Overcoming Persistent Dependency on Androgen Signaling after Progression to Castration-Resistant Prostate Cancer

Masuo Yamaoka, Takahito Hara, and Masami Kusaka

Abstract
Prostate cancer is the most common form of cancer in males in the United States, and the second leading cause of cancer death. Initially, most cases of prostate cancer respond well to hormone therapy; however, resistance often develops rapidly, leading to castration-resistant prostate cancer (CRPC). Several mechanisms for castration resistance have been proposed, of which the most significant seems to be the "intracrine" production of androgens from adrenal androgen or intratumorally via the de novo route. This mechanism stimulates disease progression through reactivation of androgen receptor signaling in patients who have previously undergone castration therapy. 17,20-lyase is essential for androgen synthesis in both the adrenal glands and CRPC tissue, and some 17,20-lyase inhibitors and second-generation anti-androgens that were developed to treat CRPC are currently under clinical investigation, with encouraging preliminary data reported so far. However, resistance to some of these therapies has already been noted. The study of circulating tumor cells will likely be important not only to identify patients likely to receive benefit from this therapeutic approach, but also to further understand the molecular mechanisms of resistance.

Background
Prostate cancer is among the most common of solid tumors in Western countries and the second leading cause of cancer death in men in the United States (1). The hormonal dependency of the majority of prostate tumors was established more than 50 years ago, when it was shown that prostate tumors were susceptible to castration and that this was due to the withdrawal of androgens (2). Since then, hormone therapy has been a mainstay of medical treatment for recurrent prostate cancer after radical prostatectomy and locally advanced or metastatic prostate cancers, for which surgical treatment is not an option. As the testes are the source of most circulating androgens, most prostate cancers initially respond well to castration, which can be achieved by surgery (surgical castration) or gonadotropin-releasing hormone (GnRH) analog treatment (chemical castration). However, resistance to the therapy develops within 12 to 33 months and is often recognized by an increase in prostate-specific antigen (PSA) levels (3). This stage of prostate cancer is called castration-resistant prostate cancer (CRPC), and symptomatic CRPC is associated with poor prognosis (4, 5). Currently, docetaxel is the only chemotherapeutic compound that has been shown to prolong overall survival in this patient population (6). However, it has only moderate efficacy and is associated with a high incidence of severe adverse events such as anemia, neutropenia, diarrhea, and sensory neuropathy (6). Thus, new treatment options for CRPC are needed.

Mechanisms of Castration Resistance
Historically, there has been much debate about the mechanisms of castration resistance, but recent studies have shown the importance of androgen receptor (AR) signaling (7). Consequently, several, likely interconnected, mechanisms of castration resistance have been proposed: (i) AR activation by androgens converted from adrenal androgens or synthesized intratumorally via the de novo route (8, 9); (ii) hypersensitivity of ARs due to overexpression of AR proteins and/or changes in cofactor expression levels (10, 11); (iii) promiscuous activation of AR signaling by various ligands following AR mutation (12–16); and (iv) constitutive activation of AR signaling by truncated ARs lacking ligand-binding domains (17–20).

Among these mechanisms, the first, AR activation by androgens converted from adrenal androgens or synthesized intratumorally via the de novo route, seems the most likely target for inhibition by a small molecule. GnRH analogs that suppress testicular androgen synthesis do not block the production of adrenal androgens because they only suppress the secretion of luteinizing hormone (LH) through desensitization of the GnRH signal, and the production of adrenal androgens is not under the
control of LH (21). Hence, following castration, residual adrenal androgens remain or can be synthesized intratumorally (Fig. 1). Androgens are synthesized from cholesterol via multiple enzymatic steps, most of which are catalyzed by members of the cytochrome P450 (CYP) family in the testes and adrenal glands (Fig. 1). As a first step, pregnenolone is generated from cholesterol via a side-chain cleavage reaction catalyzed by CYP11A1. In the zona reticularis of the adrenal cortex, where most adrenal androgens are synthesized, pregnenolone is converted to 17-hydroxypregnenolone and then to dehydroepiandrosterone (DHEA) by the 17-

hydroxylase and 17,20-lyase activities, respectively, of CYP17A1. CYP17A1 is also involved in the synthesis of glucocorticoids. Together with the type II 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme (22), the 17-hydroxylase activity of CYP17A1 is involved in the conversion of pregnenolone to 17-hydroxyprogesterone. The 17,20-lyase activity of CYP17A1 is then involved in the conversion of 17-hydroxyprogesterone to androstenedione. However, the CYP17A1 enzyme has no role in the synthesis of mineralocorticoids.

