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Review

Cancer Research

A Road Map to Comprehensive Androgen Receptor Axis Targeting for Castration-Resistant Prostate Cancer

Nicholas Mitsiades

Abstract

Gonadal androgen suppression (castration via orchiectomy or gonadotropin-releasing hormone analogues) suppresses circulating testosterone levels but does not achieve adequate androgen ablation within the prostate cancer microenvironment because it does not address adrenal and intratumoral steroid contributions. These residual extragonadal sources of androgens allow prostate cancer cells to survive, adapt, and evolve into castration-resistant prostate cancer (CRPC). The persistent significance of the androgen receptor (AR) axis in CRPC was recently validated by the clinical efficacy of androgen synthesis inhibitors (abiraterone) and novel, second-generation AR antagonists (enzalutamide). The appreciation that conventional therapeutic approaches achieve a suboptimal ablation of intratumoral androgens and AR axis signaling output opens transformative therapeutic opportunities. A treatment paradigm of comprehensive AR axis targeting at multiple levels (androgen synthesis, metabolism, and action) and at all relevant sites (gonadal, adrenal, intratumoral) simultaneously at the time of initiation of endocrine therapy (instead of the current approach of sequentially adding one agent at a time and only after disease progression) deserves examination in clinical trials to explore whether maximal first-line AR axis suppression via combination therapy can achieve maximal induction of cancer cell apoptosis (before they have the chance to adapt and evolve into CRPC) and thus, improve patient outcomes. *Cancer Res; 73(15); 4599–605. ©2013 AACR.*

Introduction

The androgen receptor (AR) is a transcription factor that plays critical roles in prostate adenocarcinoma pathophysiology (1). Gonadal androgen suppression [surgical or chemical castration via orchiectomy or gonadotropin-releasing hormone (GnRH) analogues, respectively] is an effective systemic treatment for advanced prostate cancer in use for the past 7 decades (2). However, it is not curative, as, almost universally, resistant disease eventually emerges, which had been described in the past as "hormone-refractory" and "androgen-independent" prostate cancer (1). These terms have now been confirmed to be inaccurate, as AR and its transcriptional output most frequently remain expressed and critically important in prostate cancer cells even in this state (1), leading to the adoption of the more appropriate term, "castration-resistant" prostate cancer (CRPC). The latter indicates a clinical state in which, despite suppressed circulating testosterone levels (<50 ng/dL), the AR axis has been reactivated because of a plethora of signaling mechanisms that operate within the prostate cancer cell and its local milieu (1). This revised view of the persistent role of AR signaling in CRPC led to the development of novel therapeutic agents such as the androgen synthesis inhibitor abiraterone (3) and the second-generation AR antagonist enzalutamide (MDV3100; ref. 4), and, in turn, was validated by their clinical efficacy.

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Mechanisms Promoting Persistent AR Axis Output in CRPC and Clinical Validation of Its Significance

Several mechanisms that promote persistent AR axis activation in CRPC cells, despite castrate levels of peripheral testosterone, have been reported (Fig. 1), including the following:

1. Persistence of intratumoral androgens via *in situ* synthesis and metabolism (refs. 5–11; and discussed in more detail below);
2. *AR* overexpression (frequently due to *AR* gene amplification) and missense mutations in the AR ligand-binding domain (LBD) that sensitize the receptor to even low androgen concentrations and/or broaden ligand specificity, leading to promiscuous interactions with alternative ligands (e.g., progesterone or the first-generation AR antagonists, flutamide, bicalutamide, and nilutamide, that can be converted into agonists under these conditions; refs. 1, 12–16);

