber et al. 1999) were stably transfected with AR short hairpin RNA (AR shRNA) that can be used to silence or knock down endogenous AR to generate WPMY1-ARsh cells. The influence of WPMY1-ARsh and vector-transfected WPMY1-v cells on invasion abilities of PC-3-v and PC-3-AR9 cells was studied using coculture: stromal cells were placed in the bottom chamber and cancer cells were placed in the upper chamber of a Boyden chamber. The results indicated that both PC-3-v and PC-3-AR9 cells were less invasive when cocultured with WPMY1-ARsh cells than WPMY1-v cells. These results indicated that AR signaling in stromal cells functions as a stimulator of cancer cell invasion regardless of the expression of the endogenous AR in cancer epithelial cells. Furthermore, PC-3-v or PC-3-AR9 cells and WPMY1-v or WPMY-ARsh cells were orthotopically coinoculated into the anterior prostate of nude mice to investigate the role of the stromal AR in tumor progression and metastasis (Niu et al. 2008b). Both cancer cell lines produced smaller primary tumors and PLN metastases when combined with WPMY1-ARsh compared with WPMY1-v, although tumors derived from PC-3-AR9 cells were smaller than those derived from PC-3-v cells in either combination. These observations suggest that AR signaling in stromal cells can provide growth or paracrine factor(s) for growth of AR-positive and AR-negative prostate cancer cells and influence their metastasis. In contrast, the smaller tumors observed with PC-3-AR9 cells compared with PC3-v cells suggest that AR expression in prostate cancer epithelial cells promotes differentiation, controls growth, and inhibits metastasis of epithelial prostate tumors regardless of the presence of stromal stimulation effects. These observations support the concept that stromal AR functions as a stimulator of prostate cancer epithelial cells by promoting proliferation and inhibiting apoptosis. In addition, the stromal AR appears prometastatic as opposed to the epithelial AR, which appears antimetastatic.

An inducible ARKO model of TRAMP (ind-ARKO-TRAMP) (Niu et al. 2008b) was produced by mating female TRAMP mice carrying heterozygous floxed AR with male *Mx1*-Cre mice (Kuhn et al. 1995). Following injection of pI-pC to induce cre expression and knockout of AR gene at 12 weeks of age, we found that the AR mRNA levels in the prostate of ind-ARKO-TRAMP mice at 16- and 20 weeks of age were knocked down 40–50% when AR mRNA expression was assessed in prostate epithelium and stroma isolated with laser capture microdissection. Knockdown of AR expression resulted in smaller and less differentiated primary prostate tumors in ind-ARKO-TRAMP than in control Wt-AR-TRAMP mice 16–24 weeks of age. The tumors of ind-ARKO-TRAMP mice had lower proliferation rates and higher apoptosis rates than tumors of the control TRAMP mice. The tumors from

ind-ARKO-TRAMP mice had decreased CK8-positive epithelial luminal cells and increased CD44-positive and CK5/CK8-double positive basal intermediate cells compared with control TRAMP mice. Although pes-ARKO-TRAMP and ind-ARKO-TRAMP mice both demonstrated increased epithelial apoptosis and expanded intermediate cells, pes-ARKO-TRAMP mice produced larger and ind-ARKO-TRAMP mice produced smaller metastases compared with control mice. Together, those data support the concept that epithelial luminal ARKO would alter the cell population of prostatic epithelial cells by reducing more differentiated luminal cells and increasing CD44-positive and CK5/CK8-double positive intermediate cells. Epithelial ARKO or AR signal blocking may reduce the survival of luminal epithelial tumor cells, yet would promote the expansion of more malignant intermediate tumor cells.

In addition, metastatic tumors were also evaluated when primary tumors reached 1 cm³ in TRAMP (20 weeks of age), pes-ARKO-TRAMP (18 weeks of age), and ind-ARKO-TRAMP (36 weeks of age) mice and found that the well-differentiated primary tumors of TRAMP mice developed small metastases in the PLN. The poorly differentiated tumors of pes-ARKO-TRAMP mice developed much larger PLN metastases and metastasized to multiple organs, whereas those of ind-ARKO-TRAMP mice were smaller and more often invaded into the seminal vesicle and migrated to the liver. Thus, loss of the epithelial AR could promote prostate cancer growth and metastasis. The concurrent knockdown of the stromal and epithelial AR could override partially the affects of the epithelial AR knockout, thereby retarding growth of the primary prostate tumor and suppressing metastasis. Furthermore, ind-ARKO-TRAMP mice with early induction of ARKO had longer survival time than wild-type TRAMP and pes-ARKO-TRAMP mice (Niu et al. 2008b). However, the apparent dominance of stromal AR over epithelial AR function diminished if induction of ARKO happened after the primary tumor had progressed for a period of time in ind-ARKO-TRAMP mice. Together, these observations support the concept that the epithelial AR can function as a differentiation factor and a tumor metastasis suppressor and also suggest that the stromal AR functions as a stimulator of prostate cancer growth and metastasis.

