Published in final edited form as:
Horm Cancer. 2014 October ; 5(5): 265–273. doi:10.1007/s12672-014-0190-1.

Androgen Receptor Splice Variants in the Era of Enzalutamide and Abiraterone

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Abstract

The FDA approvals of enzalutamide and abiraterone have rapidly changed the clinical landscape of prostate cancer treatment. Both drugs were designed to further suppress androgen receptor (AR) signaling, which is restored following first-line androgen deprivation therapies. Resistance to enzalutamide and abiraterone, however, is again marked by a return of AR signaling, indicating a remarkable “addiction” of prostate cancer cells to the AR pathway. Several mechanisms of castration resistance have been uncovered in the past decades, featuring a wide spectrum of molecular alterations that may explain sustained AR signaling in castration-resistant prostate cancers (CRPC). Among these, the androgen receptor splice variants (AR-Vs), particularly variant 7 (AR-V7), have been implicated in resistance to enzalutamide and abiraterone in preclinical studies, and they cannot be targeted by currently available AR-directed drugs. Drug development for AR-V–associated CRPC may therefore be necessary to augment the preexisting treatment repertoire. In this mini-review, we will discuss general mechanisms of resistance to AR-directed therapies, with a focus on the role of androgen receptor splice variants in the new era of treating advanced prostate cancer with enzalutamide and abiraterone.

1. Introduction

Prostate cancer (PCa) is the most common non-skin cancer in men, responsible for approximately 258,000 deaths annually worldwide [43]. Clinical management of advanced PCa hinges on the use of surgical or medical castration, an approach known as androgen deprivation therapy (ADT) [81]. ADT exploits the cancer’s dependence on androgen signaling, first revealed by Huggins in 1941 [42]. Today, ADT is not only used for those who are diagnosed with (or develop) advanced disease, but also in a subset of patients as an adjuvant to local therapy. The main goals of ADT are to decrease tumor burden, enhance quality of life, and improve survival [8].

While the vast majority of patients with advanced prostate cancer respond to initial ADT, given time virtually all patients progress to a disease state known as castration-resistant PCa

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(CRPC) [45, 75]. CRPC describes the most advanced state of PCa, when patients demonstrate serial rising PSA values or radiographic/clinical progression despite castrate levels of androgen (usually defined as serum testosterone <50 ng/dL) [34]. In the CRPC state, androgen signaling remains active and is mediated through the androgen receptor (AR) [30, 80]. A whole spectrum of molecular alterations, both primary and acquired, has been identified that results in sustained AR signaling in the castrate setting [37, 21].

Until 2004, the only Food and Drug Administration (FDA)-approved therapy for CRPC was the chemotherapeutic agent, docetaxel [27]. As such, until recently, the treatment paradigm for PCa was to use luteinizing hormone releasing hormone (LHRH) agonists/antagonists and/or anti-androgens (AR antagonists), followed by docetaxel after development of metastatic CRPC. In the last decade, a variety of other therapies have been approved or are in development, the most notable of which are abiraterone and enzalutamide, two FDA-approved AR-targeted therapies demonstrating significant improvement in survival in the CRPC setting (both before and after docetaxel use) [77, 24, 7, 70]. Enzalutamide is a second-generation AR antagonist with properties superior to its first-generation counterparts such as flutamide, bicalutamide, and nilutamide. Enzalutamide was developed to bind to the AR-LBD with high affinity, to reduce AR nuclear translocation efficiency, to disrupt AR binding to androgen response elements (ARE) on DNA, and to impair recruitment of coactivators to the AR transcriptional complex [90, 74]. Abiraterone is a CYP17A1 inhibitor designed to target steroid biosynthesis in CRPC, and acts to inhibit androgen synthesis from alternative sources, such as from within the tumor or the adrenal gland [5]. The efficacy of these next-generation endocrine treatments confirms that the AR-signaling axis is still a viable target after progression to CRPC. While the current CRPC drugs were designed for and approved for late-stage patients, the prospect of using enzalutamide and abiraterone for hormone-sensitive patients is gaining attention. Data from a phase II study involving the administration of abiraterone in combination with androgen deprivation therapy prior to radical prostatectomy showed robust PSA responses and a high rate of pathologic complete (or near-complete) responses in high-risk localized PCa patients [67, 87]. In another phase II trial, enzalutamide was used (without androgen deprivation therapy) in men with hormone-sensitive disease, resulting in a very high rate of PSA responses [89]. The use of these next-generation agents could possibly provide for better AR ablation in the early stages of prostate cancer and stall progression to the lethal CRPC phenotype.

