Androgen Receptor Signaling in Androgen-Refractory Prostate Cancer

Michael E. Grossmann, Haojie Huang, Donald J. Tindall

Prostate cancer is the second most prevalent cancer in males in the United States. Standard therapy relies on removing, or blocking the actions of, androgens. In most cases, this therapy results in a regression of the cancer because the prostate and most primary prostate tumors depend on androgens for growth and the avoidance of apoptosis. However, a portion of the cancers eventually relapse, at which point they are termed “androgen refractory” and can no longer be cured by conventional therapy of any type. The precise molecular events that lead from androgen-sensitive prostate cancer to androgen-refractory prostate cancer are, therefore, of great interest. This review seeks to identify specific molecular events that may be linked directly to the progression to androgen-refractory cancer. Some of the mechanisms appear to involve the androgen receptor (AR) directly and include mutations in, or amplification of, the AR gene in a manner that allows the AR to respond to low doses of androgens, other steroids, or antiandrogens. In a less direct manner, coactivators may increase the sensitivity of the AR to androgens and even other nonandrogenic substances through a number of mechanisms. Additional indirect mechanisms that do not result from mutation of the AR may involve activation of the AR by peptide growth factors or cytokines or may involve bypassing the AR entirely via other cellular pathways. Identification of the role of these mechanisms in the progression to androgen-refractory prostate cancer is critical for developing therapies capable of curing this disease. [J Natl Cancer Inst 2001;93:1687–97]

The second most common cancer diagnosed in U.S. males, after nonmelanoma skin cancer, is prostate cancer. Estimates are that, in 2000, 180,400 cases of prostate cancer were diagnosed in the United States and 31,900 men died of the disease (1–3). Withdrawal of androgens through physical or chemical castration often leads to regression of the disease. This regression is, however, often transient, and there is no known cure for prostate cancer after it has become metastatic and androgen refractory. It is still unclear why many prostate tumors eventually become androgen refractory. This review will describe the molecular mechanisms that may be involved in the progression to androgen-refractory prostate cancer.

ANDROGENS AND THE ANDROGEN RECEPTOR IN NORMAL PROSTATE

Androgens are produced primarily in the form of testosterone by Leydig cells in the testes and are generally found circulating throughout the body (4). In addition, adrenal androgens, such as androstenedione, dehydroepiandrosterone (DHEA), and its sulfate, are secreted by the adrenal cortex; although not as potent as testosterone, adrenal androgens do contribute to androgenic effects in the body. Production of androgens in the Leydig cells is regulated through the hypothalamic–pituitary–gonadal axis. The hypothalamus secretes pulses of gonadotropin-releasing hormone (GnRH) every 90–120 minutes. GnRH binds to gonadotropes in the anterior pituitary and stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone. When LH reaches the Leydig cells, it stimulates production of androgens, which, in turn, feed back on the pituitary to inhibit the secretion of GnRH and LH.

The androgen receptor (AR) is a phosphoprotein that mediates the actions of testosterone and dihydrotestosterone (DHT) by acting as a transcription factor (4). The AR is found in many tissues of both sexes but is most abundant in male sex tissues. The best characterized functions of the AR are to promote the growth and differentiation of the male urogenital structures. It is also essential for the initiation and maintenance of spermatogenesis. The AR is a member of the steroid receptor superfamily (5) and is composed of three major domains: an N-terminal transcriptional activation domain, a central DNA-binding domain, and a C-terminal steroid-binding domain. Once testosterone has entered the cell, it is usually converted to DHT by 5α-reductase. The AR is capable of binding to both testosterone and DHT, although DHT has a higher affinity for the AR (approximately twofold to 10-fold) and is consequently the primary androgen bound by the AR. The AR is also capable of being phosphorylated, and reversible phosphorylation appears to play a role in both ligand-dependent and ligand-independent AR activation (4). Before binding its ligand, the AR is thought to be in an inactive state, in which it is bound to at least three heat-shock proteins (hsp90, hsp70, and hsp56) (6). Once the AR has bound DHT, some of these proteins dissociate, and there is a conformational change in the AR. The AR then interacts with coactivators, such as the AR-associated 160-kd protein, ARA70, ARA55, ARA54, and cyclic adenosine monophosphate (cAMP) response element-binding protein-binding protein (CBP). It then binds as a homodimer to a specific DNA site, the androgen response element, in the promoter of androgen-responsive genes, to activate transcription of these genes.

Affiliations of authors: M. E. Grossmann, H. Huang (Department of Urology), D. J. Tindall (Departments of Urology and Biochemistry/Molecular Biology), Mayo Clinic, Rochester, MN.