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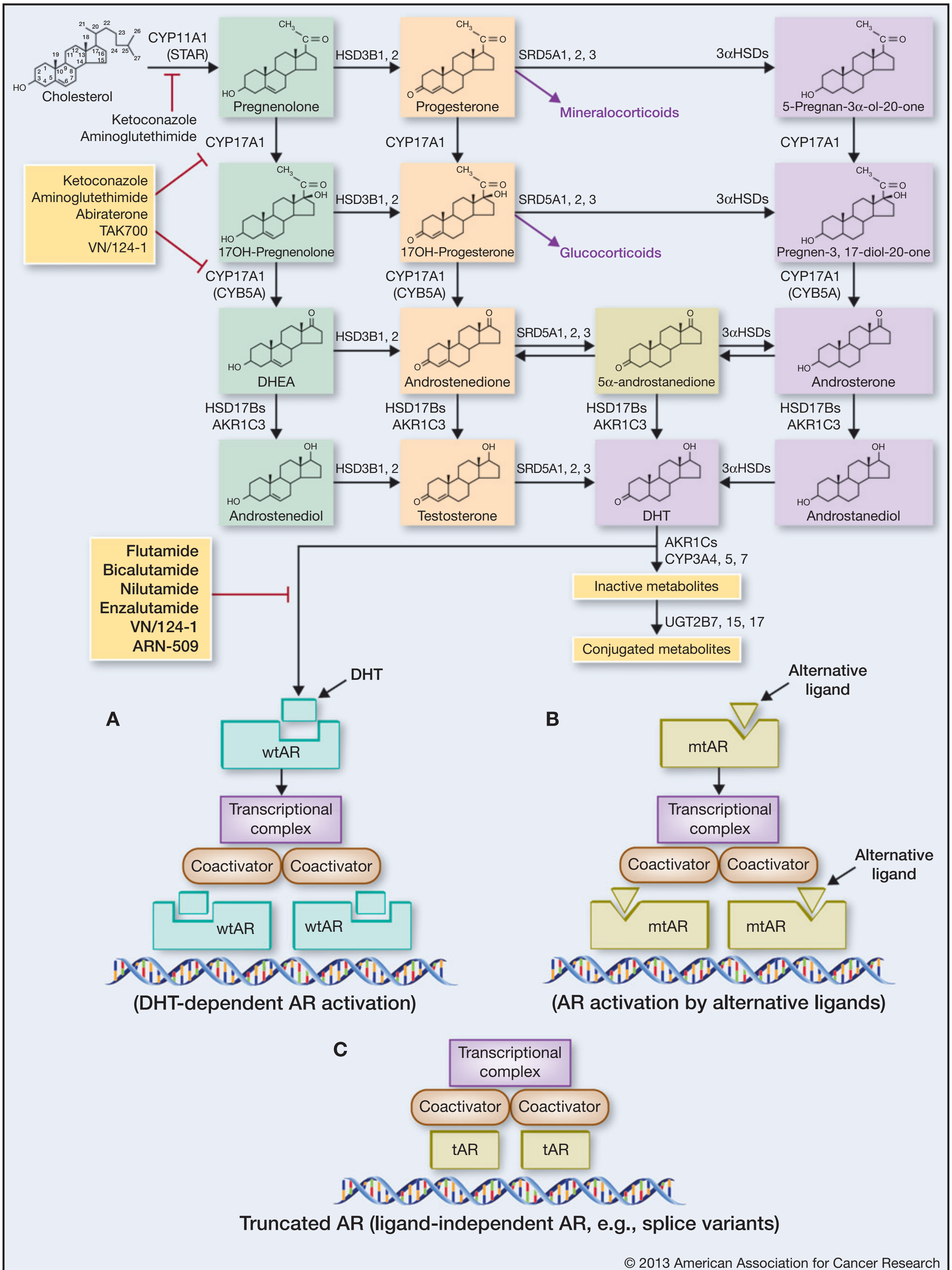

Cancer Research Reviews

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3. Expression of AR variants that lack the LBD and can signal in a ligand-independent, constitutively active manner (17–18);
4. Stoichiometric and qualitative changes in the coregulatory components of the AR complex, including the AR coactivators and corepressors that modulate the transcriptional response. For example, all three steroid receptor coactivators (SRC) of the p160 family (SRC-1, SRC-2, and SRC-3) have been reported to be overexpressed in prostate cancer and this is linked to inferior clinical outcomes (1, 19–21). In particular for SRC-2, encoded by *NCOA2*, gene amplifications and point mutations have been detected in prostate cancer and are associated with increased AR transcriptional activity (22); and
5. Activation of the AR complex via cross-talk with other signaling pathways, such as HER2, insulin-like growth factor-1 receptor, Src, and Akt pathways that phosphorylate AR or its coactivators (1, 21, 23–26).

These mechanisms are not mutually exclusive, and several of them may operate simultaneously in CRPC cells and synergistically enhance AR transcriptional output (1).