The new paradigm of treating CRPC with enzalutamide and abiraterone provides opportunities to dissect the drug resistance mechanisms to these agents. It is now established that a proportion of patients are primary refractory (*i.e.* show no response) to therapy or develops resistance despite showing an initial response. Because resistance is often marked by a return of AR signaling, it is critical to dissect mechanisms of sustained AR signaling in the presence of potent AR pathway suppressors. The extent to which the spectrum of molecular alterations acquired after first-line therapy contributes to resistance in this new treatment setting remains largely uncharacterized. The constitutively active AR splice variants (AR-Vs) represent a conceptually simple and biologically plausible mechanism of resistance to abiraterone and enzalutamide. These AR-Vs contain an intact N-terminal domain (NTD) and DNA-binding domain (DBD) but lack the c-terminal ligand binding

domain (LBD), the intended therapeutic target of abiraterone and enzalutamide (Figure 1). In addition to AR-Vs, a number of novel mechanisms of resistance have been recently investigated in the specific setting of treatment with enzalutamide and abiraterone (summarized in Figure 2), including drug-specific molecular alterations acquired during treatment with the two drugs. In this review, we will provide a succinct summary of these resistance mechanisms, with a particular focus on the androgen receptor splice variants.

2. General Mechanisms of Resistance and Progression to CRPC

2.1. AR alterations

In the normal prostate gland, dihydrotestosterone (DHT) is the primary androgen mediating AR signaling. Androgen binding to the ligand-binding domain (LBD) of the AR causes receptor dimerization, translocation to the nucleus, and subsequent transcription of androgen-responsive genes; this signaling in PCa cells sustains tumor growth and proliferation. Upon initiation of ADT (and withdrawal of these ligands), PCa cells undergo apoptosis or are held in G1 cell cycle arrest. Cells with primary resistance to androgen ablation, or surviving cells held in cell cycle arrest that eventually adapt to low androgen conditions, later emerge to resume proliferation [76]. The transition from hormone-sensitive to castration-resistant PCa is also consistently coupled with an increase in AR mRNA expression, as demonstrated by microarray profiling [20]. Multiple mechanisms involving the AR signaling axis have been proposed to account for the emergence of CRPC [21, 65, 79]. Among these, alterations involving the AR gene and its products (including AR amplification [92, 58, 14], gain-of-function mutation [86, 82], and overexpression [20]) are the most specific to CRPC.

2.2. Sustained androgen levels

Incomplete ablation of circulating androgens can cause direct activation of the AR, resulting in sustained tumor growth. Studies have shown that surgical and medical castration often do not eliminate androgens from the tumor tissue [76, 88]. In fact, residual levels of both testosterone and DHT in CRPC patients were found to be adequate to sustain androgen signaling [59, 88]. The adrenal gland is a contributor to levels of circulating androgens in the CRPC state [103]. In addition, testosterone and DHT synthesis may also occur via *de novo* pathways, possibly involving the conversion of acetic acid, a cholesterol precursor to steroids including DHT [57]. Thus, the continued presence of androgens (at lower but sufficient levels), coupled with more abundant androgen receptor levels, appear to be a prevailing explanation for CRPC.

2.3. Growth factors and AR coregulators

The AR can also be activated via alternative pathways and molecules in a low-androgen state. Several growth factors, namely insulin-like growth factor 1 (IGF-1), keratinocyte growth factor (KGF), and epidermal growth factor (EGF), are able to activate the AR directly [23]. In addition, coactivators that enhance AR function may be involved in increasing the sensitivity of the AR to low levels of androgens or alternative ligands [80]. For example, a coactivator protein known as ARA70 was shown to increase AR

responsiveness to estradiol, suggesting that ARA70 can induce AR activity using an alternative ligand [97].

3. Novel mechanisms of enzalutamide/abiraterone resistance

3.1. Reciprocal regulation of AR and PI3K

Studies have also shown that AR inhibition could lead to upregulation of other oncogenic pathways to sustain PCa growth. Along with AR, the phosphatidylinositol 3-kinase (PI3K) pathway is another signaling pathway that promotes prostate carcinogenesis. The PI3K enzymes play a role in mediating signal transduction across the cellular membrane [73]. The AR and PI3K pathways are known to be cross-regulated by reciprocal feedback. That is, the inhibition of AR signaling through androgen deprivation and other manipulations results in a heightened activation of the PI3K pathway, and vice versa [17].

