Understanding the Mechanisms of Androgen Deprivation Resistance in Prostate Cancer at the Molecular Level

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Abstract

Context: Various molecular mechanisms play a role in the development of resistance to androgen deprivation therapy in castration-resistant prostate cancer (CRPC).

Objective: To understand the mechanisms and biological pathways associated with the progression of prostate cancer (PCa) under systemic androgen depletion or administration of the novel antiandrogens abiraterone, enzalutamide, and ARN-509. This review also examines the introduction of novel combinational approaches for patients with CRPC.

Evidence acquisition: PubMed was the data source. Keywords for the search were castrate resistant prostate cancer, abiraterone, enzalutamide resistance mechanisms, resistance to androgen deprivation, AR mutations, amplifications, splice variants, and AR alterations. Papers published before 1990 were excluded from the review, and only English-language papers were included.

Evidence synthesis: This review summarizes the current literature regarding the mechanisms implicated in the development of CRPC and the acquisition of resistance to novel antiandrogen axis agents. The review focuses on androgen biosynthesis in the tumor microenvironment, androgen receptor (AR) alterations and post-transcriptional modifications, the role of glucocorticoid receptor, and alternative oncogenic signaling that is derepressed on maximum AR inhibition and thus promotes cancer survival and progression.

Conclusions: The mechanisms implicated in the development of resistance to AR inhibition in PCa are multiple and complex, involving virtually all classes of genomic alteration and leading to a host of selective/adaptive responses. Combinational therapeutic approaches targeting both AR signaling and alternative oncogenic pathways may be reasonable for patients with CRPC.

Patient summary: We looked for mechanisms related to the progression of PCa in patients undergoing hormonal therapy and treatment with novel drugs targeting the AR. Based on recent data, combining maximal AR inhibition with novel agents targeting other tumor-compensatory, non–AR-related pathways may improve the survival and quality of life of patients with castration-resistant PCa.

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1. Introduction

Prostate cancer (PCa) remains the second leading cause of death due to cancer in Western societies [1]. Usually PCa is diagnosed as localized disease, and its management includes surveillance or radical prostatectomy, radiation therapy, or even combination approaches such as hormonal therapy prior to prostatectomy. Few patients undergoing PCa screening present with metastatic disease, which is also present in \( \geq 10\% \) of unscreened populations at first presentation—usually in bone, the predominant site of advanced and lethal PCa [2]—highlighting critical differences between screened and unscreened populations.

Despite the obvious increase in overall survival of patients with PCa over the past decade, recent data indicate that improvement of survival among patients with metastatic PCa has not significantly contributed to this decline in mortality [3]. Patients with metastatic disease usually receive hormonal therapy that decreases the production of testosterone by the testes. However, after an initial response, which varies significantly among patients, the disease eventually progresses despite the low levels of testosterone in the systemic circulation (<20 ng/dl) [4]. This stage of disease is known as metastatic castration-resistant PCa (mCRPC); the average overall survival is 1.5 yr, with significant variability among patients with lymph node metastasis, bone metastasis, and metastasis in both lymph nodes and bone [3]. Docetaxel and cabazitaxel are the only chemotherapy regimens approved for this stage of the disease [5,6]. Radium Ra 223 (Xofigo) injection was recently approved for the treatment of patients with castration-resistant PCa (CRPC), symptomatic bone metastases, and no known visceral metastatic disease [7].

Numerous studies during the last decade have highlighted the role of androgen receptor (AR) in the development of mCRPC, showing that despite systemic androgen depletion, AR signaling remains active and supports the survival and growth of PCa cells. Based on these results, two novel agents have been recently evaluated in clinical trials: abiraterone acetate (AA), an inhibitor of androgen synthesis, and enzalutamide, a potent antiandrogen.

AA is a CYP17A1 inhibitor blocking the production of androgens in the testes, adrenal glands, and tumor microenvironment by inhibiting both 17α-hydroxylase and 17,20 lyase activities of the CYP17A1 enzyme [8]. AA has been recently approved for chemotherapy-naive patients with mCRPC, improving overall survival by 4 mo [9].