Collectively, these findings support the role of the AR axis as an important therapeutic target in CRPC and led to the development of novel second-line endocrine therapies for prostate cancer. The CYP17 enzymatic inhibitor abiraterone prolonged median overall survival in men with chemotherapy-refractory CRPC by 3.9 months (3) and also showed clinical activity in chemotherapy-naïve CRPC (27), thus validating the importance of residual intratumoral androgens in CRPC pathophysiology. The second-generation AR antagonist enzalutamide (MDV3100; ref. 4), which was rationally designed to overcome the antagonist-to-agonist conversion of first-generation AR antagonists (28), prolonged median overall survival in men with chemotherapy-refractory CRPC by 4.8 months (4) and is currently being tested in chemotherapy-naïve CRPC.

In Situ Steroid Synthesis and Metabolism in CRPC

Several groups have reported that, compared with primary prostate cancers or normal prostate tissue, CRPC exhibits increased expression of enzymes involved in androgen synthesis and substantial tissue androgen levels that should be sufficient to stimulate AR (and may be even higher than levels present within primary prostate cancers from untreated eugonadal men; refs. 5–11). Collectively, these findings raise the hypothesis that, despite peripheral castration, prostate cancer cells may never (yet) encounter a completely androgen-free local milieu, and that the term "androgen deprivation therapy" that is frequently used to describe first-line therapy with GnRH analogues or orchiectomy may actually be a misnomer (at least at the level of the tumor microenvironment). The clinical efficacy of the CYP17 inhibitor abiraterone validates this hypothesis.

In a recent comprehensive integrated oncogenomic analysis of primary and metastatic prostate cancer specimens, we documented aberrant expression of enzymes involved in androgen synthesis and metabolism. As a group, metastatic prostate cancers expressed higher average transcript levels for *AR* and several steroidogenic enzymes, including *SRD5A1*, *SRD5A3*, and *AKR1C3*, whereas expression of *SRD5A2*, *CYP3A4*, *CYP3A5*, and *CYP3A7* was decreased (11). The latter three enzymes are involved in phase I of dihydrotestosterone (DHT) inactivation (oxidation), and their decreased expression is predicted to increase *in situ* androgen levels and enhance AR activation. More importantly, we detected high intertumor variability of expression of individual enzyme transcripts in primary and metastatic prostate cancers, raising the hypothesis that within each individual tumor, enhanced activation of the androgen axis may occur at the level of a different enzyme, but with a common end result, i.e., increased tissue androgen levels and stimulation of AR (11).

These data thus highlight the androgen synthesis pathway *en block* as a mechanism of CRPC resistance to androgen deprivation therapy and can help explain the significant variability between lists of upregulated enzymes found in prior studies (5, 9, 29). One related question has been whether CRPC metastatic sites express the complete panel of enzymes necessary for *de novo* steroidogenesis using cholesterol as a precursor. Although it is generally accepted that prostate cancers can locally convert adrenal precursors to more active androgens (testosterone and DHT) via their own *AKR1C3* and 5α-reductase (*SRD5A1*; 29), it has been proposed that they lack adequate CYP17A1 expression, and as a result, they remain dependent on the contribution of adrenal precursors (as a two-site, "adrenal-prostate cancer" steroidogenic unit; ref. 29). In our study, we found that this is the case for the majority, but not all, prostate cancers. A small subset of prostate cancers overexpresses *CYP11A1*, *CYP17A1*, 3β-hydroxysteroid dehydrogenase (*HSD3B1*), *HSD3B2*, and *STAR* (which are necessary for the conversion of cholesterol to androstenedione), and thus may be self-sufficient for *de novo* steroidogenesis (11).

Mechanisms of Dysregulated Androgen Metabolism in Prostate Cancer: Does Acute Adaptation Precede (and Allow for) Clonal Selection?

The mechanism(s) underlying this aberrant expression in CRPC of transcripts involved in androgen metabolism remain (s) to be fully characterized. Obviously, the full elucidation of

Figure 1. Mechanisms of persistent AR transcriptional activity in CRPC cells and target sites of therapeutic agents: extragonadal (adrenal and/or intratumoral) steroidogenesis can serve as a source of residual intratumoral androgens (A). AR overexpression (frequently due to *AR* gene amplification) and/or AR LBD mutations (B) can increase sensitivity to low androgen levels and/or broaden ligand specificity, leading to promiscuous activation with alternative ligands. Constitutively active AR variants lacking the LBD, for example, alternatively spliced variants, can signal in a ligand-independent manner (C). Other mechanisms include changes in expression and posttranslational modification of AR coactivators and corepressors and enhanced activation of the AR complex via cross-talk with other growth and survival pathways (e.g., kinases/phosphatases, acetyltransferases/deacetylases, etc). mtAR, mutant AR (LBD mutation); tAR, truncated AR (constitutively active); wtAR, wild-type AR (full length).