Correspondence to: Donald J. Tindall, Ph.D., Departments of Urology and Biochemistry/Molecular Biology, Mayo Clinic, Guggenheim 17, 200 1st St., S.W., Rochester, MN (e-mail: Tindall@mayo.edu).

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Prostate Cancer Progression and the AR

More than 99% of prostate cancers develop from glandular epithelial cells in the prostate and are, therefore, described as prostatic adenocarcinomas. A number of molecular events occur in the progression to initial tumor formation in most cancers (7). These events include abnormal methylation, proto-oncogene activation, inactivation of DNA repair mechanisms, inactivation of tumor suppressors, and an increase in the synthesis and activity of growth factors and growth factor receptors (8). These events lead to an infinite growth potential for the tumor cells. It is possible that prostatic intraepithelial neoplasia is an early stage of prostate cancer, although definitive proof of this hypothesis is still lacking. Benign prostatic hyperplasia (BPH), however, is clearly not related to prostate cancer (9). As prostate cancer progresses, it eventually escapes the prostatic sheath and metastasizes to lymph nodes and bone. During the time that the tumor cells are escaping the sheath and metastasizing, they must develop the abilities to evade apoptosis, invade tissues, and produce new blood vessels.

A unique requirement for prostate cancer is the initial reliance on androgens for growth and to avoid apoptosis (10). Because of this requirement, standard therapies block the action of androgens or remove the testicular androgens from the patient (endocrine therapy). These therapies include orchietomy to physically lower testosterone levels and injections of LH-releasing hormone analogues to pharmacologically lower testosterone levels (androgen ablation); treatment with antiandrogens, such as flutamide or bicalutamide, to block testosterone binding to the AR (antiandrogen therapy); and maximal androgen blockade (MAB), in which antiandrogen treatment and androgen ablation therapy are combined. However, although many tumors initially regress after such therapies, most of the tumors eventually begin to regrow at various rates in an androgen-refractory manner. This change to androgen-refractory growth may be due to an evolution of the cancer, whereby the minority of cells that are androgen refractory before antiandrogen or androgen ablation therapy have a selective advantage relative to the androgen-sensitive cells. Of interest, studies on patient specimens (11–16) show that the AR is expressed in nearly all cancers of the prostate, both before and after androgen ablation therapy. In fact, prostate-specific antigen (PSA), which is encoded by an androgen-responsive gene, has been detected in the majority of hormone-refractory cancers, indicating that the AR-signaling pathway is still functional in these cancers.

To investigate the progression to androgen-refractory prostate cancer, a number of androgen-refractory model systems have been established (17–26) (Table 1). These cell lines, xenograft models, and transgenic animals are being utilized currently to elucidate the details of the progression to androgen-refractory prostate cancer. There is much that these models can reveal. For instance, a recent complementary DNA microarray analysis of 5184 genes in both hormone-refractory CWR22R xenografts and the hormone-sensitive parental line CWR22 revealed that only 37 genes increased in expression in the xenografts more than twofold (27). Corroboration that the genes are expressed at higher levels in androgen-refractory prostate cancer through further array analysis by use of other androgen-refractory prostate cancer models should provide a clearer picture of the progression to androgen-refractory prostate cancer.

Mechanisms for Progression to Androgen-Refractory Prostate Cancer

Prostate tumor cells appear to have several possible mechanisms by which they could become androgen refractory. First, mutations in the AR hormone-binding domain or amplification of the AR gene could increase tumor cell sensitivity to the very low levels of androgens that are produced by the adrenal glands. Second, mutations of the AR could allow it to respond to other steroids or even to antiandrogens. Third, alterations in the interactions between the AR and some of its coactivators could allow unmutated or mutated AR to become activated by adrenal androgens, other steroids, or antiandrogens. Fourth, alterations in the expression or function of genes in regulatory pathways involving peptide growth factors or cytokines could cause inappropriate activation of the AR. Fifth, the AR could be bypassed entirely, possibly as a result of constitutive activation of regulatory molecules downstream of the AR. For example, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) inactivation, p53 mutations, Bcl-2 pathway alterations, neuroendocrine (NE) factors, and alternative growth factor regulation and utilization could all bypass the need for activation of the AR. A careful examination of these five mechanisms reveals that the AR, or at least the AR-signaling pathway, is a critical component of all of them. In addition, these mechanisms to androgen-refractory prostate cancer are not necessarily mutually exclusive; indeed, it is likely that no single mechanism will be utilized in every case of androgen-refractory prostate cancer. Therefore, the remainder of this review will elucidate these mechanisms to androgen-refractory prostate cancer, the possible interactions among these mechanisms, and the potential roles of these mechanisms in the development of androgen-refractory prostate cancer.

