# PROSPECTS

# Molecular Basis for the Antiandrogen Withdrawal Syndrome

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**Abstract**     In patients with prostate cancer who manifest disease progression during combined androgen blockade therapy, discontinuation of antiandrogen treatment might result in prostate-specific antigen decline, often associated with clinical improvement. The response called antiandrogen withdrawal syndrome is thus acknowledged as a general phenomenon. However, molecular mechanisms responsible for this syndrome are not completely understood. This article outlines the proposed mechanisms, including alterations of androgen receptor gene and its coregulatory proteins and activation of the signal transduction pathway, and the potential therapeutic approaches based on the specific mechanisms. J. Cell. Biochem. 91: 3–12, 2004.     © 2003 Wiley-Liss, Inc.

**Key words:** prostate cancer; combined androgen blockade; androgen receptor; androgen receptor coregulator; prostate-specific antigen

Androgens play a major role in the development and progression of prostate cancer. Since the first observation by Huggins and Hodges [1941], hormonal therapy remains the critical therapeutic option for advanced forms of prostate cancer. Although multiple strategies have been used to reduce serum levels of androgens or interfere with their function via the androgen receptor (AR), combined androgen blockade (CAB; also called maximal androgen blockade) is a standard treatment [Hellerstedt and Pienta, 2002]. CAB usually consists of surgical castration or a luteinizing hormone-releasing hormone analogue combined with a non-steroidal antiandrogen. This treatment produces a brief clinical response in most of the patients, but the majority of them eventually develop symptomatic recurrences. In this state, termed androgen-independent or hormone-refractory prostate cancer, hormonal therapy is no longer effective and cancer cells continue to proliferate. Indeed, prostate cancer is the second leading cause of cancer-related death among men in the United States [Jemal et al., 2003].

The AR, a member of the nuclear receptor superfamily [Chang et al., 1988], functions as a ligand-inducible transcription factor that regulates expression of target genes, such as prostate-specific antigen (PSA), which is clinically used for the detection and monitoring of prostate cancer recurrence and progression [Chang et al., 1995]. Upon binding of androgens, the androgen-AR complexes form homodimers, and they translocate into the nucleus and bind to androgen responsive elements located on target genes. Androgens thus activate AR transcription in target cells, such as prostate cancer. Recent studies have revealed that transcription coregulators also modulate this AR transcriptional activation [Heinlein and Chang, 2002].

Antiandrogens include a number of compounds that competitively inhibit binding of androgens by the AR in target cells, which ultimately prevents the activation of AR pathways and decreases PSA values in prostate cancer patients. However, in 1993 Kelly and Scher first reported several patients treated with the antiandrogen flutamide, which might have lead to cancer progression showing an increase in their PSA levels [Kelly and Scher, 1993]. Consequent reports have demonstrated a similar effect by a variety of antiandrogens as well as other hormonal agents [reviewed in Kelly et al., 1997; Paul and Breul, 2000]. These patients with disease progression experience a decline of PSA value after discontinuation of the antiandrogenic compounds. These phenomena have been called "antiandrogen withdrawal syndrome." In this article, we review clinical and molecular evidence of antiandrogen withdrawal syndrome and the future prospects of the syndrome, especially potential treatment strategies based on the proposed molecular mechanisms.