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these mechanisms could reveal novel therapeutic targets for inhibition of the AR axis in CRPC. In our study of integrated gene expression and comparative genomic hybridization datasets, these aberrant expression patterns were only rarely associated with respective copy-number alterations (CNA; ref. 11). On the contrary, AR overexpression was, in agreement with previous studies (13), frequently associated with *AR* gene amplification, an event that possibly would require a process of clonal selection. In the absence of frequent CNAs, we examined whether the dysregulation of androgen metabolism enzymes occurs at the mRNA level (11). Expression of several enzyme transcripts, in particular of the *AKR1C* family, is induced by androgen deprivation (11, 30) within a timeframe (24–48 hours) that is too fast for clonal selection. Conversely, androgen treatment can suppress the expression of steroidogenic enzymes. The finding that androgen deprivation rapidly upregulates the mRNA levels of *AKR1C3*, an enzyme that can convert androstenedione to testosterone, raises the hypothesis that androgen deprivation triggers an acute adaptation feedback loop that enhances the ability of the prostate cancer cell to metabolize adrenal precursors into testosterone and DHT, thus sustaining tissue androgen levels and AR stimulation.

This hypothesis is also consistent with studies of neoadjuvant medical castration therapy in men with localized prostate cancer that exhibited suboptimal suppression of intratumoral androgens (by only about 70%–80%, in contrast with the >90% concomitant reduction in serum androgens) and AR-dependent gene expression (31–32), suboptimal induction of prostate cancer apoptosis (33), and disappointingly low rates of pathologic complete response (<3%; ref. 34). Once again, the term "androgen-deprivation therapy" may be misleading, as it overestimates the impact of the systemic treatment on androgen levels and the AR axis within the tumor microenvironment (5–10). Instead, a noncommittal term such as "medical castration therapy" would be more appropriate.

Combination-Based Endocrine Therapy for Prostate Cancer: Finally Moving Beyond Proof-of-Concept

The appreciation of the importance of extragonadal contributions to AR signaling in prostate cancer is not recent. Second- and third-line hormonal manipulations have previously been used in CRPC, yielding small successes that provided proof-of-principle. Efforts to suppress adrenal steroids by surgical adrenalectomy and hypophysectomy date back 5 decades with anecdotal responses reported. Glucocorticoids, via suppression of adrenocorticotropic hormone and adrenal androgen synthesis, also have documented activity in CRPC with reported prostate-specific antigen (PSA) responses as high as approximately 60% in small phase II studies. Chemical adrenalectomy with aminoglutethimide or ketoconazole has provided PSA responses, but a survival benefit was never formally shown. The concept of adding an antiandrogen to gonadal suppression for "combined androgen blockade" or "maximal androgen blockade" has been proposed before (35). However, clinical trials conducted in the pre-abiraterone/pre-enzalutamide era had failed to show a consistent, clinically meaningful benefit from the addition of a first-generation antiandrogen (or any other second-line hormonal agent) to gonadal suppression (a meta-analysis of 27 randomized trials including a total of 8,275 patients revealed, at best, a nonsignificant gain of 1.8% in 5-year survival; ref. 36). Similarly, despite a very strong preclinical rationale, a use for 5α-reductase inhibitors has not yet been found in CRPC (although the combination of dutasteride with ketoconazole and hydrocortisone provided promising PSA responses in a recent study; ref. 37).

So why did abiraterone and enzalutamide achieve a survival benefit, whereas earlier second-line hormonal approaches did not? This could be because of more effective targeting of androgen synthesis and AR LBD, respectively, with fewer side effects. Abiraterone is a more specific and better-tolerated inhibitor of steroidogenesis than aminoglutethimide and ketoconazole. Enzalutamide seems to lack the AR agonistic effects of first-generation antiandrogens. With these advanced therapeutic agents in our armamentarium now, and with a galvanized interest in the AR axis as a therapeutic target in prostate cancer, the next steps will be to optimize their use for maximal therapeutic benefit. For example, the best timing, setting, and sequence for these novel agents to maximize clinical benefit and minimize the risk of cross-resistance remain to be defined.

The "Androgenic Set Point" of Prostate Cancer Cells and Maintenance of Cell Survival

In studies of neoadjuvant medical castration therapy, the probability of early biochemical recurrence was inversely correlated with the degree of pathologic effect (38) and positively correlated with the residual expression of AR-dependent genes, including PSA, identified as early as 3 months after the initiation of hormonal therapy (39). Therefore, failure to adequately suppress the AR axis (or early reactivation of AR signaling) is associated with inferior clinical outcomes.