3.2. Alternative Steroid Receptors

Recently, there has been increasing interest in the role of the glucocorticoid receptor (GR) in PCa. GR activation has been shown to drive the expression of AR target genes, suggesting that AR signaling inhibition through antiandrogens can be bypassed via glucocorticoid signaling[3]. Studies from clinical samples showed that GR expression is only present in approximately 38% of untreated PCa, but exists in higher proportion (78%) in androgen-deprived PCa [85]. Indeed, in enzalutamide-resistant patients, GR expression is heightened [3]. Earlier studies also identified an AR mutation, L701H (or L701H coupled with T877A) which allows the AR to respond to glucocorticoids to sustain tumor growth [102]. Further, it is known that both the AR and GR bind to a common response element on DNA and regulate the same genes. This could imply that both the GR and its ligands may sustain AR signaling in the CRPC state [72], depicted in Figure 2, pathway 4 and 5.

The progesterone receptor (PR), another member of the steroid receptor family, was shown to be increased in CRPC [13] and may be yet another resistance mechanism due to continued progesterone production. Two isoforms of the PR (isoforms A and B) are expressed in stromal fibroblast and smooth muscle cells of the prostate and are known to regulate cell proliferation [98, 32]. While evidence supporting a role of PR in resistance to abiraterone and enzalutamide is currently weak, further studies could implicate the role of PR signaling in CRPC in the absence of circulating androgens, depicted in Figure 2, pathway 6.

4. Therapy-Specific Mechanisms of Resistance

4.1. Resistance to enzalutamide

An AR gain-of-function mutation known to confer resistance to enzalutamide has been identified in PCa cell lines. Specifically, the missense mutation F876L (depicted in Figure 2, pathway 2) in the LBD of the AR confers agonist properties to enzalutamide, sustaining AR signaling in the presence of the drug [6]. This has been verified as an acquired mechanism of resistance in patients receiving enzalutamide [44]. Further, ARN-509 (a newer AR antagonist with a higher therapeutic index compared to enzalutamide [22]) also demonstrates agonist properties to the F876L mutation [46]. The clinical impact of these

findings will need to be determined in larger collections of patient samples, but the F876L mutation will likely account for a small proportion of cases of acquired resistance to enzalutamide (and ARN-509).

4.2. Resistance to abiraterone

Studies with CRPC xenografts have shown that various genes in the androgen biosynthesis pathway are upregulated as a result of treatment with abiraterone, notably the target gene CYP17A1 itself [60]. Another study has shown that DHT can be synthesized from 5α-androstanedione instead of testosterone, the usual precursor to DHT [18, 19]. Further, recently a gain of function mutation (N367T) was shown to produce 3β-hydroxysteroid dehydrogenase type 1 (3βHSD1) resistant to ubiquitination, which results in the accumulation of this enzyme responsible for DHT synthesis [18]. Depicted in Figure 2, pathway 3, all of these represent molecular adaptations of the AR signaling pathway to evade the effects of abiraterone-mediated downregulation of androgen synthesis. Similarly, the clinical impact of these findings needs to be confirmed in large-scale studies.

5. Androgen receptor splice variants

5.1. Overview

Since 2008, multiple AR splice variants (AR-V) lacking the AR ligand-binding domain have been identified and characterized [25, 44]. Some AR-Vs, notably AR-V7 and AR^{v567es}, are known to be constitutively active, allowing for the activation of AR signaling in the absence of natural ligand binding [38, 84, 69], depicted in Figure 2, pathway 1. Levels of AR-V are known to correlate with PCa progression and CRPC [49, 38, 31]. Additionally, expression of AR-V7 increases markedly upon androgen deprivation [99, 40, 95]. AR-V expression is found to be common in tumor metastases; furthermore, levels of variant expression directly correlate with accelerated disease progression and shorter cancer-specific survival [36, 101]. Experiments on mice have shown that AR^{v567es} can induce autonomous tumor formation and proliferation [54], and AR-V7 expression leads to the expression of tumor promoting growth factors such as TGFβ2 and IGF1 [83]. These findings suggest that AR-Vs are biologically significant and may contribute to clinical progression of prostate cancer.