Castration does not affect androgen synthesis in the adrenal glands; therefore, the adrenal androgens DHEA and...
Androgen Synthesis Pathway as a Therapeutic Target

CYP17A1 is a CYP family enzyme that is essential for androgen biosynthesis and has both 17,20-lyase and 17-hydroxylase activities (23). As outlined above, 17,20-lyase converts 17-hydroxyprogrenolone to DHEA, and 17-hydroxydecoy to pregnenolone to 17α-hydroxyprogrenolone (Fig. 1). Among enzymes involved in androgen synthesis, 17,20-lyase has been reported to be highly upregulated in CRPC metastases (9). Furthermore, DHEA has been shown to have substantial agonistic activity to support tumor growth in a newly established CRPC model cell line generated from the androgen-sensitive cell line MDA PCa 2b (12) and to activate transcriptional activity of CWR22 and LNCaP mutant ARs (with H874Y and T877A mutations, respectively; ref. 16). Thus, inhibition of 17,20-lyase seems a suitable target for inhibition of CRPC progression by ligand-activated AR. Many steroid-metabolizing enzymes and drug-metabolizing enzymes belong to the CYP family and have a heme moiety in the active center, which is a common feature of CYP family enzymes, including CYP17A1; therefore, new 17,20-lyase inhibitors should show selectivity for inhibition of 17,20-lyase over other CYP enzymes. Indeed, ketoconazole, which has 17,20-lyase inhibition activity (24), has been used clinically in patients with CRPC. However, ketoconazole treatment is often discontinued because of toxicities that arise owing to its low specificity for 17,20-lyase (5). More potent and specific 17,20-lyase inhibitors may be expected to show greater efficacy for at least a subset of CRPC.

17,20-lyase activity seems to vary by species. For example, in humans, the Δ 5 steroid 17-hydroxyprogrenolone is the favored substrate by approximately 50 fold compared with the Δ 4 steroid 17-hydroxyprogesterone, which is in contrast to observations in rats (23, 25). Δ 5 steroids have a common 3β-hydroxy, 5-ene structure, whereas Δ 4 steroids have 3-oxo, 4-ene structures. Pregnenolone, 17-hydroxyprogrenolone, and DHEA are key examples of Δ 5 steroids; and progesterone, 17-hydroxyprogesterone, and androstenedione are important examples of Δ 4 steroids. Owing to the substrate preference of human 17,20-lyase activity, together with the low expression level of the HS3B2 enzyme, which converts Δ 5 steroids to Δ 4 steroids in the zona reticularis, the Δ 5 route is thought to be the main route by which adrenal androgens are synthesized in humans. This information might be important in the development of 17,20-lyase inhibitors. For example, use of 17-hydroxyprogrenolone as a substrate in a human 17,20-lyase enzyme assay would be preferable to use of 17-hydroxyprogesterone.

Clinical-Translational Advances

Several small-molecule inhibitors of 17,20-lyase are currently under investigation. The rationale of this approach is supported by recent reports that abiraterone acetate (the prodrug of abiraterone), an orally active, irreversible inhibitor of CYP17A1 with a steroidal structure (26, 27), has clinically relevant antitumor activity (≥50% PSA decline) in 67% of patients with CRPC (28). Efficacy has also been reported in patients who had received prior ketoconazole therapy, with a response rate of 47% reported (29). Abiraterone acetate is currently being investigated in phase 3 clinical trials.