Enzalutamide is a novel antagonist of AR, inhibiting nuclear translocation, chromatin binding, and interactions with AR coregulators [10]. Enzalutamide prolongs the survival of patients who failed chemotherapy [11], and more recent data suggest that in chemotherapy-naive mCRPC patients, enzalutamide increases overall and progression-free survival and delays the need for chemotherapy [12].

ARN-509, a next-generation antiandrogen, was found to be more effective than enzalutamide in CRPC preclinical models in terms of tumor growth [13]. According to a recent phase 1 clinical trial, ARN-509 is safe and well tolerated and displays dose-proportional pharmacokinetics demonstrating pharmacodynamic and antitumor activity across all dose levels examined [14].

Despite the significant advances in the targeting of AR that have been translated into survival improvement for patients with mCRPC, this stage of disease remains incurable and is associated with significant morbidity and mortality [15]. While the introduction of novel antiandrogens has provided survival benefits through tumor growth inhibition, two critical clinical concerns arise: (1) Which patients really benefit from these agents, and which biomarkers can be used to identify these patients? (2) What alternative approaches can be used if the disease progresses during treatment with these agents?

The aim of this review is to summarize the recent advances in the evaluation of the multiple levels of development of resistance to androgen deprivation and AR inhibition that occur before and after the introduction of novel antiandrogen axis agents. In PCa, the heterogeneity of the diversity of pathways involved demands a critical consideration of numerous possible explanations of these biological events.

2. Evidence acquisition

PubMed was the data source. Keywords for the search were castrate resistant prostate cancer, abiraterone, enzalutamide resistance mechanisms, resistance to androgen deprivation, AR mutations, amplifications, splice variants, and AR alterations. Papers published before 1990 were excluded from the review, and only English-language papers were included.

3. Evidence synthesis

3.1. Paracrine/autocrine androgen synthesis as a mechanism of resistance to systemic hormonal therapy and novel inhibitors of androgen biosynthesis

It is well documented that in normal prostate tissue and low-grade PCa, the prostate stroma secretes active androgens (eg, dihydrotestosterone [DHT]) and other growth factors supporting the survival and proliferation of overlying epithelium by a paracrine loop [16]. During PCa progression, this paracrine dependence is lost and converted to an autocrine phase in which cancer cells produce numerous factors, including androgens, supporting their own growth and survival [16]. Although circulating androgens are initially the driver of disease progression, which explains the initial response of metastatic disease to systemic androgen depletion, this treatment selects for cancer cells capable of surviving and growing by virtue of a variety of paracrine and autocrine mechanisms resulting in AR activation despite the reduced systemic testosterone below castration levels (<20 ng/dl).

Montgomery et al found that intratumoral levels of testosterone and DHT are higher in mCRPC compared with primary localized disease derived from untreated patients [17]. The intratumoral testosterone levels in patients with mCRPC were found to be in a range known to stimulate AR and promote PCa growth and proliferation [17]. Chang et al
showed that in mCRPC, androstenedione is converted to 5α-androstenedione, which is finally converted to DHT, leading to PCa progression despite systemic androgen depletion [18]. The authors showed that the expression of key enzymes required for the metabolism of progestins to adrenal androgens and their subsequent conversion to testosterone, such as HSD3B1 and CYP17A1, was significantly higher in mCRPC compared with primary tumors [18]. Recently, Chang et al found that CRPCs harbor a gain-of-stability N367T variant in the gene encoding for the 3β-hydroxysteroid dehydrogenase type 1 enzyme, which catalyzes the conversion of dehydroepiandrosterone to DHT, which then accumulates and activates AR despite systemic androgen deprivation [19].

It is important to note that CYP17A1, which mediates the synthesis of 17-OH progesterone and 17-OH pregnenolone from progesterone and pregnenolone, respectively, and promotes the synthesis of androgen mediators, was found to be upregulated in mCRPC. This finding led to the introduction of AA, which suppresses testosterone concentrations in the blood and tumor microenvironment to levels lower than picograms per milliliter; increased nuclear localization of AR and induced CYP17A1 are predictors of good response to this agent [20].

