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Pathogenesis of Prostate Cancer: Lessons from Basic Research

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ABSTRACT

In the United States, prostate cancer is the second most common cause of cancer-related deaths in men. While the importance of androgens and androgen receptors (ARs) in primary prostate cancer is well established, the role of ARs in prostate cancers that emerge despite androgen ablation therapies remains poorly understood. The aim of this article is to illustrate the fundamental biology of prostate cancer. We focus mainly on the AR because of its critical role in the progression and metastatic spread of prostate cancer. We also summarize the alternate pathways that may potentially contribute to the progression of prostate cancer. Identifying the underlying mechanisms of androgen independence is crucial in the design of appropriate therapies for hormone-refractory neoplasms.

INTRODUCTION

Adenocarcinoma of the prostate is a prevalent malignant disease in men and a leading cause of death in the United States. According to the American Cancer Society estimates, 186,320 men will be diagnosed with prostate cancer and 28,600 will die in 2008, representing approximately 10% of all cancer deaths in the United States. With adequate management, the 5-year relative survival rate of men with localized prostate cancer is almost 100%; however, the rate drops to 32% if a distant metastasis is found at the time of diagnosis.[1]

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ANDROGEN RECEPTOR STRUCTURE AND FUNCTION

Prostate differentiation and function, as well as prostate cancer growth and progression, are critically dependent upon androgen receptor (AR) signaling. The human AR is encoded by a single copy gene located on the X-chromosome (Xq11.2-q12). It is a protein of 919 amino acids in length, but this can vary because it contains poly-glutamine, poly-glycine, and poly-proline repeats of variable lengths. The length of the poly-glutamine repeats has been associated with levels of receptor activity. The length ranges from 9 to 36 residues, with a normal range of about 18 to 22 repeats. Extremely long repeats (≥40) are associated with spinal and bulbar muscular atrophy.[2,3] Although there is some evidence that the length of the poly-glutamine repeat correlates with prostate cancer risk, epidemiological studies have not found a strong relationship.[4]

Like other members of the nuclear receptor family of ligand-activated transcription factors, AR has three main domains: an amino-terminal transcriptional activation domain (NTD), a DNA-binding domain (DBD) containing 2 zinc finger motifs that determine the DNA sequences recognized by the receptors, and a carboxyl terminal ligand-binding domain (LBD) that provides the regulatory switch by which androgens control the transcriptional activity of the receptor (Figure 1). The hinge region (H) connects the DBD with the LBD and contains a nuclear localization signal. A part of the hinge region is also involved in high-affinity DNA binding.[4]

In the absence of androgens, AR is sequestered in the cytoplasm bound to heat shock proteins (HSP-70 and -90), which function to stabilize the protein and protect it from degradation (Figure 2). AR activity is regulated by its 2 major ligands, testosterone and dihydrotestosterone (DHT). Testosterone is produced by testicular Leydig cells and is converted to the more potent metabolite DHT by 5α-reductase in the prostate. DHT has almost 10 times higher binding affinity for AR than testosterone and is the primary androgen bound by AR. DHT binding to the AR promotes the recruitment of protein kinases, resulting in phosphorylation of several serine residues. Phosphorylation of the AR appears to serve many functions, including protection from proteolytic degra-

Figure 1. Schematic representation of the human androgen receptor. The relative locations of the amino-terminal domain (NTD), the DNA-binding domain (DBD), the Hinge region (H), the ligand binding domain (LBD), and activation function domains (AF1, AF2) are shown. The numbering is based on an assumed length of 919 amino acids.[4]

dation, stabilization, and transcriptional activation.[5] The transactivation of AR involves several coregulatory proteins that are able to differentially respond to a changing microenvironment to regulate specific gene targets involved in cell growth and survival.[6] In the normal prostate epithelium, there is a balance between the rate of cell proliferation and the rate of apoptosis; however, in prostate cancer this balance is lost, leading to tumor growth.[7]

EMERGENCE OF HORMONE-REFRACTORY PROSTATE CANCER

Because prostate cancer is highly dependent on androgens for growth, androgen-deprivation therapy has been the mainstay of treatment for decades for advanced metastatic disease. Androgen deprivation is achieved either by surgical castration or chemical castration by suppressing androgen production with luteinizing hormone-releasing hormone agonists (eg, goserelin, leuprolide, and buserelin). Because low levels of androgens are also produced outside the testes (primarily by the adrenals), adjuvant or second-line therapies are used that include the use of an AR antagonist (eg, steroidal cyproterone acetate and megesterol acetate; and nonsteroidal flutamide, nilutamide, and bicalutamide) to diminish AR activity to achieve maximal androgen blockade. Despite the initial, often dramatic response, these therapies only

