

# Interplay Between Genomic Alterations and Androgen Receptor Signaling During Prostate Cancer Development and Progression

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Received: 16 November 2012 / Accepted: 2 January 2013 / Published online: 10 January 2013  
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**Abstract** Advanced prostate cancer (PCa) treated with androgen deprivation therapy (ADT) eventually relapses to an ADT-resistant disease referred to as castration-resistant PCa (CRPC). Recent integrative analyses of PCa genomes have led to the elucidation of potential subtypes that are revelatory to the development of PCa as well as the mechanisms of resistance to ADT and CRPC progression. These studies have confirmed that alterations in the androgen receptor (AR) signaling axis are central to CRPC progression, and have uncovered complex mechanisms by which AR and other components of the AR signaling axis affect, and are affected by, genomic changes and epigenetic transformations. Among the most frequent alterations in CRPC are direct alterations in the AR gene. These AR gene alterations include AR amplification, point mutations, and more recently AR gene rearrangements leading to expression of truncated, constitutively active AR splice variants that are impervious to ADT. In this review, we will highlight genomic alterations that are important for development and progression of PCa, with a focus on how these alterations affect, and are affected by, activity of the AR signaling axis.

## Introduction

Prostate cancer (PCa) is the most frequently diagnosed male cancer and second leading cause of male cancer deaths in the USA [1]. The androgen receptor (AR) is a master regulator transcription factor in cells of prostatic lineage, and this master regulator function is maintained in PCa cells [2]. Therefore, the frontline treatment for locally advanced or metastatic PCa involves androgen deprivation therapy (ADT), which is achieved by blocking androgen production (surgical or chemical castration with LHRH agonists) or directly antagonizing the AR (antiandrogens). A major limitation is that castration-resistant or castration-recurrent prostate cancer (CRPC) results within 2–3 years due to alterations in the androgen/AR signaling axis. This limitation has begun to be addressed through mechanistic understanding of the changes to androgen/AR signaling that occur during disease progression. For example, it is now established that AR activity persists in CRPC despite ongoing ADT, and the AR signaling axis remains a viable therapeutic target for treating CRPC patients.

New therapies that have been developed to re-target AR ligand binding and extend the lifespan of patients with CRPC include the cytochrome P450 c17 (CYP17) inhibitor abiraterone acetate [3], which inhibits androgen synthesis in testes, adrenals, and tumor tissue as well as the next generation antiandrogen enzalutamide/MDV3100 [4] which suppresses AR transcriptional activity even under conditions of AR overexpression. Despite these clinical successes, the development of resistance to abiraterone and enzalutamide, and ongoing disease progression for many patients, remains a major challenge. A greater appreciation of the complex genomic events involved in the initiation and progression of PCa is expected to yield insights into risk and likely routes of therapy resistance taken by individual cells in heterogeneous tumor populations. This could facilitate the individualization and

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optimization of treatment regimens, which are cornerstones in the concept of precision medicine. To this end, many studies investigating the PCa genome have identified disease- and/or progression-specific alterations in genes that impact the signaling and regulation of the androgen/AR signaling axis. Importantly, included in this list are a myriad of alterations in the AR gene at Xq11-12, which directly alter the regulatory dynamics and biological function of the AR.

### Recurrent Alterations in the PCa Genome

Gene fusions between the AR-regulated *TMPRSS2* gene promoter and the coding region of ETS family transcription factors, including *ERG* and *ETV1*, are among the most frequent recurrent alterations in PCa. These fusions render ETS transcription factors androgen-responsive and under direct control of the AR signaling axis in PCa cells. Their existence in the earliest precursor lesions indicates that these are initiating drivers of PCa and efforts are being made to translate this knowledge into better diagnostic tools and new targeted therapies [5]. Knowledge of the broader genomic landscape of PCa has been greatly expanded in recent years by integrative whole-genome analyses using algorithms that coordinate datasets derived from studies of genome-wide copy number, exome sequence, mRNA expression, and epigenetic modification. These analyses have provided insights into the mechanisms by which mutations, epigenetic changes, and genome rearrangements conspire to dysregulate genes and pathways in PCa. For example, a recent integrative study demonstrated that the p53/RB pathway was disrupted in 34 % of primary, hormone-naïve PCa and 74 % of CRPC metastases, the PTEN/PI3K pathway was disrupted in 42 % of primary PCa and 100 % of CRPC metastases, and the RAS/RAF pathway was disrupted in 43 % of primary PCa and 90 % of CRPC metastases [6]. Similar integrative studies have found that *MYC*, *WNT*, *FOXA1*, *SPOP*, *MLL2*, *CHD1*, and *NCOA2* are frequently affected by copy number alterations or point mutations [6–10]. Moreover, these integrative studies have revealed that AR copy number alterations and point mutations do not occur in primary PCa but occur in 58 % of CRPC metastases [6]. However, when the broader AR signaling axis was considered, alterations were observed in 56 % of primary PCa and 100 % of CRPC metastases, confirming that this master regulator is among the most frequently altered pathways in PCa.