Mutation or Amplification of the AR to Increase Its Sensitivity to Androgens

It has been suggested that mutations in the AR may allow it to bind and be activated by ligands that are normally present in

<table>
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<tr>
<th>Model system</th>
<th>Species of origin</th>
<th>Tumor type</th>
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<tr>
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<td>Xenograft, cell line</td>
<td>Thalmann et al., 1994 (17)</td>
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<td>Rat</td>
<td>In vivo tumor</td>
<td>Isaacs et al., 1978 (23)</td>
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<td>Mouse</td>
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<td>Probasin SV40* large T</td>
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<td>In vivo tumor</td>
<td>Kasper et al., 1998 (25); Green et al., 1998 (26)</td>
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*SV40 = simian virus 40.
the body (e.g., adrenal androgens) but that do not normally cause substantial activation of the AR (28). These gain-of-function mutations would allow the prostatic epithelial cells to grow in an androgen-refractory manner. The gain-of-function mutations found in prostate cancer can be contrasted to the loss-of-function mutations that occur in androgen-insensitivity syndrome and that prevent specific developmental and growth events from occurring. The adrenal androgen DHEA is bound by two AR mutants, T877A and H874Y (29). T877A was initially described in the LNCaP cell line, and H874Y is found in the androgen-refractory prostate cancer xenograft CWR22. Both mutations lead to a transcriptional response to DHEA that is severalfold higher than that of wild-type AR.

A number of mutant ARs that allow cells to respond to adrenal androgens have also been identified directly in tumors of patients who have failed to respond to antiandrogen therapy (30). These mutations appear to occur in only a minority of patients but may account for some cases of androgen-refractory prostate cancer. One such mutation, V715M, results in activation of the AR by the adrenal androgens DHEA and androstenedione (31). Therefore, although the clinical implications of the in vitro data remain undetermined at this time, it is possible that, in a minority of cases, the utilization of adrenal androgens may provide the prostate cancer cells that have these types of mutations with a selective growth advantage during antiandrogen therapy.

Another means by which prostate cancer cells seem to be able to gain a growth advantage that does not involve mutation of the AR gene itself is through amplification of the AR gene. Several studies (32,33) have now used fluorescence in situ hybridization to show that, whereas the AR is rarely amplified in primary prostate cancer, it is amplified in 22%–30% of androgen-refractory prostate cancers. The amplification can take place during treatment with antiandrogens such as bicalutamide (34). In one example, a tumor was found to have an amplified AR after the patient had undergone treatment with the 5α-reductase inhibitor finasteride to relieve the problems associated with the enlargement of the prostate that occur with BPH (35). In another study (36), fluorescence in situ hybridization analysis of 371 tumor specimens revealed that the AR was amplified in 22% of metastatic prostate cancers and in 23% of local recurrent androgen-refractory prostate cancers but in fewer than 2% of the primary prostate cancers. Moreover, other genes examined (cyclin-D, ERBB2, N-MYC, and MYC) showed a much lower prevalence of amplification (0%–8%), indicating that the AR amplification is not a generalized occurrence. These data illustrate that, following the progression from an androgen-sensitive to an androgen-refractory state, the percentage of tumors with AR amplified at the genomic level increases.

The biologic role of the amplified AR gene is currently under investigation. Initial evidence indicates that the increased levels of AR DNA are associated with an increase in AR messenger RNA (33). Increased levels of AR protein associated with AR gene amplification have been implicated in the ability of cells to more effectively use the low levels of androgens that are still available during androgen deprivation therapy (37). A surprising finding was that AR amplification is often seen in recurrent tumors of patients who initially respond well to androgen deprivation therapy and in patients whose responses last more than 12 months (33). It was also reported that one patient with AR amplification after tumor recurrence had undergone MAB, which resulted in good initial treatment response that was, however, short-lived. Similar short-term responses after MAB have been documented in 20%–35% of unselected patients who received MAB therapy after failure of castration (38,39). It is possible that these patients had similar AR amplification, although this has not been determined.