Increased copy numbers of genes encoding enzymes implicated in testosterone synthesis, such as hydroxysteroid (17-beta) dehydrogenase 3 (HSD17B3), and decreased copy numbers of genes encoding enzymes promoting the conversion of testosterone to the less active androstenedione have been reported in mCRPC [21]. In a recent report, Ishizaki et al demonstrated that androgen deprivation leads to upregulation of HSD17B6, which was associated with increased incidence of biochemical recurrence [22]. HSD17B6 catalyzes the conversion of androgen metabolites such as 5α-androstane-3α/β,17β-diol (3α/β-diol) to dihydrotestosterone, and human PCa xenografts produce this metabolite from acetate and cholesterol [23]. Lee et al found that increased cholesterol synthesis through downregulation of ABCA1 is associated with increased PCa aggressiveness [24]. Thus, the combination of androgen deprivation and cholesterol synthesis inhibition may be a reasonable approach for some mCRPC.

Collectively, on systemic androgen depletion, metastatic PCa shifts from an endocrine-driven to a paracrine/autocrine-driven disease in which the local tumor microenvironment and cancer cells produce androgens leading to persistent AR activation or the cancer cells become less dependent on androgen ligand altogether. The paracrine and autocrine androgen biosynthesis pathways associated with the development of CRPC are summarized in Figure 1.

Given the critical role of CYP17A1 in the paracrine androgen biosynthesis, novel inhibitors targeting this
enzyme have been introduced in the clinical setting. AA inhibits the 17α-hydroxylase and 17,20 lyase activities of this enzyme, while orteronel (TAK700) inhibits mainly the 17,20 lyase activity of the enzyme (Fig. 1). It is interesting to note that treatment of castration-resistant VCaP xenografts with AA generated relapsed tumors with upregulated CYP17A1 [25], while LNCaP cells express a mutant form of AR (T877A) activated by progesterone, which is the product of CYP11A1 in the initial step of steroidogenesis, rendering AR sensitive to steroid metabolites produced upstream of CYP17A1 [26]. This concept could explain the failure of orteronel (TAK700), which affects mainly 17,20 lyase, to provide significant survival benefits in patients with mCRPC [27]. On the contrary, AA affects both 17,20 lyase activity and the upstream 17α-hydroxylase activity, decreasing the accumulation of steroid metabolites that can induce PCA growth through binding to AR (Fig. 1).

The transcriptional activity of AR has been found to be significantly altered in CRPC, and many of the androgen-regulated genes become upregulated during the progression of the disease to CRPC [28]; these findings support the idea that AR signaling remains active in this stage of disease. Molecular events implicated in the induction of AR signaling and their impact on the development of resistance to androgen depletion are critical and will be analyzed in the following sections of this paper.

3.2. AR mutations and resistance to androgen ablation and AR inhibition

AR mutations, which are exceedingly uncommon in primary, hormonally naive disease, have been reported to occur at a comparatively high incidence (>10%) in patients with CRPC, especially in tumors progressing under systemic hormonal therapy [29–32]. Grasso et al found that the AR gene (AR) is among the nine genes that are significantly mutated in mCRPC [33]. Of note, treatment with AR antagonists increases the incidence of mutations in the ligand-binding domain (LBD) of AR in metastatic PCa compared with hormonal therapy alone, and the T877A mutant is one of the most frequently observed variants [34]. This mutant broadens the ligand-binding specificity of AR, sensitizing it to other steroid hormones such as progesterone and estrogens [35,36] and, strikingly, to some antiandrogens that are converted to strong agonists [37].

The H874Y mutation, initially identified in CRPC tumors treated with flutamide [32], enhances the binding of AR coregulators and increases AR transcriptional activity through conformational change of AR protein [38]. AR in metastatic disease treated with AR antagonists carries mutations in the N-terminal domain, such as W435L, affecting its interaction with AR coregulators promoting androgen-independent AR activation [39]. Korpala et al [40] and Joseph et al [41] found that the F876L mutation of AR was present in all the enzalutamide and ARN-509 strongly resistant clones. This mutation switches the effect of these novel AR inhibitors from antagonistic to agonistic. Finally, circulating tumor DNA from patients with progressive disease under ARN-509 presented a higher incidence of F876L-encoding mutations [41]. These results suggest that much like mutations in other therapeutically targeted oncogenes, AR mutations provide a survival advantage to PCA cells and promote resistance to novel antiandrogens [42].