Short-term (24–48 hours) exposure of prostate cancer cells to androgen-depleted medium *in vitro* stimulates the expression of steroidogenic enzymes (11, 30), AR itself (30, 40), and its coactivators (19, 41). In the case of the *AR* gene, it has been found that agonist-bound AR protein negatively regulates gene transcription via recruitment of the histone demethylase and transcriptional corepressor lysine-specific demethylase 1 (LSD1) to a highly conserved site in the second *AR* gene intron (30). Similar LSD1-dependent mechanisms have been reported for the rapid androgen-mediated downregulation of the steroidogenic enzyme AKR1C3 by agonist-bound AR (30). Collectively, these data suggest that agonist-bound AR directly mediates a physiologic intracellular feedback loop to negatively regulate AR axis activity. Conversely, androgen withdrawal is proposed to trigger an acute adaptive response as a preprogrammed attempt of the prostate cancer cells to retain AR transcriptional activity, restoring it toward a predetermined "androgenic set point," which is critical for their survival. According to this hypothesis, these adaptive cellular responses would allow a sizeable pool of prostate cancer cells to maintain adequate intratumoral androgen levels and AR axis activity and survive, despite peripheral castrate androgen levels, eventually leading to the emergence of castration-resistant disease (possibly after later acquiring clonal genetic lesions, such as *AR* gene amplification).

Therefore, as current approaches achieve a suboptimal inhibition of AR signaling output, a more comprehensive AR axis targeting at multiple levels (androgen synthesis, metabolism, and action) and at all relevant sites (gonadal, adrenal, intratumoral) simultaneously at the time of initiation of endocrine therapy, deserves examination in clinical trials to explore whether it can improve patient outcomes over the current treatment paradigm of sequentially adding one agent at a time and only after disease progression.

Personal Opinion: What Will the Landscape of Advanced Prostate Cancer Treatment Be in the Next 5 Years?

The road map to comprehensive AR axis targeting

The above concepts lay the framework for new directions in the treatment of advanced prostate cancer. Both CYP17 inhibitors and second-generation AR antagonists are being tested in pre-CRPC disease states (hormone-naïve advanced prostate cancer and in the neoadjuvant setting), and it is reasonable to anticipate that they will be active there as well, by enhancing the efficacy of conventional medical castration therapy. In addition, the consecutive use of these agents, including identifying the optimal sequencing approach to augment clinical benefit and minimize the risk of cross-resistance, remains to be established by clinical evidence (so far, the phase III clinical trials of both abiraterone and enzalutamide have excluded patients previously treated with the other agent).

It is my personal opinion that the most promising approach would be the early application of combination systemic therapy at the initiation of medical castration rather than after the onset of CRPC. Combinations of both classes of agents with GnRH analogs are being explored in clinical trials, and I am optimistic that as part of a frontline comprehensive AR axis-targeting approach, they could move us closer to our goal of a completely androgen-free prostate cancer microenvironment. Further toward that goal, additional steroidogenic enzymes are being explored as therapeutic targets: inhibitors of AKR1C3 (42–45), *HSD3B1* and *HSD3B2*, and *SRD5A1* would also be interesting choices to be included in future clinical trials of multidrug combination regimens aiming at maximal frontline inhibition of the AR axis to augment prostate cancer cell apoptosis and deplete the pool of surviving prostate cancer cells that can later accumulate additional genetic events and resurge as CRPC.

Additional future directions

Several other CYP17 inhibitors (TAK-700/orteronel) and AR antagonists (ARN-509) are in clinical development. Galeterone (TOK-001 or VN/124-1) has been reported to be a CYP17 inhibitor, an AR antagonist, and also to promote AR degradation. Additional exciting opportunities have emerged in the field of combining AR axis–targeting agents with inhibitors of other oncogenic signaling pathways. For example, recent data suggest that AR inhibition derepresses phosphoinositide 3-kinase/Akt signaling (46), thus providing a strong rationale for combinations of inhibitors of both pathways.

Emerging technical developments can also address present needs in this field: Accurate, standardized, inexpensive assays with a reasonably rapid turnaround time for measurement of intratumoral steroids and AR signaling output (e.g., in biopsy material) will help select patients and guide therapy with these novel agents. Furthermore, interpatient variations in intracrine steroid signaling can be evaluated by noninvasive, real-time monitoring of the expression of AR (full-length and alternatively spliced constitutively active variants) and steroidogenic enzymes in circulating tumor cells (CTC; ref. 11) and serve as potential basis for individualized therapy.