5.2. Diversity of AR-Vs

The transcript structures of 18 androgen receptor splice variants have been fully characterized [44]. The majority of these splice variants arise through splicing of intronic sequences (*i.e.*, cryptic exons). In addition to the sequence features that distinguish the different variants, other features critical for clinical translation have been characterized [69, 26, 38, 31, 95, 39], including their relative abundance, functional activity, and evidence for the corresponding protein product. On the basis of these studies, AR-V7 was determined to be the most important AR splice variant because it is the most abundant AR-V, its expression increases by ~20-fold in CRPC specimens, it is constitutively active in a ligand-independent manner, and it is detectable as a protein using variant-specific antibodies.

5.3. Genomic functions of AR-V7

Relative to the full-length AR (AR-FL), AR-V7 was initially described as a low-abundance transcript, at ~1-5% of the full-length AR in the vast majority of CRPC specimens (as measured by quantitative RT-PCR) [38]. This observation has been confirmed by independent investigators [94]. Because AR-V7 coexists with the full-length AR, it is critical to dissect the differential functions of AR-V7 and AR-FL, respectively. To determine the transcriptional output of AR signaling mediated by AR-V7 in the context of suppressed AR-FL signaling, transcriptional changes driven by forced expression of AR-V7 in the presence or absence of AR-FL signaling identified a set of cell cycle genes enriched for upregulation independent of the presence or absence of ligand-mediated AR-FL signaling [40]. On the other hand, top gene sets enriched for upregulation by ligand-dependent AR-FL signaling are dominated by those related to biosynthesis, metabolism, and secretion [40]. These findings suggest that although AR-V7 and AR-FL both regulate canonical AR target genes, they are associated with distinct transcriptional programs in CRPC.

In addition, it is known that AR-V7 can be acutely induced following suppression of AR-FL in two cell lines, VCaP and LNCaP95 [40]. Corroborating the functional distinctions between AR-FL and AR-Vs, endogenous induction of AR-V7 was accompanied by genome-wide changes of gene expression consistent with a shift of AR signaling mediated by AR-Vs [40]. Therefore, constitutively active AR-Vs appear to mediate a broader function than simply another mechanism to “rescue” canonical AR signaling. To this end, the emergence of AR-Vs in CRPC may drive a lethal mitotic phenotype while maintaining certain components of AR signaling. This notion is supported by other correlative studies in clinical specimens as well as animal xenografts [40, 101, 36]. Collectively, these studies show that although ARV7 is expressed at levels that are substantially lower than those of AR-FL, it is sufficient to mediate a shift toward a transcriptional program mediated by AR-V7 when AR-FL is suppressed. Therefore, targeting the AR-V and its transcriptome is an area of priority for discovery and development of novel approaches to overcome resistance to abiraterone and enzalutamide.

5.4. Biphasic and context-specific functions of AR-Vs

In a recent study, AR-V-dependent genes were found to be induced at low receptor levels but repressed at high receptor levels [49]. This observation mirrors the canonical biphasic androgen-stimulated (*i.e.*, ARFL-mediated) growth response observed in cell line models. Study findings also suggest that AR-Vs mediate enzalutamide resistance by re-activating the AR-FL transcriptional programs, rather than by targeting a unique set of AR-V–associated genes [49]. These study findings are in contrast to those from our previous study [40], in which we showed distinct transcriptional programs mediated by AR-FL and AR-Vs, using two different cell line models in which AR-V7 can be induced following suppression of AR-FL. AR-regulated genes are known to be cell-context specific [12]. Because the two studies were performed in different cell lines, cell-context differences may explain the seemingly opposing findings, although different methodologies utilized in the studies may also be a factor. Nevertheless, we posit that the relative lower AR-V levels (relative to AR-FL) may not fully “rescue” the androgen-regulated genes suppressed by potent AR-FL inhibition.

Instead, AR-Vs may mediate genomic functions distinct from those mediated by the AR-FL, although the underlying mechanism remains unclear. It is possible that a distinct set of co-regulators, target genes, and other DNA regulatory elements may collectively mediate AR-V signaling.