3.3. AR variants as a mechanism of resistance to androgen deprivation therapy and antiandrogens

The finding that CWR-22Rv1 cells express two different AR protein species at 112kDa and 75–80kDa while the smaller form lacked the AR LBD and was constitutively localized in the nucleus and remained active [43] suggested that variant forms of AR could be implicated in the development of resistance to hormonal therapy. AR species with a similar mobility to this AR isoform were frequently expressed in human PCA tissues [44], while increased levels of particular AR variants in prostatectomy samples are associated with increased incidence of relapse [45].

Androgen-dependent gene expression and cell growth are mediated by the full-length AR, while androgen-independent transcriptional activity and cell growth are attributed to COOH-terminal truncated LBD lacking AR variants [46–50]. Sun et al identified the presence of an AR variant lacking the exons 5, 6, and 7, which encode the LBD of the AR (ARv567es) in xenografts derived from mCRPC, after prolonged exposure to androgen deprivation therapy (ADT) [50]. This AR variant was found to be constitutively active in PCA cell lines promoting the expression and the activity of the full-length AR (ARf) [50]. The authors demonstrated that castration selected for ARv567es variant expression in PCA xenografts models, while this variant was found to be upregulated in mCRPC [50]. Finally, the ARv567es variant presented distinct transcriptional activity compared with the ARf [50]. Consistently, Hörnberg et al showed that ARv567es mRNA levels are higher in mCRPC compared with hormone-naive metastatic disease and were associated with higher nuclear AR and shorter survival [51].

It was shown that inhibition of AR by small interfering RNA or enzalutamide promotes the expression of the AR-V7 variant [52]. This particular AR variant is expressed at a higher level in mCRPC compared with hormone-naive metastatic disease and was found to be associated with disturbed cell cycle regulation and shorter survival [51]. It was also discovered that ARf mainly induces genes related to biosynthesis and metabolism, while AR-V7 promotes the expression of genes related to cell cycle progression, including the ubiquitin-conjugating enzyme E2C gene (UBE2C) [52]. These results were confirmed in vivo, since treatment of LuCaP35CR with abiraterone upregulated both AR and AR-V7 but the expression of only the latter was found to be associated with UBE2C induction [52].

It was also found that the expression of AR variants in CWR-22Rv1 cells is sufficient to promote growth under enzalutamide, while knockdown of the AR variant increases the sensitivity to AR inhibitors [53]. Nadiminty et al showed that NF-kB2/p52 promotes resistance to enzalutamide in LNCaP C4–2B and CWR-22Rv1 through upregulation of AR variants [54]. The authors found that knockdown of full-length AR and AR-V7 increased the sensitivity of these two
cell lines to enzalutamide [54]. Liu et al, using luciferase activity assay to determine the activity of AR-V7 after treatment with various compounds, demonstrated that niclosamide, an antihelminthic drug approved by the US Food and Drug Administration, significantly downregulated AR-V7 protein levels through protein degradation and decreased AR-V7 transcriptional activity [55]. The addition of niclosamide significantly enhances the activity of enzalutamide in vitro and in vivo in the androgen-insensitive C4–2B and CWR22Rv1 cells [55]. Finally, Antonarakis et al recently showed that the detection of AR-V7 mRNA in circulating tumor cells (CTCs) in patients with mCRPC predicts primary resistance to enzalutamide and abiraterone [56]. In particular, detectable AR-V7 mRNA from CTCs is associated with decreased progression-free survival and absence of prostate-specific antigen (PSA) response [56].

It should be noted that all current therapies for CRPC that target AR, such as enzalutamide and ARN-509, are dependent on the presence of LBD, which, as previously analyzed, is missing in numerous AR variants upregulated under novel antiandrogens. The majority of mutations related to the development of resistance to these agents, such as T877A, are located in the LBD. These conclusions led to the introduction of small molecules targeting the N-terminal AR domain, such as EPI-001, as promising novel therapeutic candidates [57].