It was unexpected that recurrent tumors with AR amplification would follow those primary tumors that had initially responded better to endocrine therapy. One explanation is that the recurrent tumors are dependent on androgens and, therefore, an increase in AR copy number allows them to once again proliferate in response to low levels of androgens. Therefore, if these recurrent cells are still highly hormone dependent, that would explain their response to MAB. The data also suggest that more than one survival mechanism is used by the prostate cancer cells in this setting and, therefore, probably in other settings, since not all patients responded similarly. AR amplification is not seen in all androgen-refractory prostate cancers, but it may play a role in a minority of cases.

While initial studies linked AR amplification alone to androgen-refractory prostate cancer, additional research has shown that AR amplification is also positively associated with immunostaining for mutant p53. One study (40) showed that 75% of androgen-refractory prostate cancers that had p53 mutations also exhibited AR amplification, whereas only 27% of androgen-refractory prostate cancers that had wild-type p53 did so. It is possible that the inactivation of p53 may lead to amplification of the AR through genetic instability. However, an alternative explanation may be that AR amplification leads to mutation and inactivation of p53. Further studies into the basic role of the AR in normal prostate, androgen-sensitive prostate cancer, and androgen-refractory prostate cancer will be necessary to fully elucidate the biologic role of amplified AR in androgen-refractory prostate cancer and the clinical implications. However, it is clear that AR is more highly amplified in androgen-refractory prostate cancer as compared with androgen-sensitive prostate cancer, and it seems likely that this difference, in turn, results in androgen-refractory prostate cancer cells being able to better utilize low levels of androgens.

**Mutation of the AR to Permit AR Activation by Other Steroids and Antiandrogens**

Mutations in the ligand-binding domain of the AR could not only increase sensitivity to normal ligands, such as adrenal androgens, which are present at low levels, but also cause the AR to be responsive to other molecules, such as antiandrogens, which are not normal ligands. For example, in the early 1990s, an interesting phenomenon was reported in a few patients who had been undergoing therapy with the antiandrogen flutamide. In those patients, PSA levels declined after treatment was discontinued. Discontinuation of treatment with other antiandrogens, including bicalutamide, chloralidione acetate, megestrol acetate, diethylstilbestrol, and estramustine phosphate, has also been found to lead to PSA declines (41–44). This effect is now defined as endocrine withdrawal syndrome or antiandrogen withdrawal syndrome (41–44). The biologic consequences of the fall in PSA after antiandrogen withdrawal are unknown. However, although PSA levels are generally indicative of tumor burden, it is clear from bone scans that tumor progression can occur, even when PSA levels are declining (45).

Point mutations in the AR may account for the antiandrogen withdrawal syndrome (31,42); some of these mutations are in-
cluded in a database of AR mutations on the Internet (46). Some mutations in the AR can result in the AR being activatable by both adrenal androgens and by antiandrogens and other steroids. For example, a number of cases of androgen-refractory prostate cancer have been reported to contain a mutation of AR at codon 877 (T877A) (47,48), the same mutation that is found in the human prostate cancer cell line LNCaP (49). This mutant AR not only is activatable by adrenal androgens but also binds the antiandrogen hydroxyflutamide in a manner that leads to fourfold to sevenfold greater agonistic activity than hydroxyflutamide binding to wild-type AR. Of interest, the same mutation can also render cells more responsive to both estradiol and progesterone than cells with wild-type AR. Some patients with this mutation have shown remarkable declines in PSA levels after androgen withdrawal (49). Therefore, these data support the hypothesis that point mutations in the AR, particularly in the hormone-binding domain, may be responsible for some cases of androgen-refractory prostate cancer by allowing the AR to continue to be activated by antiandrogens.

Coactivators and Androgen-Refractory Prostate Cancer

A number of coactivators interact directly with the AR and enhance AR-dependent gene transcription. A comprehensive list of proteins that interact with the AR is available on the Internet (46). Some of the better characterized coactivator proteins are members of the 160-kd nuclear receptor coactivator (p160) family, including glucocorticoid receptor-interacting protein 1, the steroid receptor coactivator-1, and the receptor-associated coactivator-3 (50). These coactivators interact with the N-terminal activation domain of the AR (51). The p160 coactivators can also bind to the ligand-binding domain of the AR, thereby enhancing ligand-dependent, AR-mediated transcription of target genes (52). It has recently been shown that the BRCA1 protein can interact physically with the p160 coactivators and the AR, although whether these interactions occur simultaneously is not known (53). In addition, BRCA1 can enhance AR-dependent transactivation of an AR-responsive promoter by an unknown mechanism (53). The possible roles of the p160 coactivators and BRCA1 in androgen-refractory prostate cancer have yet to be determined, but one possibility is that an increase in the protein levels of the p160 coactivators or BRCA1 may allow adrenal androgens to function more efficiently as AR ligands.