5.5. Molecular origin of AR-Vs

Multiple mechanisms may account for elevated expression of AR-Vs in CRPC. Complex CRPC-specific genomic alterations involving the *AR* locus have been characterized by genome-wide copy number analysis [56]. However, the relationship between these alterations and the genesis of AR-V is presently not fully characterized. In the CWR22Rv1 cell line [48] as well as the LuCaP86.2 xenograft [50], unusually high levels of the constitutively active AR-V7 and AR^{v567es} have been linked to genomic alterations including duplication and deletion of segments of the *AR* DNA. DNA rearrangements have been modeled using genome engineering [66]. A recent study has also shown that ADT can induce AR gene transcription and recruitment of RNA splicing factors at the specific 3′ splice site, which together contribute to the formation of AR-V7 [4, 55]. However, these specific alterations are yet to be demonstrated in clinical CRPC specimens. Alternatively, generation of AR-Vs may be coupled to the transcriptional output from *AR* locus, possibly regulated by negative feedback mediated by AR-FL [15]. Indeed, in CRPC specimens as well as in cell lines with induced AR-V7, AR-V7 expression is often (but not always) associated with elevated AR-FL levels, though the AR-V7/AR-FL ratios trend up in CRPC specimens and cells with induced AR-V7 [38, 94, 99]. These studies also suggest that the adaptive shift to AR-V expression is regulated at the mRNA level, and that the magnitude of AR-V mRNA change is greater than that of AR-FL. Therefore, in the setting of AR-directed therapies targeting the AR-LBD, ARV expression is associated with that of AR-FL but may not strictly parallel that of AR-FL, and AR-V levels may be negatively regulated by AR-FL signaling.

5.6. Clinical and therapeutic implications of the AR-V concept

Investigations encompassing the full spectrum of molecular characterization and clinical validation of the AR-V concept are still at a nascent stage, requiring carefully designed, multi-pronged approaches to dissect the biological and clinical significance of AR-Vs in the context of the coexisting (and often more abundant) AR-FL as well as other competing mechanisms of resistance in the setting of men treated with abiraterone and enzalutamide. In the *in vitro* setting, AR-Vs have been implicated in resistance to enzalutamide and abiraterone [49, 60, 40, 16, 63]. This concept, even in the absence of clinical validation, is beginning to fuel the development of novel agents that target all AR molecules to overcome resistance [1, 71, 78, 51, 47, 96, 64, 11, 33, 52, 53, 61, 63] (also see most recent reviews on this topic [29, 93]). We envision that conceptual advances in the field will provide the sustained impetus to drive and guide clinical development of novel agents designed to overcome resistance to AR-LBD-targeting agents, much like the compelling and rational evidence establishing the functional mechanism of sustained AR signaling in CRPC that marked the successful clinical development of abiraterone and enzalutamide. Development of methodologies for detecting these variants in the relevant patient population has significant implications for the future of drug development and clinical decision-making.

6. AR-independent mechanisms

As PCa is being aggressively managed by different androgen deprivation strategies, it is important to note the effects of therapy to the disease itself. As novel, more potent strategies are developed to target the AR, we could anticipate the rise of tumors that show new mechanisms of resistance, or even become AR-independent [100, 65]. Neuroendocrine prostate cancer (NEPC) is an aggressive manifestation of PCa that arises due to neuroendocrine differentiation of prostate adenocarcinoma [68, 28]. It is associated with very poor prognosis, characterized by low PSA (despite high disease burden) and the development of nonosseous (visceral) metastases [62]. The median survival is only 6-12 months [2]. NEPC does not express AR and is thus unresponsive to AR-directed therapies [9], but does show transient responses to chemotherapy [91]. The development of NEPC correlates with progression of disease [10], as well as the use of ADT [35]. A study has shown that NEPC cells express interleukin-8, a molecule with demonstrated involvement in androgen-independent growth [41]. While NEPC is only found in about 5-10% of CRPC patients [2], its emergence after androgen deprivation strategies is crucial to consider in the clinical setting.

Conclusions

With multiple emerging therapies for CRPC, the treatment paradigm for PCa is ever evolving. The new range of treatment options has also given rise to the need to assess mechanisms of resistance to these therapies; an understanding of their limitations will allow for better use of these options. The various resistance mechanisms suggests molecular heterogeneity and individual differences among patients, both of which need to be accounted for in the clinical setting. It is evident that the availability of drugs like enzalutamide and abiraterone has facilitated studies aimed at understanding the general mechanism of sustained AR signaling under castrate conditions. Future biomarker-driven clinical trials may help to achieve the dual goal of drug development and overcoming drug resistance mechanisms. It is worth noting that the successful clinical development of enzalutamide, a potent AR antagonist designed to target the LBD (a target which is missing in AR-Vs), is a direct result of laboratory mechanistic studies establishing AR protein overexpression as the key molecular determinant of castration resistance [21]. Along these lines, the recent appreciation that AR-Vs may drive resistance to enzalutamide (and abiraterone) in a subset of patients may fuel a new wave of AR-pathway–directed drugs focusing on inhibiting or eliminating AR splice variants or their transcriptional programs. Further understanding of additional molecular mechanisms of resistance should greatly facilitate drug development for CRPC moving forward.