3.4. Combinational approaches targeting AR

As previously analyzed, alterations of androgen levels in the tumor microenvironment and alterations of the AR gene and AR protein levels are implicated in the development of resistance to hormonal therapy and novel antiandrogens. AA decreases the testosterone levels in both the systemic circulation and the bone microenvironment [20], but soon after the initiation of treatment the AR copy number is increased, while enzalutamide is associated with increases of testosterone levels in both the systemic circulation and the bone microenvironment [58]. When 57 patients with mCRPC received AA and enzalutamide and were monitored every 4 wk, significant declines in PSA levels were observed in the majority of patients [58]. These results suggest that combinational therapy including an androgen synthesis inhibitor and an AR inhibitor may lead to more effective inhibition of AR signaling.

3.5. Post-translational alterations of AR and transcriptional activity: alternative oncogenic signaling implications

Tyrosine phosphorylation is significantly higher in hormone-resistant xenografts compared with their hormone-sensitive counterparts [59]; it also was associated with increased AR transcriptional activity and increased PCa cell growth under androgen depletion and clinical progression of PCa [59]. Ueda et al found that AR activation is induced by steroid receptor coactivator-1 (Src-1) and interleukin 6 (IL-6) in the absence of androgens, while inhibition of mitogen-activated protein kinase (MAPK) abrogated this effect [60]. Downregulation of Src-1 reduces PCa growth and transcription of AR target genes, while higher Src-1 expression in localized PCa is associated with higher Gleason score, extracapsular extension, and pelvic lymph node metastases [61]. IL-6 promotes resistance to bicalutamide, an AR inhibitor, through upregulation of the AR coactivator TIF2 [62], which has been related to increased incidence of biochemical recurrence after radical prostatectomy [63]. These results highlight the critical role of Src-1 and IL-6 in the regulation of AR at multiple levels and demonstrate that these effects are mediated by MAPK signaling.

Epidermal growth factor receptor (EGFR) and its dimerization partner HER2 have been implicated in activation of AR and promotion of PCa growth [64]. Mellinghoff et al found that knockdown of HER2 and not EGFR inhibits the AR transcriptional activity in LNCaP and LAPC4 cells, while HER2 and HER3 stabilize AR and increase its binding to androgen-responsive elements in the promoters of AR-regulated genes, such as PSA and KLK2 [65]. Chen et al found that androgen depletion increases HER2 and ERBB3, promoting AR stabilization and PSA production, while dual inhibition of HER2 and EGFR with trastuzumab and erlotinib abrogated these events, sensitized the LNCaP cells to androgen depletion, and showed synergistic effects with castration, decreasing the growth of the androgen-independent CWR22 PCa cells [66]. It is interesting to note that the inhibition of PI3K signaling by BEZ235 leads to increased AR protein levels and transcriptional activity through an induced HER2/HER3 pathway [67]. These data suggest that the inhibition of HER2/HER3 may sensitize PCa cells to androgen depletion, providing a rationale for combination therapy.

The loss of the tumor suppressor gene phosphatase and tensin homolog (PTEN) and activation of PI3K signaling take place in almost 70% of metastatic PCa [68]. Akt-mediated AR phosphorylation at the Ser 81 residue increases the interaction of AR with the transcriptional factor p300, inhibiting AR ubiquitination and degradation [69]. Also, deletion of p300 in a prostate-specific PTEN deletion transgenic animal model decreases the incidence of high-grade intraepithelial neoplasia and invasive cancer, thus increasing the survival of these mice [69]. Finally, high expression of p300 is critical for the androgen-dependent and androgen-independent transactivation of AR [70] and was correlated with higher AR protein levels in human PCa specimens [69]. The implications of alternative oncogenic pathways in the post-transcriptional activation of AR under low androgen levels are summarized in Figure 2.

3.6. The role of glucocorticoid receptor in the development of resistance to novel AR inhibitors

The glucocorticoid receptor (GR) has been implicated in cancer progression, while dexamethasone has been used in the treatment of CRPC associated with PSA response [71]. Glucocorticoids were found to inhibit lymphangiogenesis through vascular endothelial growth factor downregulation [71], while induction of GR led to inhibition of PCa cell proliferation associated with upregulation of p21 and p27 and downregulation of oncogenic molecules such as MAPKs, nuclear factor-κB, and STAT1 [72]. Sahu et al
evaluated the AR and GR target genes in different PCa cells and concluded that these two receptors present overlapping sets of gene targets, suggesting that GR may be implicated in the development of resistance to ADT [73].