Members of a second group of coactivators alter the ligand specificity of AR activation. These coactivators include the AR-associated proteins ARA54, ARA55, and ARA70 (also known as RFG and ELE1) (54–56). ARA55 and ARA70 both activation of the AR by 17β-estradiol (E2), with ARA70 being the most effective coactivator for conferring androgenic activity for E2 (6,55,57). In addition, ARA70 can function as a coactivator of AR in the presence of androst-5-ene-3β,17β-diol (Adiol), a precursor to testosterone. The activation is Adiol dependent and is not related to its metabolism to testosterone (57).

The use of a two-hybrid assay in mammalian cells has demonstrated that the antiandrogens hydroxyflutamide, bicalutamide, cyproterone acetate, and RU58841 can promote the interaction between AR and ARA70 in a dose-dependent manner, substantially enhancing AR transcriptional activity (58). ARA55 also enhances AR transcriptional activity in the presence of hydroxyflutamide (55). Thus, one way for progression to androgen-refractory prostate cancer to occur may be through an increase in coactivator RNA or protein levels or mutations in coactivators that provide a means for antiandrogens to activate the AR.

Another way for androgen-refractory prostate cancer formation to occur may be by the recruitment of coactivators that alter the steroid specificity of mutant AR activation. For example, ARA54, in conjunction with the T877A mutant AR found in the LNCaP cell line, is able to enhance transcriptional activity of the AR in the presence of both E2 and hydroxyflutamide (54). ARA54 does not have this effect with wild-type AR or with a different AR mutant (E708K), which is associated with partial androgen insensitivity syndrome but has not been documented to be related to androgen-refractory prostate cancer.

A final way for androgen-refractory prostate cancer formation to occur may be by AR coactivators, taking advantage of the adrenal androgens that remain available following hormone ablation therapy. Transfection of LNCaP cells with the proto-oncogene Her2/Neu induces PSA through the mitogen-activated protein kinase pathway at low androgen levels (59). Furthermore, AR-sensitive promoters are activated when ARA70 and the AR are expressed in conjunction with the overexpression of the Her2/Neu proto-oncogene (59). The mechanism by which the interaction increases AR activity is not clear, but it may provide a novel pathway for AR transactivation with low levels of androgens.

Thus, given current knowledge, there are several possible ways by which AR coactivators may be involved in the progression of androgen-sensitive prostate cancer to androgen-refractory prostate cancer. First, overexpression of certain coactivators may cause activation of AR by nonandrogenic steroids. Second, overexpression of other coactivators may cause activation of the AR by antiandrogens. Third, AR mutations may result in conformational changes of the AR that, in combination with certain coactivators, can result in activation of the AR. These three mechanisms, alone or in combination, may provide a means for prostate cancer cells to overcome their initial dependence on androgens. However, there is as yet no direct evidence of altered expression levels of the coactivators or of altered interactions between the coactivators and AR in androgen-refractory prostate cancer.

Activation of the AR by Peptide Growth Factors and Cytokines

The AR exists as a phosphoprotein, and the functional status of the AR is associated with its phosphorylation status (60). Induction of AR transcriptional activity by the factors that mediate AR phosphorylation may provide one mechanism for progression to androgen-refractory prostate cancer. These factors include peptide growth factors and cytokines. For example, serum levels of insulin-like growth factor-I (IGF-I) have been reported to be associated with prostate cancer risk, although whether elevated levels of IGF-I are associated with androgen-refractory prostate cancer has not been determined (61). Blockade of IGF-I signaling, by reduced expression of its cognate receptor, inhibits growth of prostate cancer cells in vitro and in vivo (62,63). Transgenic mice expressing IGF-I in prostate epithelium exhibit activation of IGF-I receptor and spontaneous tumorigenesis in the prostate (64). In the absence of androgen, IGF-I is also able to promote AR transcriptional activity in vitro (65). In addition, the antiandrogen bicalutamide can inhibit the activation of the AR by nonsteroidal factors such as IGF-I (65),
which suggests that this activation requires the AR. Taken together, these results suggest that IGF-I may play a role in the progression from androgen-sensitive to androgen-refractory prostate cancer in a manner that is independent of androgen. That the wild-type AR can be activated by IGF-I indicates that the activation of the AR by IGF-I may be mediated through the signaling cascade initiated from ligand binding of the IGF-I receptor rather than through mutations in the AR itself.