Unanswered questions

The advances in the field of AR targeting in prostate cancer have opened new opportunities and, simultaneously, generated new questions. Despite their documented clinical activity, neither CYP17 inhibitors (abiraterone) nor second-generation AR antagonists (enzalutamide) are curative as single agents (at least in the clinical states in which they have been tested so far), and refractory disease eventually develops. Although the mechanisms of resistance (*de novo* or acquired) to these novel agents are not yet fully studied, it is of critical significance that these refractory tumors usually continue to produce PSA (at least to some degree). Because PSA (*KLK3*) gene expression requires AR transcriptional activity, this preliminary observation (which needs to be supported by more comprehensive gene expression profiling analyses in the near future) suggests that the AR axis remains active (at least to some degree) even after treatment with these novel AR-targeting agents. In the case of abiraterone, preclinical models of resistance have suggested that abiraterone treatment upregulates the expression of AR (both full-length and splice variants; ref. 47) and several steroidogenic enzymes, including *CYP17A1* (abiraterone's own target) and *AKR1C3* (47–48), and promotes *in situ* accumulation of pregnenolone and progesterone (that are synthesized upstream of CYP17; ref. 48). Pregnenolone and progesterone are putative AR agonists (especially in the case of AR harboring certain LBD mutations; ref. 48). These putative mechanisms of resistance to CYP17 inhibitors, which await confirmation in clinical specimens, suggest that despite treatment with these agents, some intratumoral steroids persist and may be available to drive AR activity. This lends further rationale to combinations of CYP17 inhibitors with other enzymatic inhibitors (e.g., of AKR1C3) or with potent second-generation AR antagonists (e.g., enzalutamide). The constitutively active AR splice variants have been proposed as mechanisms of resistance to both abiraterone (47) and enzalutamide (49) and will need to be addressed in the future with a new treatment paradigm.

Other important questions in this field include the following:

- What are the mechanisms underlying the feedback loops that lead to the increased expression of steroidogenic enzymes in CRPC? What other transcription factors, beyond

AR, control this process, and can they possibly serve as therapeutic targets in the future?
- What is the role of the bone microenvironment in *in situ* steroidogenesis in prostate cancer metastasis? Steroidogenic enzyme expression has been reported in the epithelial compartment (carcinoma cells), via immunohistochemistry in tissues (1) and quantitative real-time PCR in CTCs (11), implying capacity for cell-autonomous steroidogenesis and intracrine signaling. However, steroidogenic enzyme expression has also been reported in the stroma, suggesting that (at least some) enzymatic reactions may occur outside the prostate cancer cell, in a two-compartment model involving paracrine signaling. Moreover, can the bone microenvironment promote steroidogenesis in prostate cancer cells at the metastatic site? Conversely, what is the impact of high local tissue androgen levels on bone remodeling in prostate cancer bone metastasis?
- What is the role of other steroids (e.g., progesterone, mineralocorticoids, glucocorticoids) and their nuclear receptors (PR, MR, GR, respectively) in CRPC?
- Although generally the importance of the overexpression of steroidogenic enzymes for CRPC is attributed to increased androgen synthesis, what other, nonendocrine roles may these enzymes play? For example, several of these enzymes may have roles in drug detoxification, prostaglandin metabolism, N-linked glycosylation, etc.