5 Molecular Mechanisms Promoting Metastases Following Depletion of AR Function

In addition to characterizing the differential AR roles in the prostate cancer metastasis using cell and mouse models, gene microarrays have been applied to understand the mechanistic changes in metastatic tumors of pes-ARKO-TRAMP and of PC-3-v cancer cell xenografts, and found that several prometastasis genes such as cyclooxygenase-2 (*Cox-2*) (Attiga et al. 2000), matrix metalloproteinase-9 (*MMP-9*) (Aalinkeel et al. 2004; Corey et al. 2002; Saleem et al. 2006), interleukin-6 (IL-6)

(Hammacher et al. 2005; Saleem et al. 2006), and tumor necrosis factor- α (TNF- α) (Hammacher et al. 2005; Michalaki et al. 2004) were elevated, while antimetastasis genes such as neutral endopeptidase (NEP) (Nelson and Carducci 2000; Tanimura et al. 2005) and P27(kip1) (Baldassarre et al. 2005; Belletti et al. 2005; Papatreou et al. 1998) were decreased in the tumors of pes-ARKO-TRAMP mice and PC3-v orthotopic grafts compared with the tumors of their AR-expressing counterparts (Niu et al. 2008a). Thus, due to the dual functions of the AR, treatment of prostate cancer patients with ADT should have good responses at an early stage of cancer but the prognosis may worsen when ADT is given at later stages when tumors are less dependent on stromal AR-derived signals for growth, a scenario apparently observed in castrated TRAMP mice (Johnson et al. 2005). These findings may also explain why some patients can benefit from intermittent cycles of ADT and androgen supplementation (Akakura et al. 1993; Feltquate et al. 2006), which may allow the tumor cells to remain sensitive to ADT without pushing the adaptive phenotypic changes.

6 Adaptive Phenotypic Changes via AR Somatic Mutations in Prostate Cancer After ADT

In addition to managing androgen activity on the prostate cancer patients, the AR is subjected to somatic mutations in prostate cancer, and most of the mutations involve single base change substitution of an amino acid residue and are found more frequently in castration-recurrent, metastatic bone tumors than in primary tumors (Bentel and Tilley 1996; Buchanan et al. 2001a, c; Chen et al. 2005; Gottlieb et al. 2004; Linja and Visakorpi 2004; Marcelli et al. 2000; Taplin et al. 1995; Tilley et al. 1996). Many of these AR mutants display gain-of-function with equal sensitivity toward androgen, increased sensitivity toward dehydroepiandrosterone (DHEA), and a broader range of ligand activation by 17 β -estradiol, progesterone, corticosteroids, and/or antiandrogens (Yeh et al. 1997, 1998; Buchanan et al. 2001c; Chen et al. 2005; Fenton et al. 1997; Monge et al. 2006; Shi et al. 2002). Other AR mutants exhibit decreased transactivation, no activity (Shi et al. 2002), or altered interaction with AR coactivators (Bentel and Tilley 1996; Duff and McEwan 2005; Li et al. 2005). Some AR mutations produce nonsense codons that lead to expression of truncated AR proteins with significantly altered transcriptional activity (Lapouge et al. 2007). The nonsense AR mutants were detected at higher frequency in metastatic cells, where several of the nonsense mutations coexist with other AR mutants, particularly the promiscuous T877A mutation, and one of the nonsense mutants, Q640X, appeared capable of androgen-independent stimulation of the activity of the AR(T877A) mutant (Alvarado et al. 2005).

Somatic mutation of AR also occurs spontaneously in TRAMP mouse prostate tumors at a high rate and the rate of mutation increases further after castration (Han et al. 2001), which suggests an adaptive response. In clinical prostate cancer, AR

mutants are detected more frequently in castration-recurrent disease and after treatment with antiandrogens (Linja and Visakorpi 2004), which also suggests an adaptive change. Expression of the murine AR mutant, AR (E231G), in mouse prostate resulted in development of prostate intraepithelial neoplasia (PIN) (Pinkas and Teicher 2006), which progressed to invasive and metastatic disease (Han et al. 2005; Yeh et al. 1997, 1998). In view of this observation and those gain-of-function AR mutants, which are considered as providing growth advantages for prostate cancer cells, AR may be regarded as a proto-oncogene. Although the linkages of phenotypic changes via AR somatic mutations in prostate cancer cells remain unclear, these changes may contribute to prostate cancer progression and influence the response to ADT.

In addition, AR interacts with a group of proteins, AR coregulators, including coactivators and corepressors. To date, there are numerous AR coregulators identified (Heinlein and Chang 2002; Heemers and Tindall 2007). AR requires the proper interaction with its associated proteins to activate the target gene expression. Several AR coregulators are upregulated in advanced prostate cancer (Culig and Bartsch 2006; Fujimoto et al. 2007; Heemers and Tindall 2007; Hu et al. 2004; Kahl et al. 2006; Nishimura et al. 2003; Yang et al. 2007a, b) and increase androgen sensitivity and ligand promiscuity of wild-type AR and some AR mutants (Yeh et al. 1997, 1998; Heinlein and Chang 2002; Rahman et al. 2004). AR-interacting proteins could alter the AR functions both in the prostate cancer cells and in cancer-associated stromal cells. Regulating the AR protein complex in prostatic stromal or epithelial cells could possibly be applied as an alternative therapeutic strategy in combination with ADT to treat prostate cancer. Together, the studies on AR mutation and AR-associated proteins have added more complexity to the understanding of AR functions in prostate cancer initiation and progression.