## CLINICAL FEATURES OF ANTIANDROGEN WITHDRAWAL SYNDROME

In some patients with prostate cancer who manifested disease progression during CAB therapy, discontinuation of antiandrogen treatment might result in a significant fall in serum PSA, often correlated with clinical improvement. This withdrawal response was first reported with flutamide [Kelly and Scher, 1993], but similar phenomena have been observed after cessation of other non-steroidal antiandrogens, including bicalutamide [Small and Carroll, 1994] and nilutamide [Huan et al., 1997] or steroidal antiandrogens such as cyproterone acetate [Sella et al., 1998] and chlormadinone acetate [Akakura et al., 1995], as well as a semi-synthetic estrogen diethylstilbestrol [Bissada and Kaczmarek, 1995] and a progestational agent megestrol acetate [Dawson and McLeod, 1995]. Therefore, what initially was called "flutamide withdrawal syndrome" was renamed "antiandrogen withdrawal syndrome" or even more recently "steroid hormone withdrawal syndrome" [Kelly et al., 1997]. However, this phenomenon has been observed more often with non-steroidal antiandrogens. Moreover, in patients on antiandrogen monotherapy, withdrawal response apparently does not occur, possibly due to the ability of androgens to promote tumor growth after discontinuation of antiandrogen treatment. Although few randomized trials (i.e., comparison between continuing and discontinuing antiandrogen treatment) have been conducted, many, mostly retrospective, studies have suggested that, as determined by PSA decline of more than 50%, a significant number of treated patients (15–80%) display withdrawal responses. A prospective study with flutamide withdrawal has revealed that 16 of 57 cases (28%) show a greater than 50% decrease in PSA levels and up to one third of the patients have measurable disease regression and/or symptomatic improvement [Scher et al., 1995]. Accordingly, the withdrawal phenomenon is more general than had been thought. In addition, it is noted that this response may occur quickly (within 6 weeks). Therefore, when prostate cancer patients under CAB therapy present with an increase in PSA level, it is worthwhile discontinuing the antiandrogen before initiating second-line treatment. However, the duration of response is usually limited (4–8 months), and tumors then progress into an androgen-independent state. One of the largest retrospective reviews [Small and Srinivas, 1995] identified no significant difference of survival time after discontinuation of antiandrogen in patients who did and did not respond to the antiandrogen withdrawal (13 months for responders vs. 12 months for non-responders). This may indicate that responders had a lower proportion or number of truly androgen-independent cells at the time of commencing hormonal therapy or may reflect the presence of more advanced or aggressive cancers in non-responders or both. There are no established prognostic parameters to separate patients who will respond to antiandrogen withdrawal from those with androgen-independent cancer. It has been suggested that quicker response to initial hormonal therapy (e.g., normalization of PSA values within 3 months) or longer duration of exposure to the antiandrogen may be predictive of developing antiandrogen withdrawal syndrome [Small and Srinivas, 1995; Kelly et al., 1997; Furuya et al., 1998]. Thus, it is likely that prostate cancer cells in patients with antiandrogen withdrawal syndrome are still hormone-sensitive and the antiandrogen induces cell proliferation or prevents cell death. Alternatively, withdrawal response could be a reliable predictor of the transition to androgen-independent prostate cancer.

## MOLECULAR EVIDENCE FOR THE ANTIANDROGEN WITHDRAWAL SYNDROME

A number of molecular mechanisms may underlie androgen-independent growth of prostate cancer, but exact mechanisms are still far from being fully understood. Similarly, molecular mechanisms responsible for the antiandrogen withdrawal syndrome have not yet been determined. Some possible mechanisms are shown in Table I and are described below.

### AR Gene Alterations

Progression of androgen-dependent and some androgen-independent prostate cancers has been indicated to strongly associate with AR activation. Therefore, the first possibility is *AR* gene alterations that change binding specificity and sensitivity to ligands and thereby enable antiandrogens to function as AR agonists.

Flutamide has been shown to stimulate AR transcription and cell growth in the LNCaP prostate cancer cell line with a point mutation of codon 877 (Thr → Ala) in the ligand-binding domain (LBD) of the *AR* gene [Wilding et al., 1989; Veldscholte et al., 1990]. This type of mutation was indeed found in patients with the antiandrogen withdrawal syndrome [Suzuki et al., 1996]. A number of different mutations in the *AR* gene, mainly occurring in the LBD, have been reported with higher frequency (up to 50%) in advanced prostate cancer specimens [Gottlieb et al., 1998]. Significantly, most mutations were isolated from the patients treated with flutamide in conjunction with castration, but not from those treated with castration alone [Taplin et al., 1999], while other studies showed relatively frequent AR mutations in metastatic tumors even prior to hormonal therapy [Tilley et al., 1996; Marcelli et al., 2000]. Recently, Hara et al. [2003] demonstrated that 6–13 weeks culture of LNCaP cells in the presence of bicalutamide could generate novel sublines harboring additional point mutation at codon

### TABLE I. Potential Mechanisms for Antiandrogen Withdrawal Syndrome

(1) *AR* gene alterations: mutation, amplification
(2) AR coregulatory protein alterations: increased/decreased expression, mutation
(3) Activation of MAPK pathway (not through AR pathway) by antiandrogen
(4) Clonal selection