As described above, CYP17A1 has both 17,20-lyase and 17-hydroxylase activities. If 17-hydroxylase activity is inhibited, cortisol levels will be decreased. However, patients with congenital CYP17A1 deficiency show pseudohermaphroditism but do not show symptoms of adrenal insufficiency, possibly owing to the existence of the glucocorticoid corticosterone, the synthesis of which does not depend on 17-hydroxylase activity (23). Indeed, in clinical studies of abiraterone acetate, symptoms of adrenal insufficiency have not been observed (30). To compensate for the reduced level of total glucocorticoid activity, secretion of adrenocorticotropic hormone (ACTH) is stimulated, leading to increased steroid synthesis. This increased steroid synthesis, concomitant with suppression of 17-hydroxylation, leads to increases in corticosterone and deoxycorticosterone levels. Although these high corticosterone levels compensate for the low cortisol levels, these steroids cause symptoms of secondary mineralocorticoid excess such as hypertension, because of their mineralocorticoid activity. Indeed, hypertension is the most common grade 3 adverse event seen with abiraterone acetate (29). Although these symptoms can often be managed by nonsteroidal mineralocorticoid receptor (MR) antagonists such as eplerenone and spironolactone, it would be preferable to avoid the use of steroidal antagonists because they have been reported to have AR affinity (31). Small doses of synthetic glucocorticoids could be used instead because they compensate for the low levels of cortisol and suppress ACTH secretion; these agents might also increase the efficacy of 17,20-lyase inhibitors because increased levels of corticosterone and progesterone by ACTH can stimulate AR transcription (32).

However, it would be preferable to avoid the concomitant use of glucocorticoids, particularly in patients with less-advanced prostate cancer. Although CYP17A1 has both 17-hydroxylase and 17,20-lyase activities, several studies have suggested differences in 17-hydroxylase and 17,20-lyase activities. For example, a clinical case of isolated congenital deficiency of 17,20-lyase activity has been reported (33), and cytochrome b5 has been reported to...
be necessary for 17,20-lyase activity through a nonelectron transfer mechanism (34, 35). Cytochrome b5 predominantly exists in the zona reticularis of the primate adrenal cortex, where most adrenal androgens are synthesized (36). Thus, it may be possible to identify 17,20-lyase inhibitors with greater specificity for 17,20-lyase versus 17-hydroxylase. In addition, specificity for 17,20-lyase over other CYP family enzymes, including other steroid-synthesis enzymes and drug-metabolizing enzymes, may also translate into minimal toxicity and drug-drug interactions.

Accordingly, novel, nonsteroidal 17,20-lyase-specific inhibitors have recently been investigated. Using a nonsteroidal scaffold to minimize the risks of drug metabolism and pharmacokinetic (DMPK) effects and the formation of metabolites that might have AR agonistic activity, the novel investigational agent TAK-700 was identified and found to show high selectivity for 17,20-lyase over 17-hydroxylase in preclinical experiments and have a favorable pharmacokinetic profile in male cynomolgus monkeys (37). TAK-700 is currently under investigation in a phase 2 clinical trial, and preliminary response data are encouraging, with 52% of patients who received TAK-700 ≥300 mg twice daily showing a PSA decrease ≥50%, including 29% who showed reductions ≥90%. Importantly, the reported incidence of hypertension is low, supporting the selectivity of 17,20-lyase versus 17-hydroxylase inhibition in humans (38, 39).

Another 17,20-lyase inhibitor in clinical investigation is TOK-001 (formerly VN/124-1; ref. 40). This compound has high structural similarity to abiraterone. However, it has been reported that, in addition to CYP17A1 inhibition, TOK-001 has a downregulatory effect on AR expression in vitro and in vivo and has AR antagonistic activity, showing higher affinity for wild-type AR than bicalutamide, unlike abiraterone acetate. The in vivo antitumor efficacy of this compound has been shown to be more potent than castration or bicalutamide therapy. TOK-001 is currently being evaluated in a phase 1 clinical trial.

An alternative approach has been the development of AR antagonists. As androgens produced by the "intracrine" mechanism outlined in Fig. 1 act on ARs, antagonists that block the interaction between androgens and ARs may be expected to show efficacy in the treatment of CRPC. However, first-generation AR antagonists such as bicalutamide show minimal efficacy for CRPC because they have agonistic activity for CRPC (5, 41, 42). For example, some first-generation AR antagonists alter the recruitment of coactivators and corepressors to the promoter-enhancer regions of androgen-receptor target genes (10), and can lead to the emergence of AR mutations, for which such antagonists act as agonists (11, 13, 14).

