Mechanisms Underlying the Development of Androgen-Independent Prostate Cancer
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Background

Prostate cancer continues to be the most common lethal malignancy diagnosed in American men and the second leading cause of male cancer mortality. The American Cancer Society estimates that during 2005, ~232,090 new cases of prostate cancer will be diagnosed in the United States and 30,350 men will die of metastatic disease (1). Approximately 1 man in 5 will be diagnosed with prostate cancer during his lifetime, and 1 man in 33 will die of this disease. As the population ages, these numbers are expected to increase. Initially, almost all metastatic prostate cancers require testosterone for growth, and the role of androgen deprivation as a first-line therapy for metastatic prostate cancer has been recognized for more than 60 years (2, 3). Hormone deprivation is accomplished by surgical (orchietomy) or medical (luteinizing hormone-releasing hormone agonists, antiandrogens) castration. Hormonal therapy leads to remissions lasting 2 to 3 years; however, virtually all patients progress to a clinically androgen-independent state resulting in death in ~16 to 18 months (4–9).

Androgens are primary regulators of normal prostate as well as prostate cancer cell growth and proliferation. During androgen-dependent progression, prostate cancer cells depend on the androgen receptor as the primary mediator of growth and survival (10–12). When testosterone enters the cell, it is converted by the enzyme 5α-reductase to its active metabolite, dihydrotestosterone, a more active hormone with a 5- to 10-fold higher affinity for the androgen receptor. Dihydrotestosterone binds androgen receptors in the cytoplasm, causing phosphorylation, dimerization, and subsequent translocation into the nucleus, thereby binding to the androgen-response elements within the DNA, with consequent activation of genes involved in cell growth and survival (10). During androgen-independent progression, prostate cancer cells develop a variety of cellular pathways to survive and flourish in an androgen-depleted environment. Postulated and documented mechanisms include androgen receptor (AR) gene amplification, AR gene mutations, involvement of coregulators, ligand-independent activation of the androgen receptor, and the involvement of tumor stem cells (Fig. 1; refs. 8, 10–14).

Recent Advances

Hypersensitive Pathway. One way in which prostate cancer cells circumvent the effects of androgen blockade is by developing the ability to use very low levels of androgen for growth (14–16). Hence, they do not become androgen independent in the classic sense, but rather castration independent. There are several proposed mechanisms that could explain response to lower levels of androgen.

One such mechanism is increased expression of the androgen receptor, allowing enhanced ligand binding. The existence of this pathway is supported by studies of hormone-refractory tumors that have shown increased expression of androgen receptor compared with androgen-dependent tumors (14, 17–22). Increased production of androgen receptor is likely secondary to gene amplification as a result of mutation or through selective pressure of the androgen-depleted environment, causing the cells with fewer androgen receptors to die off and the clonal expansion of cells with more androgen receptor. Chen et al. (14) have shown, through comprehensive gene profiling of seven human prostate cancer xenograft models, that AR gene expression was up-regulated in the progression from androgen-dependent to castration-independent growth. Chen et al. (14) also showed that androgen-independent cells require 80% lower concentrations of androgen than androgen-dependent cells for growth.

Increased sensitivity of the androgen receptor to androgens has been proposed as another mechanism for castration independence (15). Using the prostate cancer cell line LNCaP, which has a mutated androgen receptor, Gregory and co-workers have shown high level expression of the androgen receptor, increased stability of the androgen receptor, and enhanced nuclear localization of the androgen receptor in recurrent prostate tumors associated with an increased sensitivity to the growth-promoting effects of dihydrotestosterone. The concentration of dihydrotestosterone required for growth stimulation of androgen-independent cells was 4 orders of magnitude lower than that required for androgen-dependent LNCaP cells (15).