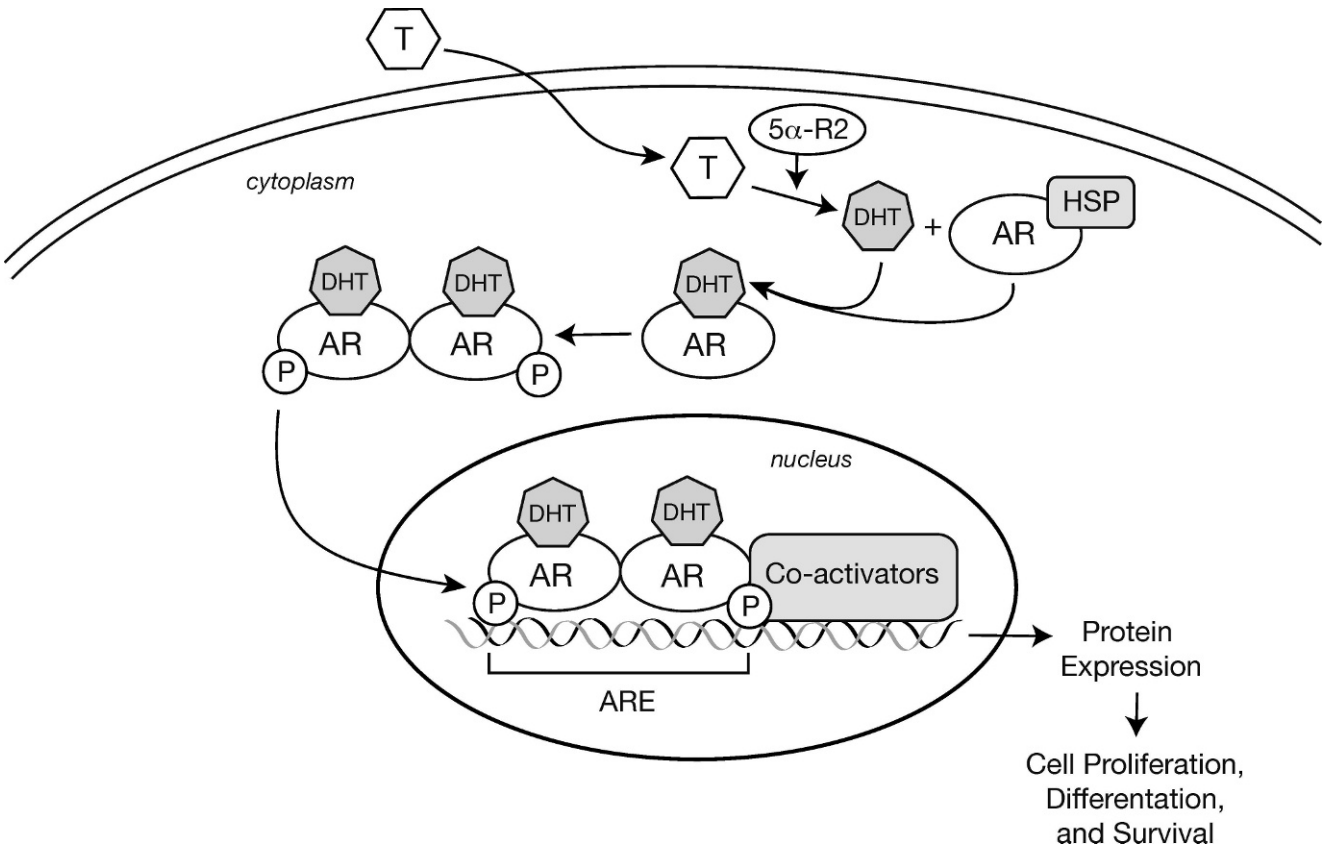

Figure 2. Mechanism of ligand-dependent gene transactivation by the androgen receptor. Testosterone (T) enters the prostate epithelial cell and is converted to dihydrotestosterone (DHT) by 5α-reductase. Binding of DHT to AR leads to dissociation of the AR-heat shock protein (HSP) complex, dimerization, and translocation to the nucleus. AR binds to specific DNA sequences termed androgen response elements (ARE) and recruits a series of co-activators to enhance transcription.

delay tumor progression by 18 to 24 months, followed by the development of a lethal drug-resistant stage called hormone-refractory prostate cancer (HRPC). Thus, understanding the mechanism of growth and proliferation in androgen-independent tumor cells is the major focus.