Recently, MDV3100, a second-generation anti-androgen has been investigated in patients with CRPC. This compound is reported to dissociate ARs from AREs of the target genes or cofactors and have very low CRPC agonistic activity (43). In addition, MDV3100 inhibits AR signaling by mutant ARs, on which steroid hormones other than androgens and first-generation anti-androgens act as agonists (43). Thus, MDV3100 might be expected to have greater efficacy than that of other AR antagonists, a group that includes bicalutamide, flutamide, and nilutamide, and other investigational agents. However, the PSA response rate seen in MDV3100 studies (44) is similar to that seen in abiraterone studies (28). One possible explanation for this is that CRPC might be associated with a low frequency of AR mutations for which steroids other than androgens act as agonists. Another possibility may involve the production of estrogens, as it has been reported that the expression of CYP19 (aromatase) is highly upregulated in CRPC tissues (9). Estrogens are synthesized from androgens by aromatase and have been reported to upregulate expression of the TMPRSS2-ERG fusion gene through binding to estrogen receptor-α (ERα; ref. 45). TMPRSS2-ERG is a recently discovered fusion of an androgen-controlled serine protease, TMPRSS2, and the erythroblast transformation-specific (ETS) family gene ERG by chromosomal rearrangement (46). Together with phosphoinositide-3 kinase (PI3K), this fusion gene is thought to be involved in the pathogenesis and progression of prostate cancer (46, 47). Although 17,20-lyase inhibitors can reduce the intracellular levels of estrogens through inhibition of androgen production, androgen antagonists generally cannot block the binding of estrogen to ERs. Initial clinical studies investigating MDV3100 in prostate cancer have shown promising results (44), and MDV3100 is now in phase 3 clinical trials.

The preliminary clinical efficacy data for abiraterone acetate, TAK-700, and MDV3100 are promising, as each of these investigational agents is associated with ≥50% PSA decreases in more than 50% of patients with metastatic CRPC (28, 38, 39, 44, 48). Also, the safety profiles of these therapies seem reasonable for this patient population with advanced-stage disease. Nevertheless, identification of patients likely to receive optimum clinical benefit from treatment is desired. Recently, circulating tumor cells (CTC) have received particular attention as a marker of disease progression and treatment response, and it has been speculated that CTC counts would not only be useful as intermediate endpoints in CRPC (49), but they may also reflect genetic information of cancer cells, especially the presence of inaccessible bone metastases (so-called liquid biopsy; ref. 50). In addition, a significant association between ERG rearrangement and decreases in PSA level has recently been reported in CRPC patients treated with abiraterone acetate (51), and it is conceivable that the TMPRSS2-ERG fusion gene is under the control of AR signaling (46). In addition to TMPRSS2-ERG, several fusion genes of the ETS transcription factor family have been reported (52–55). Expression of some of the N-terminal fusion partners is thought to be androgen-repressed or androgen-independent (55). Therefore, extensive analysis of expression of these fusion genes might allow more accurate prediction of treatment response.
Future Directions

Despite the substantial clinical efficacy reported with abiraterone acetate, including in patients with postdocetaxel CRPC (56), resistance to this therapy has already been reported (57). Resistance to MDV3100 treatment has also been observed (50). Reactivation of AR signaling following abiraterone or MDV3100 treatment might occur by several mechanisms, including the expression of truncated ARs or cross-talk with other signaling pathways, because most cases of resistance have been identified by increased PSA levels.

This resistance might also be caused by a switching of the transcription program under the control of AR signaling (58), and it might not be possible to inhibit the new AR-regulated transcription program by currently available therapies. If so, substantial downregulation of AR expression would be a promising strategy for future studies. Further understanding the mechanism of resistance remains a key issue to be overcome in the development of new therapies; this may be enabled by investigation of the genetic information (amplification, mutation, rearrangement, etc.) of CTCs.

Treatment of prostate cancer is challenging as it has developed resistance to many current therapies. However, gaining a clear understanding of the molecular mechanisms of this resistance and developing therapies that target all such mechanisms will ultimately improve the prognosis of patients with prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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