A third hypersensitive mechanism proposed is increased local production of androgens by prostate cancer cells themselves. This most likely occurs by increased rate of conversion of testosterone to dihydrotestosterone by increasing 5α-reductase activity (10). Evidence in support of this mechanism includes (a) ethnic groups with higher levels of 5α-reductase activity have a higher rate of prostate cancer (23); (b) after androgen ablation therapy, serum testosterone levels decrease by 95%, but concentration of dihydrotestosterone in prostatic tissue is reduced by only 60% (24); and (c) genes involved in steroid biosynthesis have been found to be overexpressed in recurrent human prostate tumors (22).
Promiscuous Receptor. Although the wild-type androgen receptor is activated only by testosterone and dihydrotestosterone, the specificity of the androgen receptor has been shown to be broadened by mutations. The majority of mutations discovered leading to binding promiscuity are clustered in the ligand-binding domains (25, 26). These mutations allow the androgen receptor to be activated by nonandrogenic steroid molecules normally present in the circulation as well as antiandrogens to bind and activate the androgen receptor. Antiandrogens molecules activate the androgen receptor by ligand-dependent binding or activate downstream signaling of the androgen receptor by ligand-independent mechanisms (e.g., epidermal growth factor (EGF)). Outlaw pathway. Nonsteroid molecules activate the androgen receptor by ligand-dependent binding or activate downstream signaling of the androgen receptor by ligand-independent mechanisms. Growth and proliferation of tumor cells is therefore no longer under androgen control, although the androgen receptor machinery remains active. This type of activation has been coined outlaw activation (10–13). Deregulated growth factors, including insulin-like growth factor, keratinocyte growth factor, and epidermal growth factor, and cytokines, including IL-6, have been shown to directly phosphorylate and activate the androgen receptor (36, 37).

Outlaw Pathway. It has also been determined that activation of androgen receptor can be accomplished by ligand-dependent binding of nonsteroid molecules or activation of downstream signaling of the androgen receptor by ligand-independent mechanisms. Growth and proliferation of tumor cells is therefore no longer under androgen control, although the androgen receptor machinery remains active. This type of activation has been coined outlaw activation (10–13). Deregulated growth factors, including insulin-like growth factor, keratinocyte growth factor, and epidermal growth factor, and cytokines, including IL-6, have been shown to directly phosphorylate and activate the androgen receptor (36, 37). In addition, it has been shown that androgen receptor-dependent genes can be activated by deregulation of signal transduction pathways.

**Fig. 1.** Mechanisms of androgen independence. 1, amplification. Prostate cancer cells develop the ability to use low levels of androgen for survival by increased production of the androgen receptor (AR; usually by gene amplification), increased sensitivity of the androgen receptor to androgen, and by increased local conversion of testosterone to dihydrotestosterone by 5α-reductase (5αR). 2, promiscuous binding. Mutations of the androgen receptor broaden binding specificity allowing nonandrogenic steroid molecules normally present in the circulation as well as antiandrogens to bind and activate the androgen receptor. 3, outlaw pathway. Nonsteroid molecules activate the androgen receptor by ligand-dependent binding or activate downstream signaling of the androgen receptor by ligand-independent mechanisms (e.g., epidermal growth factor (EGF)). 4, bypass pathway. Prostate cancer cells develop the ability of survive independent of the androgen receptor. The best known bypass pathway is through modulation of apoptosis by up-regulation of the molecule Bcl-2 by androgen-independent prostate cancer cells which protect them from apoptosis or programmed cell death when they are exposed to lack of testosterone. 5, coregulators. Alterations in the balance between coactivators and corepressors, which function as signaling intermediates between the androgen receptor and the transcriptional machinery, influence androgen receptor activation contributing to ability to respond to lower levels of androgen and alternative mechanisms of activation. 6, stem cell regeneration. Prostate cancer stem cells, which are not dependent on the androgen receptor for survival, continually resupply the tumor cell population despite therapy.
transduction pathways (38, 39). For example, overexpression of the receptor tyrosine kinase HER-2/neu has been shown to activate androgen receptor–dependent genes in the absence of a ligand (38, 39).

The activation of outlaw pathways spotlights the potential importance of tumor-microenvironment interactions in the development of castration-independent growth (40, 41). Paget (42) recognized that a “fertile soil” was necessary for the successful growth of metastases. For example, many factors contribute to both androgen-dependent and independent growth of prostate cancer in the bone (41). The interaction of prostate cancer cells with bone stromal cells, osteoblasts, and osteoclasts, as well as the remodeling of bone extracellular matrix, results in the release and deregulation of multiple growth factors that the prostate cancer cells can use to become androgen independent.