Arora et al in a recent study treated LNCaP xenografts expressing wild-type AR (LNCaP/AR) with novel AR inhibitors, including enzalutamide, ARN-509, and RD162, until the tumors regressed [74]. The authors discovered that numerous common targets of AR and GR were upregulated in the resistant tumors, while GR mRNA and protein levels were found to be significantly higher in enzalutamide-resistant and ARN-509-resistant tumors [74]. Knockdown of GR in cells derived from resistant tumors retained the sensitivity to enzalutamide and dexamethasone-induced resistance to enzalutamide when administrated in VCaP cells [74], supporting the idea that GR upregulation promotes resistance to novel antiandrogens. The authors examined the expression of GR in metastatic PCa obtained from patients treated with enzalutamide and showed that poor responders had higher levels of GR 8 wk after the initiation of treatment compared with good responders at the same time point and compared with baseline levels [74]. It was also shown that in a subset of PCa cells, AR represses GR expression while AR inhibition leads to GR induction because of derepression of the gene [74]. This result was consistent with a previous report by Davies et al showing that the GR expression in the ventral rat prostate is increased after castration [75]. These conclusions highlight the role of GR in the development of resistance to novel antiandrogens despite the earlier evidence that GR exhibits a tumor suppressor role in PCa.

3.7. **AR inhibition derepresses numerous genes implicated in the activation of oncogenic signaling, promoting resistance to androgen deprivation therapy**

ADT-mediated derepression of GR signaling was discussed earlier in this paper. It could be hypothesized that androgen depletion or AR inhibition in general acts as a selective pressure favoring the survival of PCa cells, maintaining this negative feedback between AR and genes implicated in oncogenic signaling promoting cancer cell survival.

Cai et al showed that numerous genes implicated in DNA synthesis and repair, DNA metabolism, and cell cycle that are upregulated in CRPC xenografts are repressed by androgens [76]. Given that AR signaling is also upregulated in CRPC, the androgen levels at this stage of disease are adequate to induce AR-regulated genes but are not high enough to stimulate AR activity on suppressor elements and inhibit the expression of genes implicated in cell cycle and DNA metabolism [76]. Thus, the maximal androgen depletion and AR inhibition may further derepress these cell cycle and DNA synthesis genes in a subset of PCa, potentially leading to resistant phenotypes associated with activation of oncogenic and growth-promoting pathways very early during treatment.

AR has also been found to inhibit the phosphorylation of Akt in PTEN conditional knockout transgenic animal models and PCa cell lines such as LNCaP and LAPC4 [67,77]. This phenomenon was mainly attributed to the upregulation of FKBP5 and PHLPP, leading to decreased Akt phosphorylation by AR [67,77]. PTEN deletion and subsequent Akt activation were also found to decrease AR protein levels and transcriptional activity through HER3 signaling alteration [67]. Akt was also found to increase AR ubiquitination and degradation, providing another reasonable mechanism to explain this phenomenon [78]. Papamycin, a mammalian target of rapamycin (mTOR) inhibitor, led to increased tumor regression in PTEN knockout models when combined with castration compared with single agents [77]. However, according to a phase 2 clinical trial in patients with CRPC RAD001, another mTOR inhibitor in combination with bicalutamide failed to improve response compared with bicalutamide alone [79]. This result may be attributed to the complex adaptive resistance pathways to PI3K/mTOR inhibition [80].

The enhancer of zeste homolog 2 (EZH2), which functions as an epigenetic gene silencer, is induced in numerous human malignancies, including PCa [81]. This molecule is downregulated by androgens while the administration of androgens in LNCaP cells decreased their migration, but knockdown of E-cadherin, a gene target of EZH2, abrogated these effects [82]. According to a recent study by Xu et al, EZH2 is more critical for the survival and growth of the castration-resistant LNCaP abl cells compared with the castration-sensitive LNCaP cells [83], providing a rationale for introducing EZH2 inhibitors as a novel therapeutic approach in CRPC, especially in combination with hormonal therapy.
Fig. 3 – Prostate cancer progression under androgen deprivation and androgen receptor (AR) inhibition. Systemic androgen depletion by hormonal therapy leads to progressive activation of numerous survival mechanisms, including androgen biosynthesis in the tumor microenvironment, while more effective AR inhibition through abiraterone, enzalutamide, and ARN-509 promotes the emergence of AR gene (AR) mutations, amplifications, and variants maintaining disease progression. Finally, sustained AR inhibition leads to alternative oncogenic signaling derepression such as Akt, enhancer of zeste homolog 2, STAT3, and c-Met and induction of glucocorticoid receptor, providing a survival advantage to cancer cells under maximum AR inhibition.