Other peptide growth factors, such as keratinocyte growth factor and epidermal growth factor, can also stimulate the transcription-promoting activity of the AR (65). That is, each of these growth factors can activate transcriptional activity from androgen-responsive reporter gene constructs, either in the absence of androgen or synergistically, in conjunction with androgens. Other nonsteroidal molecules, including cytokines, such as interleukin 6 (IL-6), and activating factors of protein kinase A (PKA), such as 8-Br-cAMP or forskolin, can also activate the AR pathway (Fig. 1). Forskolin activates the AR through a PKA-signaling pathway by way of adenylate cyclase to increase intracellular levels of cAMP (66–69). IL-6 not only activates AR-responsive reporter gene constructs in DU-145 prostate cancer cells but also increases PSA secretion by LNCaP cells (70). Clinical data show that serum IL-6 levels are elevated in men with hormone-refractory prostate cancer and that these high serum IL-6 levels are accompanied by high levels of serum PSA (71–73). However, the clinical importance of elevated serum levels of IL-6 in patients with androgen-refractory prostate cancer is unclear.

Growth factors serve as ligands for receptor tyrosine kinases and activate downstream intracellular kinase cascades. Receptor tyrosine kinases may also be involved in the progression to androgen-refractory prostate cancer through an interaction with the AR. The receptor tyrosine kinase Her2/Neu (also known as erbB2) is expressed at low levels in normal secretory epithelial cells, including prostate epithelial cells (74,75). Although the clinical importance of Her2/Neu in prostate cancer is not yet known, several studies (76–79) have demonstrated Her2/Neu protein overexpression and/or gene amplification in a subset of prostate cancer patients. Overexpression of Her2/Neu not only stimulates proliferation of LNCaP cells but also enhances AR-transactivating activity both in the absence of androgens and in the presence of androgens, in which case activation is synergistic (59,80). Moreover, Her2/Neu induces PSA expression, and this induction can be partially inhibited by blocking the MAP kinase pathway (59). Thus, MAP kinase may mediate the activation of the AR by Her2/Neu. In addition, protein kinase inhibitors can affect androgen-induced transcriptional activity of the AR (68,70).

Because Her2/Neu is a critical component of IL-6 signaling through the MAP kinase pathway in prostate cancer cells (81), it is possible that signaling pathways involving the transcriptional activity of the AR by both IL-6 and Her2/Neu may be merged through the MAP kinase cascade (Fig. 1). This hypothesis is supported by the following findings: 1) Activation of the AR by either IL-6 or Her2/Neu can be attenuated, but not completely blocked, by the antiandrogens bicalutamide or hydroxyflutamide (59,70,80); 2) stimulation of PSA secretion by IL-6 or activation of the AR by Her2/Neu can be inhibited by the MAP kinase inhibitor PD98059 (59,70); and 3) one MAP kinase, MAP kinase kinase kinase 1, can induce the promoter activity of an AR-regulated gene in both an AR-dependent and an androgen-independent manner (82).

Fig. 1. Pathways of activation of the androgen receptor (AR) in prostate cancer cells. In addition to being activated by androgens, as in normal prostate epithelial cells, the AR can also be activated in prostate cancer cells by nonsteroid factors, such as cyclic adenosine monophosphate (cAMP), produced by adenylate cyclase after stimulation by forskolin; growth factors (GF) binding to growth factor receptors (GFR); and cytokines, such as interleukin 6 (IL-6). Androgen-independent activation of the AR can also be mediated by receptor tyrosine kinases (RTKs), such as Her2/Neu, mitogen-activated protein kinase kinase (e.g., MEK), mitogen-activated protein kinase kinase kinase (e.g., MEKK1), protein kinase A (PKA), and Janus activation kinase (JAK), which act through signal transducer and transactivator of transcription-3 (STAT3). The activation of the AR by Her2/Neu and IL-6 can be inhibited by the MEK inhibitor PD98059. On activation, the AR translocates from the cytoplasm to the nucleus, dimerizes, and binds to androgen response elements (ARE) in the promoters of target genes to activate them.
The AR, in addition to being activated by peptide growth factors, cytokines, and receptor tyrosine kinases, can be regulated by many other nonsteroidal factors, including β-catenin, caveolin-1, histone acetytransferase binding to the origin replication complex, cyclin E, tumor susceptibility gene TSG 101, butyrate, and Smad3 (83–92). Thus, it appears that AR-mediated transcriptional activities can be affected in many ways at multiple levels. However, because the results that show nonsteroidal activation of the AR are all from in vitro studies, it is important for future studies to determine the clinical importance of these findings with reference to androgen-refractory prostate cancer patients.