741 (Trp → Cys or Leu) in the *AR* gene. In these LNCaP sublines, bicalutamide stimulated cell growth and increased PSA secretion both in vitro and in vivo. Interestingly, flutamide was still able to act as an antagonist for these mutated (codon 741 only) ARs. This finding is consistent with a clinical case of flutamide withdrawal syndrome with an additional PSA decline after bicalutamide treatment [Kelly et al., 1997]. These data suggest not only that different antiandrogens may be effective as a second-line therapy for the patients with an initial withdrawal response to an antiandrogen, but also that each antiandrogen has its own unique withdrawal response mechanism. Hence, antiandrogen treatment may more easily induce AR mutations, particularly at the codons 741 and 877, in tumor cells. When compared to the wild-type receptor, these mutations within the AR LBD can alter ligand binding specificity and increase the sensitivity to hormonal agents other than testosterone and 5α-dihydrotestosterone (DHT), such as adrenal androgens, estrogens, and progestins, as well as antiandrogens [Veldscholte et al., 1990; Culig et al., 1993; Taplin et al., 1995; Miyamoto et al., 1998a; Miyamoto and Chang, 2000].

*AR* gene amplification is also a mechanism for increasing receptor activity, although there are no reports showing *AR* gene amplification in the antiandrogen withdrawal syndrome. In support of this possibility, however, the *AR* gene has been shown to be amplified in approximately one-third of androgen-independent prostate cancers, but in none of the samples collected from the same patients prior to hormonal therapy [Linja et al., 2001]. Consequently, withdrawal responses may occur in cases where antiandrogens with agonist activity are the major stimulators of cell proliferation.

### AR Coregulatory Protein Alterations

Recent cloning and characterization of transcription coregulators has been a break-through in our understanding of how steroid receptors regulate gene transcription [Torchia et al., 1998; McKenna et al., 1999; Heinlein and Chang, 2002]. These coregulators, termed coactivators and corepressors, mediate receptor transcriptional activation or repression. Thus, transcriptional activity of the AR is mediated by a number of interacting proteins that function as coregulators [Yeh et al., 1999; Heinlein and Chang, 2002].

We and others have shown that AR coactivators, such as ARA70 and ARA55, can enhance the ability of antiandrogens to induce AR activity [Yeh et al., 1997, 1999; Miyamoto et al., 1998b; Fujimoto et al., 1999; Truica et al., 2000]. If expression of such AR coregulators is altered during CAB treatment, AR antagonists may function as agonists to activate the AR pathway. The increasing expression of SRC-1 and TIF-2 after androgen deprivation therapy has been observed [Gregory et al., 2001], but these coactivators only weakly promote the androgenic effect of antiandrogens. We have recently applied the yeast two-hybrid system, using codon 877 mutant AR as bait in the presence of hydroxyflutamide, in order to screen AR-interacting proteins that contribute to the development of the flutamide withdrawal syndrome. One of the positive clones, gelsolin, a multifunctional actin-binding protein known to have implications in cell signaling, apoptosis, and carcinogenesis [Kwiatkowski, 1999], significantly enhanced AR transcriptional activity in the presence of androgen and/or hydroxyflutamide [Nishimura et al., 2003]. Interestingly, after androgen depletion, expression of gelsolin was up-regulated in LNCaP cells, LNCaP xenografts, and prostate cancer specimens, whereas down-regulation of gelsolin in primary prostate cancer as a tumor suppressor was reported [Dhanasekaran et al., 2001]. Upregulation of ARA70 expression after CAB treatment was also observed [Gregory et al., 1998]. Using transient transfection assay, we showed significant enhancement of agonist effect of several antiandrogens, including hydroxyflutamide, bicalutamide, and cyproterone acetate, by ARA70 on AR transcription in prostate cancer cells [Miyamoto et al., 1998b]. Similarly, other AR coregulators, such as ARA55 and β-catenin, which can enhance AR transcription in the presence of adrenal androgens, antiandrogens, or estrogens [Fujimoto et al., 1999; Yeh et al., 1999; Truica et al., 2000], were found to be overexpressed in advanced and/or androgen-independent prostate cancers [Fujimoto et al., 2001], as well as mutated (gain of function) in some prostate cancers [Voeller et al., 1998]. These results suggest that weak agonist effects of antiandrogens may be amplified by increasing the amount of some AR coactivators after CAB treatment. Furthermore, we have recently shown that several mutant AR coactivators, including ARA54, ARA55, and ARA70, which function as dominant-negative inhibitors of AR transcription, reduce hydroxyflutamide- or 17β-estradiol-enhanced PSA expression and cell proliferation in prostate cancer [Miyamoto et al., 2002; Rahman et al., 2003a,b]. These findings confirm the involvement of AR coregulators in promoting the agonist effect of antiandrogens.