MECHANISMS OF HORMONE RESISTANCE IN PROSTATE CANCER

The proposed mechanisms to explain the emergence of HRPC despite sustained androgen ablation and/or the use of AR antagonists have been classified into 3 general categories: DNA-based alterations in the AR gene, such as amplification or point mutations, AR-growth factors crosstalk, and activation of alternative pathways of survival and proliferation.[8]

Androgen Receptor Amplification

The AR gene amplification occurs in approximately 30% of HRPC patients who were initially treated with androgen ablation monotherapy but is seen at very low rates in those with primary prostate cancer.[9] AR gene amplification leads to an increase in AR protein expression, which sensitizes prostate cancer cells to respond to low levels of ligands.[10] Patients with AR gene amplification appeared to have greater response to second-line combined androgen blockade than patients without amplification.[11]

Androgen Receptor Mutations

Mutations in the AR gene have been detected in 10% to 20% of locally advanced prostate tumor specimens[12] and with higher frequency in hormone-refractory distant metastatic tumors as compared with untreated patients who have lower-grade primary tumors or patients treated with castration alone.[13] The majority of mutations reported are in the ligand-binding domain.[14] In addition, most of the mutations that have been identified are associated with increased functional activity of the AR and produce a receptor that is more sensitive to low androgen levels or that can be activated by other steroid types such as adrenal androgens, estrogens, and progestins, as well as anti-androgens used for the management of the disease.[13]

The progression of prostate cancer was first reported in several patients treated with the anti-androgen flutamide.[15] Subsequent studies reported similar results by a variety of anti-androgens as well as other hormonal agents (reviewed in reference 16). A significant number of patients showed measurable disease regression and/or symptomatic improvement after discontinuation of the anti-androgenic compounds. The most frequent mutation found in the flutamide-treated patients is a point mutation of codon 877 (threonine → alanine) in the ligand-binding domain of the AR gene.[17] However, clinical responses to another commonly used anti-androgen bicalutamide were still observed in patients bearing flutamide-induced mutations.[18,19] Bicalutamide, on the other hand, generated point mutation in the AR at codon 741 (tryptophan → cysteine or leucine).[20] While this mutation resulted in acquisition of agonistic properties to bicalutamide, flutamide was still able to act as an antagonist, suggesting that each anti-androgen may have its own unique withdrawal mechanism. Observations like these offered clues that second-line maximum androgen blockade treatments can be effective for prostate cancer that has relapsed after first-line maximum androgen blockade therapy. A number of studies describe the usefulness of such therapies.[21] In a recent study, about 60% of the patients on alternative anti-androgen showed significantly better survival than nonresponders.[21]

Androgen Receptor-Growth Factors Crosstalk

The second category of hormone resistance mechanisms is based on the observation that most patients who do not have AR mutations or amplification retain active androgen receptor signaling despite the elimination of the requirement for high levels of androgens. There is now ample evidence that numerous growth factors and their receptors are overexpressed in tumor epithelial and stromal cells, which can contribute to ''androgen-independent'' AR activation and progression of prostatic adenocarcinoma (Figure 3). Among them, the peptide growth factors such as epidermal growth factor (EGF), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), and fibroblast growth factor (FGF) are known to stimulate the expression of androgen-responsive genes in the absence or presence of low androgen levels.[22] As an example, EGF induces the activation of AR-mediated gene transcription in AR-transfected DU145 human prostate carcinoma cells.[23] The stimulatory effect was inhibited in the presence of the selective AR antagonist bicalutamide. Enhanced expression of EGF receptor (EGFR) and its ligands (EGF, TGF-α, HB-EGF, and amphiregulin) has been correlated with high grades of prostate cancer malignancies (reviewed in reference 24). Targeting EGFR with an EGFR-selective tyrosine kinase inhibitor gefitinib suppressed growth and invasion of androgen-dependent and -independent prostate cancer cell lines.[25,26]

Several cytokines are elevated in the sera of patients with prostate cancer and appear to be associated with the development of more malignant forms of the disease. For instance, clinically elevated plasma levels of interleukin 6 (IL-6) and its soluble