**Coactivators and Corepressors.** A large number of coactivators and corepressors involved in the regulation of androgen receptor–driven transcription have been identified (43). They function as signaling intermediaries between the androgen receptor and general transcriptional machinery. Alterations in the balance between coactivators and corepressors have been shown to influence androgen receptor activation, although the precise mechanisms remain unknown (10, 44–47). An increase in coactivator levels has been shown in androgen-independent disease (44, 48–51). Coactivator proteins have been shown to enhance the activity of the androgen receptor to alternative ligands, sensitize the receptor to lower levels of native and

**Fig. 2.** Targeting critical pathways and the tumor microenvironment of androgen-independent prostate cancer. Targeted therapies are being developed that block outlaw signal transduction pathways to prevent ligand-independent phosphorylation of the androgen receptor, including agents that inhibit growth factor receptors directly (e.g., epidermal growth factor receptor by gefitinib) or indirectly through inhibition of downstream signal transduction (e.g., inhibition of ras by reolysin or b Raf by BAY43-9006). The bypass pathway is being targeted by chemotherapeutic agents such as docetaxel as well as specific inhibitors of Bcl-2 (gossypol and the antisense molecule G3139) as well as clustatin (the antisense molecule OGX-011). Geldanamycin is an antibiotic that destabilizes heat shock protein 90 and targets the hypoxia pathway as well as the androgen receptor signaling pathway. The tumor microenvironment is now actively targeted by agents which bind bone hydroxyapatite and inhibit osteoclast function, including zoledronate and the radiotopes samarium and strontium. The signal transduction of supporting cells is being targeted by vascular endothelial growth factor antibodies (endothelial cells) and endothelin-1 (ET-1) receptor antagonists (osteoblasts). Stimulation of the immune system to recognize prostate cancer cells as foreign is being pursued through multiple vaccine strategies (e.g., APC8015, GVAX, and TRICOM-vaccinia). GF, growth factor; VEGF, vascular endothelial growth factor receptor; PDGF, platelet-derived growth factor receptor; EGFR, epidermal growth factor receptor; ET-R, endothelin receptor; RTK, receptor tyrosine kinase; mTOR, mammalian target of rapamycin.
nonnative ligands, and to allow ligand-independent activation (44, 52).

**Bypass Pathway.** Alternatively, the androgen receptor pathway can be bypassed completely and prostate cancer cells develop the ability to survive independent of ligand-mediated or non-ligand-mediated androgen receptor activation. The best known bypass mechanism involves modulation of apoptosis. In androgen-dependent prostate cancer cells, androgen receptor activation stimulates cell proliferation and depletion of androgen leads to apoptosis of these cells. Androgen-independent prostate cancer cells have been shown to frequently up-regulate antiapoptotic molecules (53–56). Inactivation of the tumor-suppressor gene phosphatase and tensin homologue with subsequent activation of Akt is one way in which androgen-independent prostate cancer cells escape apoptosis in an androgen-depleted environment (12). Another postulated bypass mechanism is the neuroendocrine differentiation of prostate cancer cells (12). Neuroendocrine cells have a low rate of proliferation and are more prevalent in androgen-refractory prostate cancer (12). They also secrete neuropeptides, such as serotonin and bombesin, which can increase the proliferation of neighboring cancer cells, allowing progression in a low-androgen environment (12).

**Prostate Cancer Stem Cells.** In contrast to the stochastic model of tumorigenesis, in which every cell within the tumor is potentially tumor-initiating, the stem cell model of cancer, or the hierarchical model, postulates that only a rare subset of cells are tumorigenic (54). A population of cells comprising 0.1% of prostate tumors, which are CD44+/CD133+/CD44−/CD133− and do not express androgen receptors, has been identified and is thought to be prostate cancer stem or progenitor cells (57). Another potential mechanism for survival in the androgen-depleted environment is the presence of prostate cancer stem cells that continually resupply the tumor cell population, despite therapy. Although these cells remain a small percentage of the actual percentage of the tumor, they are not affected by androgen-depletion therapy. These stem cells differentiate into androgen-dependent and independent cells, resulting in the heterogeneous androgen receptor phenotype that is observed in androgen-independent patients (8).