7 Clinical Implication and New Concept for Prostate Cancer Treatment

There are different stages of prostate cancer development: nonmalignant stage, prostatic intraepithelial neoplasia PIN, invasive prostatic adenocarcinoma, and metastatic tumor (Fig. 1). The classic clinical evidence for AR to function as a proliferation stimulator is the observation by Huggins and Hodges (1972) that treatment of prostate cancer patients with castration or injection of estrogen, to reduce levels of circulating testosterone, resulted in reduction of tumor sizes. The success of ADT for treating prostate cancer patients at earlier stages appears to support the classic view that AR functions as a proliferator that stimulates the growth of prostate cancer. However, ADT treatment eventually fails in most patients, and the cancers recur with high rates of metastasis and almost certain mortality. Current evidence of different AR roles in prostate stromal and epithelial cells using multiple *in vitro* and *in vivo* strategies indicates that AR signaling does have differential roles on prostate cancer cell proliferation, survival, and metastasis

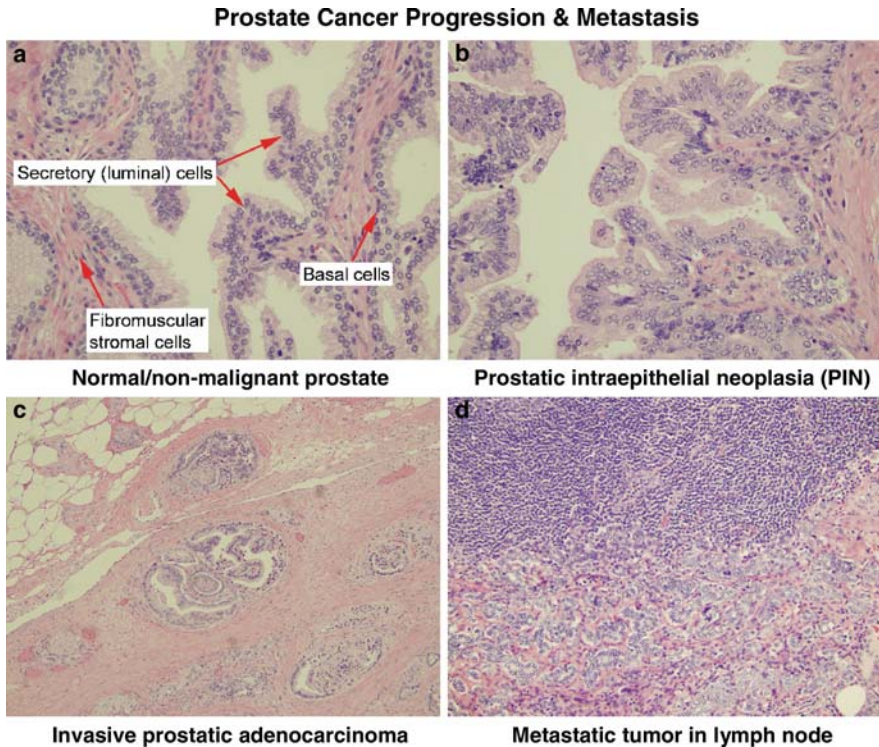


Fig. 1 Histopathology of the human prostate cancer progression. (a) normal/nonmalignant prostate, (b) prostatic intraepithelial neoplasia (PIN), (c) invasive adenocarcinoma, and (d) metastatic tumor in lymph node (See Color Insert)

(Wu et al. 2007; Niu et al. 2008a, b). These findings also provide explanations for the fact that ADT will have some positive effects on earlier prostate cancer but will eventually fail via the dual roles of AR signaling in prostate cancer progression. Treatment regimens targeting solely the AR signaling, such as intermittent ADT (Crook et al. 1999; Egawa et al. 2000; Higano et al. 1996; Hurtado-Coll et al. 2002), or secondary hormonal therapy (Sharifi et al. 2008), may have additional benefits, but may eventually fail. Therefore, new treatment regimens should be developed to use in combination with ADT for the management of prostate cancer. Strategies that selectively target the tumor stromal cells (Bouzin and Feron 2007; Hofmeister et al. 2008), and not epithelial cells, should benefit prostate cancer patients. Furthermore, patients with castration-recurrent prostate cancer usually die of metastatic disease, but their survival can be extended if tumor metastasis is delayed or prevented through additional treatment regimens. Treatments targeting metastasis-related genes downstream of the epithelial AR signaling should improve results from adjunct ADT alone. For example, treatments targeting TGF β 1 and/or its receptor (Pinkas and Teicher 2006), Akt, COX-2, MMP-9 (Miyamoto et al.

2005), or other relevant targets should produce clinical benefits for patients with prostate cancer, when used in conjunction with ADT. The discovery of the differential roles of the stromal and epithelial AR on prostate development, cancer progression, and metastasis may facilitate the development of new therapeutic approaches to battle this deadly disease.

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