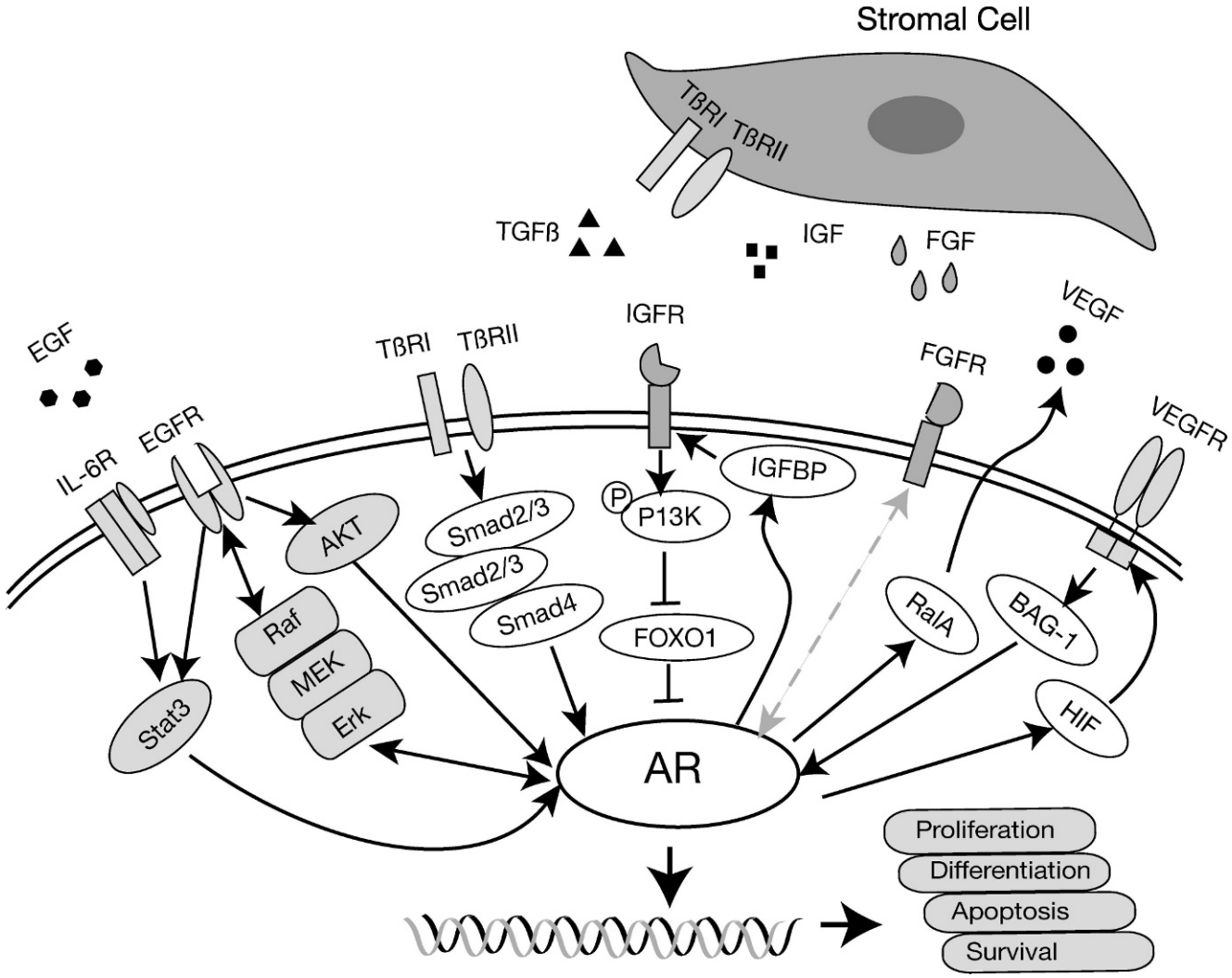

Figure 3. Crosstalk of growth factors with androgen receptor in prostate cancer cells. Major signaling pathways initiated by growth factor-AR crosstalk in the absence of or diminished levels of androgens, implicated in prostate cancer progression. Taken from Zhu and Kyprianou.[22]

receptor have been associated with prostate cancer progression and poor prognosis.[27,28] IL-6 activates AR-mediated transcription in a ligand-independent manner.[27] The stimulatory effect of IL-6 is blocked by the AR antagonist bicalutamide or by the inhibitors of Janus kinase/signal transducer and activator of the transcription-3 (STAT3) signaling pathway. Activated STAT3 levels are significantly higher in the hormone-refractory prostate cell lines than in hormone-sensitive cell lines.[29] Increased levels of STAT3 enhance STAT3-AR complex formation in response to EGF and IL-6 stimulation to induce transcriptional activation of the AR, a key factor of prostate cancer progression.[30]