Conclusions

On the basis of the clinical activity of CYP17 inhibitors and enzalutamide, AR is now a validated therapeutic target in CRPC. Still, however, current treatment approaches do not achieve complete inhibition of AR signaling in prostate cancer because they do not address all aspects of this axis (androgen synthesis, metabolism, and action) and all relevant sites (gonadal, adrenal, intratumoral) early enough in the course of hormonal therapy. Failure to adequately and comprehensively inhibit the AR axis may allow prostate cancer cells to survive, adapt, and evolve into CRPC. There is a need and opportunity to explore in clinical trials a new paradigm in the management of advanced prostate cancer: first-line regimens based on combinations of new-generation inhibitors of all these AR axis components.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Mitsiades N, Chen Y, Scher HI. The AR axis as a pathogenetic mechanism and therapeutic target throughout the clinical states of prostate cancer: Opportunities for second-line hormonal manipulations in castration-resistant prostate cancer. In: Scardino PT, Linehan WM, Zelefsky MJ, editors. Comprehensive textbook of genitourinary oncology. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2011. p. 262–73.
2. Huggins C, Hodges CV. Studies on prostatic cancer: I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1941;1: 293–7.
3. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995–2005.
4. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367:1187–97.
5. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 2006;66:2815–25.
6. Geller J, Albert JD, Nachtsheim DA, Loza D. Comparison of prostatic cancer tissue dihydrotestosterone levels at the time of relapse following orchiectomy or estrogen therapy. J Urol 1984;132:693–6.
7. Mohler JL, Gregory CW, Ford OH III, Kim D, Weaver CM, Petrusz P, et al. The androgen axis in recurrent prostate cancer. Clin Cancer Res 2004;10:440–8.
8. Gregory CW, Johnson RT Jr, Mohler JL, French FS, Wilson EM. Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res 2001;61: 2892–8.
9. Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 2008;68:4447–54.
10. Holzbeierlein J, Lal P, LaTulippe E, Smith A, Satagopan J, Zhang L, et al. Gene expression analysis of human prostate carcinoma during hormonal therapy identifies androgen-responsive genes and mechanisms of therapy resistance. Am J Pathol 2004;164: 217–27.
11. Mitsiades N, Sung CC, Schultz N, Danila DC, He B, Eedunuri VK, et al. Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. Cancer Res 2012;72:6142–52.
12. Hobisch A, Culig Z, Radmayr C, Bartsch G, Klocker H, Hittmair A. Distant metastases from prostatic carcinoma express androgen receptor protein. Cancer Res 1995;55:3068–72.
13. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, et al. *In vivo* amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 1995; 9:401–6.
14. Newmark JR, Hardy DO, Tonb DC, Carter BS, Epstein JI, Isaacs WB, et al. Androgen receptor gene mutations in human prostate cancer. Proc Natl Acad Sci U S A 1992;89:6319–23.