We also found that a C-terminal fragment of ARA55 without mutations acted as an inhibitor of AR transcription. A dominant-negative mutant ARA55 is a C-terminal fragment with a point mutation at amino acid 413 (Ala → Thr) [Rahman et al., 2003a]. We noticed that ARA55-related genes (e.g., mouse *hic-5*) contain Thr within the conserved regions [Shibanuma et al., 1994]. We then found Thr at position 413 of ARA55 in a human prostate cancer specimen. Another group also noticed that most of sequenced normal prostate/prostate cancer samples contain Thr, not Ala (from personal communication with Dr. DeFranco, University of Pittsburgh). These data may imply the existence of a polymorphism at amino acid 413 position. It is also possible that the original clone isolated from prostate cDNA library by yeast two-hybrid screening [Fujimoto et al., 1999] had a rare mutation at this position. As shown in Figure 1, the C-terminal fragment of ARA55 (413A) also suppressed AR transactivation in PC-3 cells expressing ARA55 (Fig. 1B, lanes 2 vs. 3–5) as well as that induced by exogenous full-length ARA55 (413A) in COS-1 cells (Fig. 1A, lanes 3 vs. 5–7). However, suppressive effect of a C-terminal fragment of ARA55 harboring a point mutation (mtARA55-413T) was stronger (Fig. 1A, lanes 5–7 vs. 8–10), as compared to the C-terminal fragment of wild-type (wt) ARA55-413A (Fig. 1B, lanes 3–5 vs. 6–8). Androgen-mediated interaction between AR and mtARA55-413T was then found to be weaker than that between AR and wtARA55-413A (Fig. 1C,D, lane 4). Nevertheless, the effect of full-length ARA55 with 413T mutation on androgen-mediated AR transcription was equivalent to that of wtARA55-413A (Fig. 1A, lanes 3 and 4).