Although prostatic epithelium maintains normal and malignant tissues, prostatic stroma is recognized as a major factor influencing cancer initiation and progression. Stromal cells secrete paracrine peptide growth factors such as IGF, FGF, and EGF which diffuse from the stromal compartment to the epithelium and control epithelial growth and differentiation (reviewed in reference 22). For instance, IGF-1 is principally synthesized in the prostate stroma and IGF-R1 in the epithelium, suggesting that epithelial cells are the main targets for IGF-1 effects in the human prostate.[31] The protein levels of IGF and IGF-binding proteins are significantly increased in prostate cancer compared with benign tissue.[32] Further, IGF-1–mediated AR signaling in prostate cancer cell lines is inhibited by the AR antagonist bicalutamide, suggesting direct interaction between IGF-1 and AR.[23]

ALTERNATIVE PATHWAYS

The third category of the HRPC mechanisms is based on the clear support for signaling pathways that are completely independent of the growth- and

survival-promoting functions of the AR. The PI3K/PTEN/Akt/mTOR pathway, a major pathway known for its role in mediating cell survival and neoplastic transformation, is often constitutively activated in advanced stages of prostate cancer.[33] This is mostly attributed to the tumor suppressor gene encoding phosphatase and tensin homolog deleted on chromosome 10 (PTEN), which is a negative regulator of PI3K/Akt/mTOR signaling and is lost or mutated in 50% to 80% patients with prostate adenocarcinoma.[34] PTEN negativity is associated with a high (≥7) Gleason score and with advanced pathological stage disease.[35]

In addition to the PI3K/PTEN/Akt/mTOR pathway, numerous reports have documented mutations and aberrant expression of various genes that regulate other signaling pathways. For instance, Src family kinases, Src and Lyn, are highly expressed in androgen-independent prostate cancer cell lines, as well as in most clinical specimens.[36,37] Src signaling is involved in androgen-induced proliferation of prostate cancer cells and may also participate in the transition to androgen-independent growth (reviewed in reference 38). Treatment of human prostate cancer cells with dasatinib, a Src and Lyn activity inhibitor, or with a Lyn-specific inhibitor, results in the inhibition of growth and proliferation of human prostate cancer cells.[36,39] Increased activity of stem cell embryonic developmental signaling pathways has long been implicated in tumorigenesis and in regulating critical aspects of the malignant phenotype such as survival, angiogenesis, invasion, and migration. Stem cell signaling pathways like hedgehog and Wnt have been shown to be active in human prostate cancer cells and contribute to their survival and proliferation.[40,41]

Several lines of evidence implicate aberrant cell cycle regulation in prostate cancer. Numerous cell cycle proteins are often mutated at early to late stages of prostate cancer progression, which result in defective cell cycle checkpoint control and loss of tumor suppressor activity (reviewed in reference 33). Mutations in p53 are one of the most common genetic events in malignant cells and are linked to tumor progression. Although mutations of p53 are rare in primary prostate cancer, they are found in 20% to 25% of advanced cancers.[42] The retinoblastoma tumor suppressor protein, another critical mediator of cell cycle progression, is lost or functionally inactivated in 30% to 60% of prostatic adenocarcinomas.[43]

THERAPEUTIC CONSIDERATIONS

It has long been recognized that androgens play an important role in prostate carcinogenesis. While androgen ablation therapy is highly successful for hormone-sensitive prostate cancer, the rapid emergence of hormone-resistance phenotype in most cases remains a key dilemma in treating this malignancy. As reviewed in this article, the transition of prostate cancer cells from androgen-sensitivity to androgen-independence is rather complex and involves multi-step routes, such as the AR pathway or through bypassing the AR pathway. Although recent efforts have provided several new insights into the complex molecular events involved in prostate carcinogenesis, the clinical challenges to selectively target survival signaling pathways to solve the problem of androgen escape or to inhibit disease progression remain unmet. Given the complexity and multitude of signaling networks in prostate carcinogenesis, new therapeutic strategies need to be more individualized and employ the knowledge and the tools provided by the basic research to complement the therapies that are in use today.

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