**Progression to Androgen-Refractory Prostate Cancer Via Bypassing the AR Pathway**

Even though the AR plays a major role in the progression to androgen-refractory prostate cancer, androgen-refractory prostate cancer cells may use other pathways for proliferation via bypassing the AR entirely. One potentially relevant pathway is NE differentiation in prostate tumors. The prevalence of focal NE cells in prostate adenocarcinoma varies from 30% to 100%, depending on the sources of tumor samples and the methods used to detect NE cells. NE cells, which become more prevalent after long-term antiandrogen therapy both in vitro and in vivo (93), are nonmitotic and do not express the AR. The absence of proliferation may make NE cells relatively resistant to radiation therapy and endocrine therapy (94). The prevalence of proliferating carcinoma in the vicinity of NE cells (95,96) suggests that these cells may play a role in the growth of androgen-refractory prostate cancer. NE cells may contribute to the progression to androgen-refractory prostate cancer via their production of neurosecretory products, such as parathyroid hormone-related protein, the neurotransmitter serotonin, the neuropeptide hormone bombesin, calcitonin, chromogranin A, neurotensin, and thyroid-stimulatory hormone (97–100).

Although the mechanism of NE differentiation after androgen ablation is not completely understood, recent studies (101,102) show that IL-6 treatment induces LNCaP and PC-3 cells to undergo NE differentiation. The process of NE differentiation induced by IL-6 in LNCaP cells is accompanied by the activation, through phosphatidylinositol 3 kinase (PI3K), of the non-receptor tyrosine kinase Etk/Bmx; IL-6-induced NE differentiation can be blocked by a dominant-negative mutant form of Etk. IL-6 can also activate signal transducer and activators of transcription (STATs), such as STAT3, through the Janus kinase pathway. STAT3, one of the downstream targets of Etk (103), mediates the process of IL-6-induced NE differentiation in LNCaP and PC-3 cells (104) (Fig. 2). Therefore, IL-6 may induce NE differentiation in prostate cancer cell lines by activating phosphatidylinositol 3-kinase (PI3K) (105), Etk, and STAT3 (Fig. 2).

Although no in vivo data are available to account for the enrichment in NE cells after androgen ablation therapy, circulating levels of IL-6 are elevated in patients with hormone-refractory disease (71). It is possible that the high levels of serum IL-6 activate the PI3K–Etk–STAT3 signaling cascade to cause NE differentiation in prostate tumors.

Androgen-refractory prostate cancer cells may utilize other cellular pathways for their survival. Androgen withdrawal triggers apoptosis in both normal and malignant androgen-dependent prostate epithelial cells. However, androgen-refractory prostate cancer cells do not undergo apoptosis (10). Therefore, activation of antiapoptotic signaling pathways, such as the PI3 kinase and related pathways, should be critical for the survival of androgen-refractory prostate cancer cells. PI3K is activated by a number of survival factors, such as IL-6, and by factors that activate Her-2/Neu (Fig. 3). PI3K phosphorylates phosphatidylinositol to generate D-3 phosphatidylinositol, including phosphatidylinositol-3,4,5-triphosphate and phosphatidylinositol 3,4-bisphosphate, both of which participate in the activation of the

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Fig. 2. Potential pathways to neuroendocrine differentiation (NED) in prostate cancer cells. After treatment of LNCaP prostate cancer cells with interleukin 6 (IL-6), phosphatidylinositol 3-kinase (PI3K), Etk, and signal transducer and transactivator of transcription-3 (STAT3) are activated. Alternatively, IL-6 may act directly through Janus activation kinase (JAK) to activate STAT3. Secretion of IL-6 and promoter activity of the IL-6 gene are positively regulated by the transcription factor NFκB in prostate cancer cell lines. Establishment of an IL-6 autocrine loop after androgen ablation in prostate tumors is likely mediated by loss of repression of NFκB transactivation from the competitive binding of the androgen receptor to coregulators such as CBP/p300. Unknown effectors leading from androgen withdrawal to NED are shown as a question mark, and the lack of effect on NED by endocrine and radiation treatments is shown as a circle with a slash.
downstream target, protein kinase B (PKB, also known as Akt). PKB is one of the key regulatory molecules involved in the protection of cells against apoptosis (106,107). PTEN (also known as MMAC1 and TEP1) has lipid phosphatase activity that metabolizes PIP3 (phosphatidylinositol triphosphate) (108–112). Ultimately, PTEN functions as a tumor suppressor primarily through negative regulation of the PI3K/Akt pathway (113–115).