AR = androgen receptor; EZH2 = enhancer of zeste homolog 2; GR = glucocorticoid receptor.

STAT3 transcriptional factor is associated with metastatic potential in PCa cells [84], while Schroeder et al found that AR inhibition and androgen depletion induced STAT3 activation and promoted the development of PCa stemlike cells [85]. Liu et al showed that overexpression of IL-6 promotes the development of resistance to enzalutamide through STAT3 activation, and autocrine IL-6 promotes AR transactivation through STAT3 induction, while the addition of AG490, a STAT3 inhibitor, increases the sensitivity of LNCaP cells to enzalutamide [86]. These results support the idea that STAT3 signaling may represent another example of an AR-depressed oncogenic pathway, and targeting STAT3 in combination with AR inhibition may be a reasonable approach for patients with advanced PCa.

It is known that hepatocyte growth factor derived from prostate stroma enhances PCa cell proliferation, motility, and invasion through activation of the c-Met protein [87–90]. Verras et al showed that AR represses c-Met expression directly through binding in its promoter, while castration was found to induce c-Met expression in LNCaP xenografts [91]. It is interesting to note that long-term androgen deprivation leads to a signaling pathway switch from AR to c-Met, which could be predictive of a more aggressive disease [92], while cabozantinib, a c-Met inhibitor, leads to increased progression-free survival, improvement of bone scans, and reduction of soft tissue lesions in patients with mCRPC [93]. However, phase 3 clinical trials evaluating the efficacy of cabozantinib in combination with prednisone and mitoxantrone for patients with mCRPC failed to reach their primary end point of improved survival compared with prednisone and mitoxantrone alone (COMET-1, NCT01605227, and COMET-2, NCT01522443).

ADT and AR inhibition were recently found to increase the expression of c-Myb [94], a transcriptional factor that is upregulated during the progression of breast, prostate, and head and neck cancers [95,96]. AR suppresses the expression of MYB, which mediates growth and induces the metastatic potential of PCa cells [94]. AR and c-Myb coregulate a signature of DNA damage response–related genes strongly associated with tumor recurrence, castration resistance, and metastasis [94]. These data suggest that c-Myb regulates a resistance pathway that might be targeted to develop novel combinational therapeutic approaches for patients with PCa.

Based on these observations, a critical function of AR in a subset of PCa is to repress numerous genes implicated in oncogenic signaling, promoting the development of a more aggressive form of PCa. Some of the oncogenic pathways that are derepressed by AR inhibition are presented in Figure 3. AR inhibition by novel antiandrogens may select for these cells, leading to rapid development of resistance. Further studies are needed to discover reliable biomarkers to identify this subset of patients and new targets for the introduction of novel therapeutic approaches.

4. Conclusions

Despite the introduction of novel agents targeting androgen action, mCRPC remains an incurable disease. Increased androgen biosynthesis in the tumor microenvironment and alterations of AR signaling (including AR mutations mainly in the LBD, AR variants, and AR gene amplifications) all play roles in the development of resistance to systemic hormonal therapy. Especially after the introduction of AA
and enzalutamide, which maximally decrease AR activity, these and other mechanisms related to resistance emerge (Fig. 3). The combination of these two agents is predicted to maximally inhibit AR signaling. Cancers presenting with upfront resistance to this approach should be more dependent on post-translational modifications of AR and alternative oncogenic signaling. The finding that AR represses critical oncogenic mediators explains the clinical observation that a subset of patients becomes rapidly resistant to hormonal therapy and does not respond to novel antiandrogens (Fig. 3). Finally, a new classification of patients with mCRPC based on molecular biomarkers predicting this subset of patients, as well as novel therapeutic approaches targeting these pathways, is required to change the natural history of currently incurable mCRPC.

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Study concept and design: Karantanos, Isaacs.

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