Published OnlineFirst July 25, 2013; DOI: 10.1158/0008-5472.CAN-12-4414

15. Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med 1995;332:1393–8.
16. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004;10:33–9.
17. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. Cancer Res 2008;68:5469–77.
18. Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res 2009;69:16–22.
19. Agoulnik IU, Vaid A, Nakka M, Alvarado M, Bingman WE III, Erdem H, et al. Androgens modulate expression of transcription intermediary factor 2, an androgen receptor coactivator whose expression level correlates with early biochemical recurrence in prostate cancer. Cancer Res 2006;66:10594–602.
20. Agoulnik IU, Vaid A, Bingman WE III, Erdeme H, Frolov A, Smith CL, et al. Role of SRC-1 in the promotion of prostate cancer cell growth and tumor progression. Cancer Res 2005;65:7959–67.
21. Xu J, Wu RC, O'Malley BW. Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family. Nat Rev Cancer 2009;9:615–30.
22. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22.
23. Mellinghoff IK, Vivanco I, Kwon A, Tran C, Wongvipat J, Sawyers CL. HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. Cancer Cell 2004;6:517–27.
24. Yeh S, Lin HK, Kang HY, Thin TH, Lin MF, Chang C. From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. Proc Natl Acad Sci U S A 1999;96:5458–63.
25. Mahajan NP, Liu Y, Majumder S, Warren MR, Parker CE, Mohler JL, et al. Activated Cdc42-associated kinase Ack1 promotes prostate cancer progression via androgen receptor tyrosine phosphorylation. Proc Natl Acad Sci U S A 2007;104:8438–43.
26. Liu Y, Karaca M, Zhang Z, Gioeli D, Earp HS, Whang YE. Dasatinib inhibits site-specific tyrosine phosphorylation of androgen receptor by Ack1 and Src kinases. Oncogene 2010;29:3208–16.
27. Ryan CJ, Smith MR, De Bono JS, Molina A, Logothetis C, De Souza PL, et al. Interim analysis (IA) results of COU-AA-302, a randomized, phase III study of abiraterone acetate (AA) in chemotherapy-naive patients (pts) with metastatic castration-resistant prostate cancer (mCRPC). J Clin Oncol 30, 2012 (suppl; abstr LBA4518).
28. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science 2009;324:787–90.
29. Hofland J, van Weerden WM, Dits NF, Steenbergen J, van Leenders GJ, Jenster G, et al. Evidence of limited contributions for intratumoral steroidogenesis in prostate cancer. Cancer Res 2010;70:1256–64.
30. Cai C, He HH, Chen S, Coleman I, Wang H, Fang Z, et al. Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1. Cancer Cell 2011;20:457–71.
31. Mostaghel EA, Page ST, Lin DW, Fazli L, Coleman IM, True LD, et al. Intraprostatic androgens and androgen-regulated gene expression persist after testosterone suppression: therapeutic implications for castration-resistant prostate cancer. Cancer Res 2007;67:5033–41.
32. Nishiyama T, Hashimoto Y, Takahashi K. The influence of androgen deprivation therapy on dihydrotestosterone levels in the prostatic tissue of patients with prostate cancer. Clin Cancer Res 2004;10:7121–6.
33. Ohlson N, Wikstrom P, Stattin P, Bergh A. Cell proliferation and apoptosis in prostate tumors and adjacent non-malignant prostate tissue in patients at different time-points after castration treatment. Prostate 2005;62:307–15.
34. Gleave ME, Goldenberg SL, Chin JL, Warner J, Saad F, Klotz LH, et al. Randomized comparative study of 3 versus 8-month neoadjuvant hormonal therapy before radical prostatectomy: biochemical and pathological effects. J Urol 2001;166:500–6.
35. Labrie F, Dupont A, Belanger A, Lefebvre FA, Cusan L, Raynaud JP, et al. New hormonal therapy in prostate cancer: combined use of a pure antiandrogen and an LHRH agonist. Horm Res 1983;18:18–27.
36. Maximum androgen blockade in advanced prostate cancer: an overview of the randomised trials. Prostate Cancer Trialists' Collaborative Group. Lancet 2000;355:1491–8.
37. Taplin ME, Regan MM, Ko YJ, Bubley GJ, Duggan SE, Werner L, et al. Phase II study of androgen synthesis inhibition with ketoconazole, hydrocortisone, and dutasteride in asymptomatic castration-resistant prostate cancer. Clin Cancer Res 2009;15:7099–105.
38. Kitagawa Y, Koshida K, Mizokami A, Komatsu K, Nakashima S, Misaki T, et al. Pathological effects of neoadjuvant hormonal therapy help predict progression of prostate cancer after radical prostatectomy. Int J Urol 2003;10:377–82.
39. Ryan CJ, Smith A, Lal P, Satagopan J, Reuter V, Scardino P, et al. Persistent prostate-specific antigen expression after neoadjuvant androgen depletion: an early predictor of relapse or incomplete androgen suppression. Urology 2006;68:834–9.
40. Cai C, Wang H, Xu Y, Chen S, Balk SP. Reactivation of androgen receptor-regulated TMPRSS2:ERG gene expression in castration-resistant prostate cancer. Cancer Res 2009;69:6027–32.
41. Heemers HV, Regan KM, Schmidt LJ, Anderson SK, Ballman KV, Tindall DJ. Androgen modulation of coregulator expression in prostate cancer cells. Mol Endocrinol 2009;23:572–83.
42. Penning TM, Byrns MC. Steroid hormone transforming aldo-keto reductases and cancer. Ann N Y Acad Sci 2009;1155:33–42.
43. Byrns MC, Steckelbroeck S, Penning TM. An indomethacin analogue, N-(4-chlorobenzoyl)-melatonin, is a selective inhibitor of aldo-keto reductase 1C3 (type 2 3alpha-HSD, type 5 17beta-HSD, and prostaglandin F synthase), a potential target for the treatment of hormone dependent and hormone independent malignancies. Biochem Pharmacol 2008;75:484–93.
44. Byrns MC, Jin Y, Penning TM. Inhibitors of type 5 17beta-hydroxysteroid dehydrogenase (AKR1C3): overview and structural insights. J Steroid Biochem Mol Biol 2011;125:95–104.
45. Adeniji AO, Twenter BM, Byrns MC, Jin Y, Chen M, Winkler JD, et al. Development of potent and selective inhibitors of aldo-keto reductase 1C3 (type 5 17beta-hydroxysteroid dehydrogenase) based on N-phenyl-aminobenzoates and their structure-activity relationships. J Med Chem 2012;55:2311–23.
46. Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 2011;19:575–86.
47. Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, et al. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. Clin Cancer Res 2011;17:5913–25.
48. Cai C, Chen S, Ng P, Bubley GJ, Nelson PS, Mostaghel EA, et al. Intratumoral *de novo* steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. Cancer Res 2011;71:6503–13.
49. Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. Cancer Res 2013;73:483–9.

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