### Activation of Mitogen-Activated Protein Kinase (MAPK) Pathway Which May Bypass the AR Pathway

Previous studies have demonstrated that several peptide growth factors, including epidermal growth factor (EGF) and insulin-like
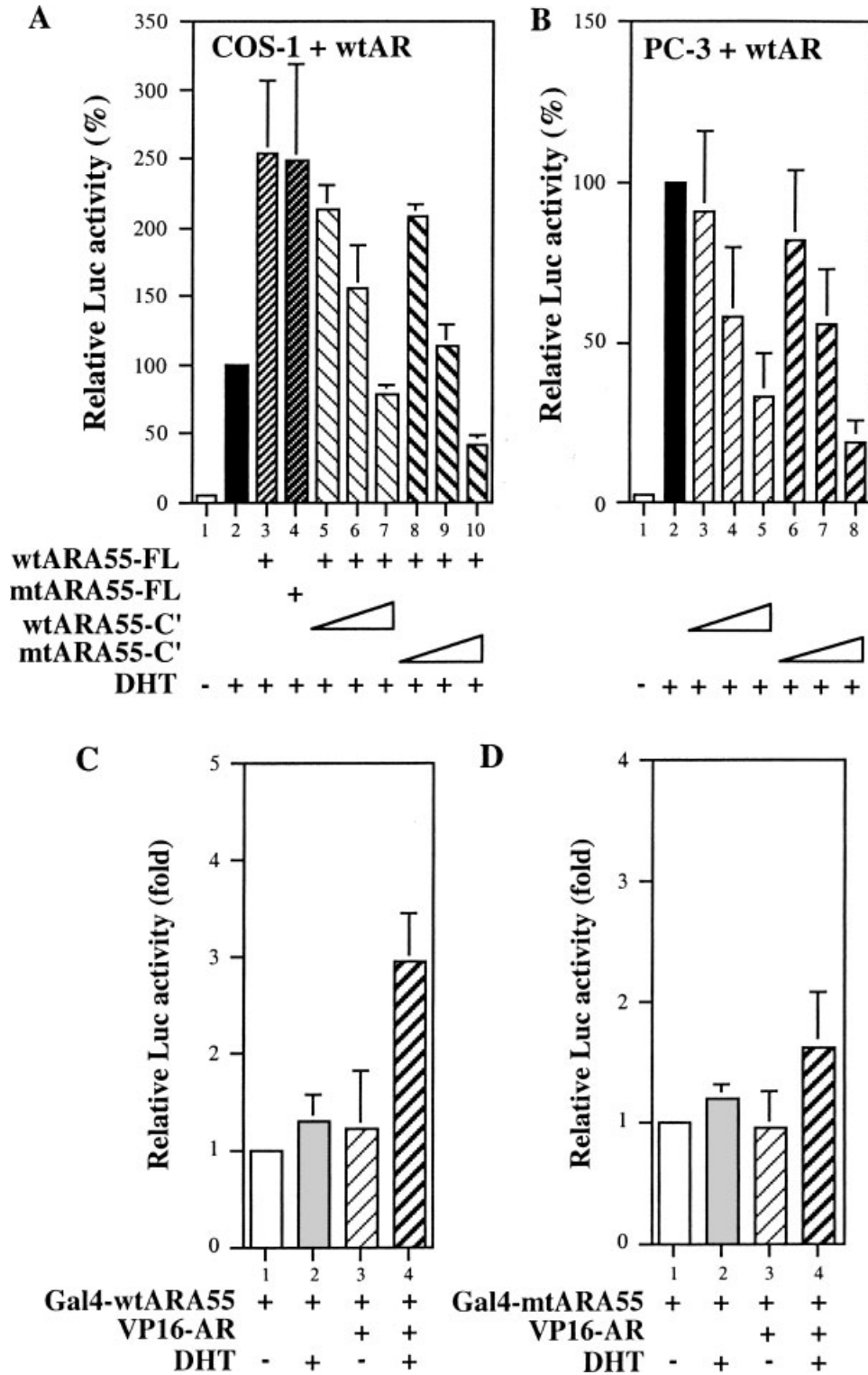
**Fig. 1.** wtARA55 versus mtARA55. COS-1 (**A**) or PC-3 (**B**) cells were transfected with the wtAR expression plasmid pSG5-AR and mouse mammary tumor virus (MMTV)-luciferase (Luc). wtARA55-full length (FL), mtARA55-FL, wtARA55-C terminal fragment (C′), and/or mtARA55-C′ were also co-transfected as indicated. After transfection, cells were cultured for 24 h in the presence or absence of 1 nM DHT. The Luc activity is presented relative to that of **lane 2** (set as 100%). Values represent the mean ± SD of at least three determinations. COS-1 cells were transfected with Gal4-hybrid expression plasmid Gal4-wtARA55 (**C**) or Gal4-mtARA55 (**D**), VP16-hybrid expression plasmid VP16-AR, and pG5-Luc reporter plasmid. After transfection, cells were cultured for 24 h in the presence or absence of 1 nM DHT. The Luc activity is presented relative to that of **lane 1** (set as onefold). Values represent the mean ± SD of at least three determinations.

growth factor-1 (IGF-1), which serve as ligands for receptor tyrosine kinases, such as the EGF receptor and HER-2/neu, increase AR transcriptional activity in the absence of androgens [Culig et al., 1994; Russell et al., 1998]. It has been postulated that this mechanism of AR activation is mediated through signal transduction pathways, such as the MAPK and Akt (also known as protein kinase B), which can specifically bind to and phosphorylate the AR [Abreu-Martin et al., 1999; Wen et al., 2000]. This ligand-independent activation of the AR could be a potential mechanism for the androgen-independent growth of prostate cancer.

Recently, Lee et al. [2002] have found hydroxyflutamide activates the MAPK pathway in prostate cancer cells. In the AR-negative prostate cancer cell line DU145, as well as AR-positive cell lines, hydroxyflutamide was found to induce MAPK activation via the Ras/Raf pathway. Hydroxyflutamide also stimulated DU145 cell proliferation, and this effect was inhibited by an EGF receptor inhibitor and an EGF receptor-neutralizing antibody. Therefore, the activation of the Ras/MAPK signaling pathway by hydroxyflutamide can be mediated in an AR-independent manner. In addition, a significant increase of activated MAPK signals in the prostate cancer specimens from patients whose tumors progressed on CAB therapy with flutamide was observed, compared to tumor specimens from the same patients prior to CAB therapy. These findings provide a potential mechanism that may contribute to the withdrawal response of antiandrogens.