PTEN is inactivated in several types of cancers, including those from prostate, brain, breast, endometrium, and kidney (116). Loss of PTEN function in prostate cancer can occur through a variety of mechanisms, including deletion, mutation, and, in a xenograft model, methylation (108,117–119). Although the frequency of PTEN mutations in prostate cancer is relatively low overall, inactivation of PTEN is more frequent in advanced stages of the tumor (117,120,121). Thus, loss of PTEN function may favor tumor cells surviving the selective pressure caused by androgen ablation therapy. In addition, one recent study (122) has demonstrated that PTEN functions as an antagonist to block AR signaling. These data suggest that loss of PTEN may also have a direct impact on androgen-independent activation of the AR.

Loss of PTEN and activation of PKB/Akt in prostate cancer cells might provide a favorable cellular environment for Bcl-2 and Bcl-XL proteins to function as inhibitors of apoptosis. PKB/Akt phosphorylates Bad, a proapoptotic member of the Bcl-2 family that, when dephosphorylated, displaces Bax from binding to Bcl-2 and Bcl-XL, resulting in cell death (107). Bad phosphorylation frees Bcl-2 and Bcl-XL, allowing them to act as antiapoptotic proteins. In the normal prostate, Bcl-2 is expressed in the basal epithelial cells but not in the luminal epithelial cells (123,124). Overexpression of Bcl-2 has been implicated in the conversion of androgen-dependent to androgen-refractory lesions (125–127). Bcl-2 is overexpressed in early-stage disease, but most studies have shown higher frequencies of overexpression in advanced prostate cancer (126). These in vivo observations are further supported by in vitro studies that show that forced expression of Bcl-2 in LNCaP cells protects cells from apoptosis caused by androgen withdrawal and enables cells to be less dependent on androgens (127). Bcl-2 may also affect the function of the AR through alteration of the subcellular distribution of the AR in prostate cancer cells (128). Thus, inactivation of Bad or expression of Bcl-2 may confer on prostate cancer cells the ability to avoid apoptosis, which is the primary consequence of androgen ablation therapy, thus enabling androgen-sensitive prostate cancer to progress to androgen-refractory prostate cancer.

CONCLUSION

A number of mechanisms have been identified that may contribute to the progression of prostate cancer from androgen sensitive to androgen refractory. Two mechanisms alter the AR directly, modulating its ability to respond to specific ligands. First, mutations in the AR hormone-binding domain or amplification of the AR gene could result in an increased sensitivity to...
adrenal androgens. Second, mutations in several portions of the AR could allow it to respond to other steroids or antiandrogens. Both mechanisms are probably responsible for some cases of androgen-refractory prostate cancer, but it is apparent from current clinical research that they are responsible for only a minority of such cases.

AR signaling may also be modulated by three indirect mechanisms. In one such mechanism, coactivators could augment the sensitivity of the AR to androgens and even other nonandrojenic compounds. Although these coactivators have yet to be linked directly to androgen-refractory prostate cancer, it seems likely that this could be an important pathway to androgen-refractory prostate cancer because of the large number of coactivators that have already been identified. In a second indirect mechanism, AR signaling may be enhanced through activation of the AR by peptide growth factors and cytokines. In a third indirect mechanism, prostate cancer may become androgen refractory if the AR is bypassed. This mechanism could reflect inactivation of PTEN or the development of alternative pathways for growth. These three indirect mechanisms are likely to be involved in the majority of cases of androgen-refractory prostate cancer because they do not require mutated or amplified AR but could result from mutation or altered expression of a large number of different genes. However, although each of these mechanisms may not involve classical AR signaling, the fact that the AR is expressed in androgen-refractory prostate cancer cells indicates that it remains an important component of such cells. Therefore, the AR must be necessary for and able to modulate some signaling pathways in the absence of its ligand.

In conclusion, many different mechanisms are emerging that may be involved in the progression of androgen-sensitive to androgen-refractory prostate cancer. This finding is not surprising, given the heterogeneous nature of prostate cancer and the large number of interconnected pathways in which the AR is involved. However, the key genes and pathways that are regulated by AR have not yet been defined clearly. It will be important to elucidate how the interconnected pathways downstream and upstream of AR contribute to the growth and maintenance of the normal prostatic cells. Once the mechanisms of progression to androgen-refractory prostate cancer are better understood, it may be possible to design rational strategies for the treatment and possible cure of both androgen-sensitive and androgen-refractory prostate cancer.

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NOTE

Manuscript received January 24, 2001; revised September 13, 2001; accepted September 25, 2001.