**Therapeutic Implications**

**Novel Strategies to Target Hormone Refractory Prostate Cancer.** Ultimately, understanding the mechanisms is not enough. Understanding the pathways to what we now know as castration-resistant prostate cancer offers specific therapeutic opportunities based on these mechanisms (40, 41, 58). As in many cancers, novel therapies are being approached with two different strategies. The first is the identification of critical pathways and targets. The second is the development of multitargeted approaches that are directed not only at the cancer cell but also at the surrounding microenvironment of the tumor (Fig. 2).

**Critical Pathways.** Bypass pathways that promote cell survival are being targeted with a variety of strategies. Targeting the Bcl-2 molecule-microtubule complex with the agent docetaxel has led to the first shown survival benefit for patients with androgen-independent prostate cancer (58). In a trial involving 1,006 men with hormone refractory prostate cancer, Tannock et al. (59) reported in 2004 that 75 mg/m² of docetaxel given every 3 weeks plus daily prednisone led to a median survival rate of 18.9 months, compared with a survival rate of 16.4 months in patients who received 12 mg/m² of mitoxantrone given every 3 weeks plus daily prednisone (P = 0.009). As compared with the mitoxantrone group, those given docetaxel every 3 weeks had a hazard ratio for death of 0.76 (95% confidence interval, 0.62–0.94; P = 0.009). The every-3-week schedule of docetaxel showed an advantage in median survival when compared with mitoxantrone (18.9 versus 16.4 months; P = 0.009). Docetaxel every 3 weeks plus prednisone therapy also resulted in improvement in pain, a decrease in prostate-specific antigen, and improved quality of life compared with mitoxantrone plus prednisone.

G3139 (oblimersen sodium) is an antisense oligonucleotide directed against Bcl-2 mRNA that seems to have clinical activity in conjunction with docetaxel (60). Several trials are under way with the polyphenol gossypol and its derivatives inhibit Bcl-2, Bcl-xl, and cyclin D1 (61). An antisense molecule to clusterin, a prosurvival gene that is up-regulated in androgen-independent prostate cancer, is currently in clinical trials (62). 1,25-Dihydroxycholecalciferol (calcitriol) potentiates the activity of chemotherapeutic agents by causing G0-G1 arrest and inducing apoptosis. The combination of calcitriol and docetaxel seems to have increased antitumor activity as compared with docetaxel alone and is the subject of ongoing investigation (63).

Although new chemotherapeutic drugs such as satraplatin and the epothilones are in clinical trials in advanced prostate cancer, the most exciting progress is being made in targeting outlay signal transduction pathways that are deregulated in prostate cancer (58, 64–66). Outlay pathways often rely on the activation of the tyrosine kinase growth factor receptors. Antibodies and small molecules that target growth factor receptors on prostate cancer cells include those directed against epidermal growth factor receptor (gefitinib, cetuximab, trastuzumab, lapatinib, and erlotinib) and platelet-derived growth factor receptors (imantinib and leflunomide; refs. 41, 67). Other tyrosine receptor pathway inhibitors include BAY43-9006, reolysin, LEraFON, and GEM 231. Many of these pathways can also be targeted in the supporting cells of the tumor. The new classic example of this is the use of agents that target the vascular endothelial growth factor pathway to interrupt the blood supply to the tumor cells (68). A phase III trial of bevacizumab (Avastin) and docetaxel is being done by the Eastern Cooperative Oncology Group. Another such agent is atrasentan, which targets the endothelin-1 axis in osteoblasts (69). This agent, in combination with docetaxel, is the subject of a phase III trial by the SouthWest Oncology Group. As the role of the androgen receptor in androgen-resistant disease has been recognized, it has become a focus of therapeutic development.

In focusing on targeting the hypersensitive pathway, one approach has been to try to lower androgen receptor protein levels. Ansamycin antibiotics, such as 17-allylamino-17-demethoxygeldanamycin, have been shown in preclinical models to induce degradation of steroid receptors, including the androgen receptor, by inhibiting heat shock protein 90, a chaperone required for the folding of these proteins, and are currently in phase I trials (70–73). In preclinical studies, Eder et al. (74) were able to inhibit androgen receptor expression in LNCaP tumor cells using antisense androgen receptor...
oligodeoxynucleotides resulting in decreased growth, decreased production of prostate-specific antigen, and increased apoptosis. Further advances are also being made to reduce testosterone beyond the currently known second-line and third-line hormonal agents; it was recognized that much less ligand is needed for androgen receptor activation in hormone-refractory prostate cancer cells (75).