### Clonal Selection Theory

Isaacs [1999] proposed the clonal expansion theory to explain androgen-independent growth of prostate cancer. Androgen-independent tumor cells may co-exist with androgen-dependent cells at the time of initial treatment, and become predominant (selected) during hormonal therapy. This theory might also explain the paradoxical growth stimulatory effect of antiandrogens in prostate cancer. CAB treatment with the antiandrogen may select for cells, for example, which have an AR mutation, amplified AR, or overexpressed/mutated AR coregulators.

### FUTURE PROSPECTS

The antiandrogen withdrawal syndrome is now a well-established phenomenon in prostate cancer, and at least 30% of patients with rising PSA will benefit from discontinuing antiandrogen treatment. However, whether antiandrogen withdrawal alone prolongs survival, compared to the patients with no response (transition to androgen-independent state), remains controversial. To improve overall survival of patients with advanced prostate cancer/antiandrogen withdrawal syndrome, we need to determine: (1) whether CAB therapy is more beneficial than castration alone or antiandrogen monotherapy; (2) precise mechanisms responsible for the emergence of antiandrogen withdrawal syndrome as well as androgen-independent prostate cancer; (3) novel drugs/treatment strategies for advanced prostate cancer to prolong the androgen-dependent state or to prevent the occurrence of antiandrogen withdrawal response; (4) treatment options as second-line therapy; and (5) predictive parameters for the emergence of antiandrogen withdrawal syndrome. Clinically, larger studies and new clinical trials are first necessary to resolve the above problems. Here we will focus on potential treatment strategies based on the molecular mechanisms for the antiandrogen withdrawal syndrome.

As noted, the majority of available antiandrogens have been reported to induce withdrawal response, possibly resulting from their partial agonist effects. Therefore, the identification of new antiandrogenic compounds with lower androgenic activity could be a potential approach. Indeed, we have screened synthetic dehydroepiandrosterone (DHEA) derivatives and found that 3β-acetoxyandrost-1,5-diene-17-ethylene ketal (ADEK) acted as a potent antiandrogen in vitro and could inhibit DHT-induced PSA expression and proliferation of LNCaP cells [Miyamoto et al., 2003]. Importantly, ADEK had only marginal agonist activity on both the wt AR and mtAR (codon 877), which could not be induced further by AR coactivators. Previously, we identified that an adrenal androgen, $\Delta^5$-androstenediol, possessed intrinsic androgenic activity in prostate cancer cells, which could not be antagonized by pharmacological concentrations of hydroxyflutamide and bicalutamide [Miyamoto et al., 1998a]. We then demonstrated that ADEK [Miyamoto et al., 2003] and other DHEA metabolites [Chang et al., 1999] could inhibit AR transactivation induced by $\Delta^5$-androstenediol. We are further screening newly synthesized compounds with structures similar

to ADEK to find more effective antiandrogenic compounds. In vivo studies are also necessary to test the anti-tumor effects of these compounds as well as toxicity, tolerance, and side effects.

Inhibition of AR function through the mechanisms interfering with AR expression, protein stability, nuclear translocation, and interactions of the $NH_2$ and COOH terminals of the AR, all of which are expected to decrease transcriptional activity of the AR, might provide another therapeutic benefit. However, only a few compounds are known to inhibit prostate cancer cell growth through these mechanisms. Resveratol [Mitchell et al., 1999], found in grapeskins and used in some Asian medicines with weak estrogenic activity, and vitamin E succinate [Zhang et al., 2002] are reported to suppress AR expression and androgen-induced LNCaP cell growth. Curcumin, a perennial herb used as a yellow coloring and flavoring agent in foods, can inhibit proliferation of both AR-positive and AR-negative prostate cancer cells through inducing apoptosis [Dorai et al., 2001; Mukhopadhyay et al., 2001]. We also found that an analogue of curcumin inhibited DHT-induced PSA expression and tumor growth only in AR-positive cells, by (1) competing with ligands for AR binding, (2) reducing AR protein expression in the presence and absence of androgens, (3) promoting AR degradation, (4) retarding androgen-induced AR translocation into the nucleus, and/or (5) interrupting androgen-mediated AR $NH_2$/COOH interaction (Miyamoto et al., unpublished data). Thus, these compounds might be expected to not only inhibit proliferation of both androgen-dependent and androgen-independent prostate cancer cells but also carry fewer risks of inducing the antiandrogen withdrawal syndrome. Further investigations are necessary to determine their ultimate therapeutic use.