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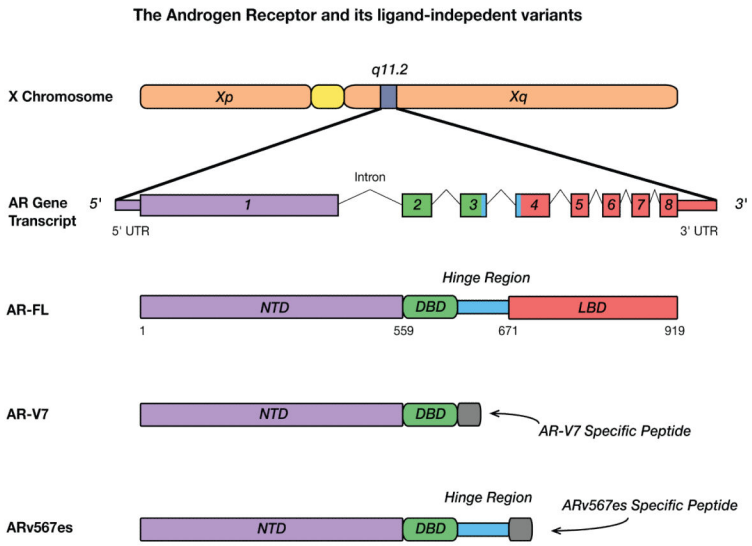

Figure 1.

Transcript structures for the full-length AR and major splice variants AR-V7 and ARV567ES. The human AR gene has 8 canonical exons, color coded in relation to the AR protein domains they encode, including the N-terminal domain (NTD (purple), DNA-binding domain (DBD) (green), the hinge region (blue), and the ligand-binding domain (red). AR-V7 retains the first three canonical exons, followed by variant-specific cryptic exon 3 within intron 3, leading to premature translation termination after 16 variant-specific amino acids (gray). ARV567ES retains the first four canonical exons, followed by exon 8. Skipping of exons 5-7 leads to disruption of the open reading frame and a variant-specific peptide of 10 amino acids (gray). Peptide positions are marked according to GRCh36/hg18 human genome sequences (not drawn to scale).

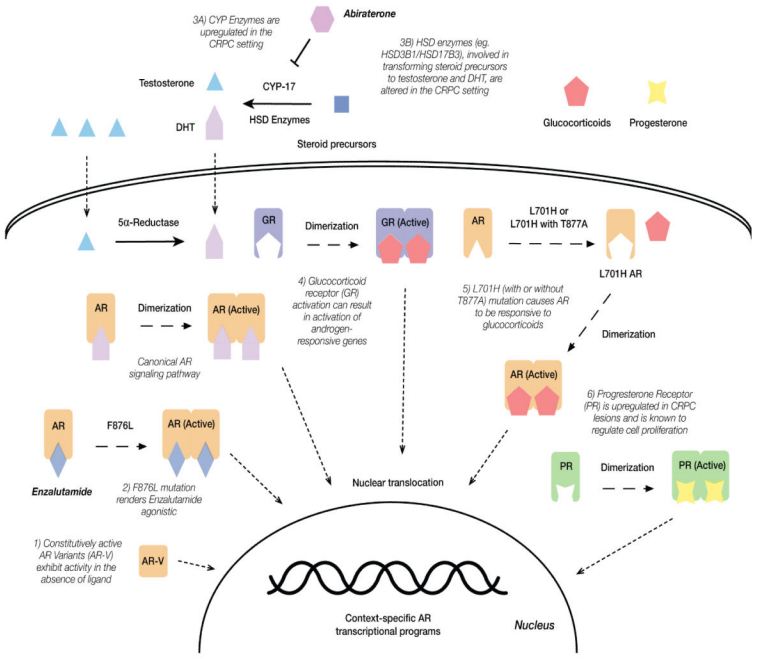

Figure 2.

A summary of candidate molecular alterations leading to the return of AR signaling in spite of treatment with enzalutamide and abiraterone. These alterations include 1) androgen receptor splice variants; 2) AR F876L mutation; 3) steroidogenic enzymes; 4) glucocorticoid receptor overexpression; 5) AR L701H mutation; and 6) progesterone receptor activation. Diverse mechanisms may operate independently or cooperatively. The list is not intended to be comprehensive and emphasis was on those under investigation in the specific setting of the two new drugs. Dissection of the major drivers of resistance to enzalutamide and abiraterone will direct future prostate cancer drug development.