A large number of coactivators and corepressors involved in the regulation of androgen receptor–driven transcription have been identified (43). Because it is known that ligand-dependent and ligand-independent androgen receptor transcriptional activity is dependent on coactivator recruitment, modification of these coregulators or their binding targets may be important therapeutically. Peptide antagonists that target specific nuclear receptor coactivator binding surfaces have been developed in the preclinical setting and have been shown to inhibit androgen receptor transcriptional activity by disrupting androgen receptor-coactivator interactions (76).

The signal transduction pathways associated with tumor hypoxia has been the subject of intense scrutiny because they involve the outlaw, bypass, and cofactor pathways (77). CCI-779 and RAD001 target the mammalian target of rapamycin and upstream regulator of hypoxia-inducible factor 1a (78–80). Hypoxia-inducible factor 1a is also regulated through its binding to heat shock protein 90, which prevents its degradation by the proteosome complex (80). Heat shock protein 90 also stabilizes multiple other enzymes that are part of tyrosine kinase signaling cascades, as well as other proteins, such as steroid receptors including the androgen receptor (81). The geldanamycin derivative 17-(allylamino)-17-demethoxy-geldanamycin is an antibiotic that inhibits heat shock protein 90 function, leading to proteosome degradation of these multiple signaling targets (77–81).

**Multitargeted Attack of the Cancer Cell and the Tumor Microenvironment.** While other molecular targets, including the mitotic kinesins, histone deacetylases, the small-molecule inhibitor suberoylanilide hydroxamic acid, and cyclic GMP phosphodiesterases, are being pursued, the combination of multiple agents that simultaneously attack the tumor and its microenvironment are being developed as well as already being used in the clinic (41, 82, 83). Prostate cancer cells in the bone interact with the extracellular matrix, stromal cells, osteoblasts, osteoclasts, and endothelial cells to coordinate a sophisticated series of interactions to promote cell survival and proliferation (41, 84). The Food and Drug Administration–approved bisphosphonate zoledronic acid binds to the hydroxyapatite of damaged bone and inhibits osteoclast function, leading to a decrease in skeletal related events, such as fractures, in patients with hormone refractory prostate cancer (85, 86). Similarly, the radioisotopes samarium and strontium have been Food and Drug Administration approved for patients with bone metastases because they, too, bind to hydroxyapatite and deliver radiation to the entire tumor microenvironment, damaging not only the cancer cells but all of the supporting cells of the microenvironment (87, 88). The benefits of these agents in decreasing the morbidity of metastatic prostate cancer have been clearly delineated. Agents that deliver radiation to all prostate cancer cells, whether they reside in bone or soft tissue sites, are in clinical trials. Prostate-specific membrane antigen is a transmembrane glycoprotein that is highly expressed by prostate cancers and trials using antibodies to prostate-specific membrane antigen conjugated to multiple different radiopharmaceuticals and chemotherapy agents are under way (89).

All of these approaches can be complemented by strategies that exploit the immune system. Several vaccine strategies that either increase the antigenicity of prostate cancer cells or sensitize dendritic cells to the presence of prostate cancer cells are in clinical trials. These include APC8015 (Provenge), an investigational therapeutic vaccine that uses autologous antigen-presenting cells loaded with a recombinant fusion protein of prostactic acid phosphatase linked to a molecule that specifically targets a receptor expressed on the surface of human antigen-presenting cells, GVAX, an autologous tumor vaccine in which cells from the patient’s tumor are transduced in vitro with an engineered adenovirus containing the GM-CSF gene and poxvirus-TRICOM combinations (90–92).

**Conclusion**

The treatment of hormone-refractory prostate cancer is evolving rapidly. The definition of the multiple mechanisms by which prostate cancer cells can become resistant to castration has led to the identification of several new leads and pathways that are being quickly exploited. Combining this knowledge with a multifaceted attack on the tumor microenvironment that includes therapy involving endothelial cells, osteoblasts, osteoclasts, tumor stroma, and immune modulation should lead to a rapid increase in survival in patients with hormone-refractory prostate cancer in the near future.

References

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