AR coregulators may play an essential role in the regulation of AR activity. Therefore, another strategy, possibly using gene therapy, to down-regulate AR activity is to block interactions of the AR with AR coregulators highly expressed in tumors from patients with antiandrogen withdrawal syndrome. We have found that several mutants of AR coactivators (e.g., ARA54, ARA55, and ARA70) inhibit AR (wt and codon 877 mutant) transcription, antiandrogen/AR-mediated prostate cancer cell proliferation, and PSA expression in a dominant-negative fashion, presumably through the disruption of AR coactivator dimerization

[Miyamoto et al., 2002; Rahman et al., 2003a,b]. Technology of RNA interference, double-stranded short interfering RNA (siRNA) which suppresses the expression of endogenous genes [Sui et al., 2002], strengthened our finding by demonstrating similar suppression of AR transactivation by siRNA-mediated silencing of the AR coactivators [Rahman et al., 2003a,b]. These results suggest that down-regulation of AR activity, by altering its necessary coregulators, may provide a new therapeutic approach in the treatment of antiandrogen withdrawal syndrome. However, since prostate cancer cells appear to be capable of overexpressing more than one coactivator simultaneously [Fujimoto et al., 2001; Gregory et al., 2001], this approach is feasible only if AR interaction with multiple coactivators can be blocked. We have data showing peptides (40–50 amino acids) from the AR LBD or DNA-binding domain could block gelsolin-enhanced AR activity. However, different AR coactivators may interact with different AR domains [Heinlein and Chang, 2002]. Therefore, it might be difficult to design enough proteins/peptides to effectively interrupt androgen- or antiandrogen-induced interactions of the AR with multiple coactivators. Further study is necessary to test whether the AR peptides also interfere with functions of other coregulators.

Several AR corepressors have been identified and characterized as being able to interact with liganded AR [Heinlein and Chang, 2002]. These corepressors, such as cyclin D1, were shown to repress AR transcription in prostate cancer cells [Reutens et al., 2001]. However, little is known about the role of AR corepressors in prostate cancer progression and antiandrogen withdrawal syndrome, as well as the mechanism of their suppressive effect. Further investigation of AR corepressors will hopefully clarify the above mystery.

Modulation of signal transduction pathways might be a useful way to induce apoptosis in both androgen-dependent and androgen-independent prostate cancer cells. It has been suggested that some growth factors can modulate apoptosis through phosphorylation of multiple target proteins. Indeed, inhibition of growth factors has been tested through decreasing the availability of growth factors, modulation of intracellular kinase activity, immunological targeting of growth factor receptors, or inhibition of receptor tyrosine kinase

activity [Agus et al., 1999; Plonowski et al., 1999]. Initial clinical studies, using IGF-1 modulators, including somatostatin analogues and vitamin D analogues, or humanized monoclonal antibody to the HER-2 receptor trastuzumab, showed some benefits in androgen-independent prostate cancer patients [Koutsilieris et al., 2001; Liu et al., 2002; Morris et al., 2002]. Since signal transduction pathways, such as MAPK and Akt, may be activated in prostate cancer, especially tumors in antiandrogen withdrawal syndrome patients and androgen-independent tumors, specific inhibitors to these pathways could be potential therapeutic agents [Wen et al., 2000; Lin et al., 2001; Lee et al., 2002]. It is also possible that these agents could be combined with second-line hormonal therapy.

### CONCLUDING REMARKS

Many different mechanisms are emerging that may be involved in the antiandrogen withdrawal syndrome. These include alterations of *AR* gene and its coregulatory proteins and activation of the signal transduction pathways that may not involve the AR pathway. It is also possible that no single mechanism is utilized in every case. Therefore, it may be necessary to explore more individualized approaches, according to each potential mechanism. Hopefully, further investigations about withdrawal response of antiandrogens might also lead to better understanding of prostate cancer, especially finding some clues to overcoming androgen-independent prostate cancer.

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