While these integrative studies have revealed important biological insights into PCa, they have relied on targeted approaches that may not reveal the full spectrum of genomic alterations that could occur during disease development and progression. Indeed, recent whole-genome sequencing of seven primary PCa specimens and matched normal tissues

demonstrated that important genomic events in PCa are likely underappreciated due to the limitations of targeted approaches such as CGH and exome sequencing. The most striking finding from PCa whole-genome sequencing was the high prevalence of copy number neutral, or balanced, translocations and inversions among the median 90 rearrangements per genome [9]. Some of these rearrangements had interrupted tumor suppressor genes such as *PTEN* and *p53*, which would be expected to activate oncogenic signaling through PI3K/Akt and RB pathways, respectively [9]. It is also interesting to note that some loci did not appear to undergo copy number loss even though inversions and/or point mutations were commonly observed, whereas other loci were frequently deleted [7, 9]. This suggests that specific chromatin configurations and/or genome architectures may be required for activation or inactivation of genes harbored within these affected regions.

### Role of AR in the Genesis of Rearrangements in the PCa Genome

These integrative and whole-genome sequencing studies have raised an intriguing question: why is there a propensity towards structural alteration in the PCa genome? For *TMPRSS2*–*ERG* rearrangement-positive cancers, rearrangement breakpoints appear to be enriched near sites of AR and/or *ERG* binding with open chromatin histone marks, indicating that active transcription driven by these transcription factors may create chromatin environments that are prone to breakage [9]. There is also gathering evidence to support the notion that AR binding strongly influences higher-order chromatin structure and three-dimensional organization of the nucleus in PCa cells, which may favor genesis of specific rearrangement events by enhancing proximity of discrete genomic domains [11–13].

The best evidence supporting a role for AR in shaping deterministic genomic events in the natural history of PCa progression comes from work investigating the mechanisms underlying recurrent *TMPRSS2*–*ERG* fusions. *TMPRSS2* expression is controlled by an AR-driven enhancer element just upstream of the *TMPRSS2* promoter [11]. This *TMPRSS2* locus is 3 Mb upstream of ETS family transcription factor *ERG* on chromosome 21. FISH-based studies with molecular probes targeting regions 5' to the *TMPRSS2* breakpoint and 3' to the *ERG* breakpoint demonstrated that these loci are brought within close proximity in fusion-negative LNCaP PCa cells upon addition of the natural AR ligand, DHT [12]. DHT-induced proximity was also observed for the chromosomes 21 and 7 break fusion junctions of the *TMPRSS2*–*ETV1* gene fusion [13]. Importantly, exposure of LNCaP or the normal prostate epithelial PrEC cell line to gamma irradiation, which induces genotoxic stress, synergized with DHT to induce genesis of

TMPRSS2–ERG and TMPRSS2–ETV1 fusions [12, 13]. Induction of these fusion events appeared to require DHT/AR-mediated recruitment of various DNA-directed enzymatic activities to the break fusion junctions, which promoted the formation of DNA double strand breaks at these sites. For example, activation-induced cytidine deaminase, the ORF2 endonuclease encoded by long interspersed nuclear elements (LINE-1), and topoisomerase IIB could all be recruited to these AR binding sites, and all have been shown to be important for generation of the fusion events [13, 14]. Importantly, DHT-induced proximity and irradiation-induced DNA damage were also shown to lead to generation of the SLC45A3:ETV1 fusion in LNCaP cells, which is a less-frequent PCA gene fusion leading to AR-mediated overexpression of ETV1 in a subset of PCA [13, 15]. These data imply that many other less frequently occurring rearrangements between androgen-regulated genes and ETS family transcription factors may arise through this same mechanism [5]. Importantly, these ETS family fusions may then play a role in promoting further instability of the PCA genome. For example, ERG overexpression increases DNA damage in PCA lines and ERG binding is enriched at rearrangement breakpoints in primary PCA, implicating ERG as a driver of subsequent genomic rearrangements and tumor progression [9, 16].

#### AR and Epigenetic Alteration of the PCA Genome

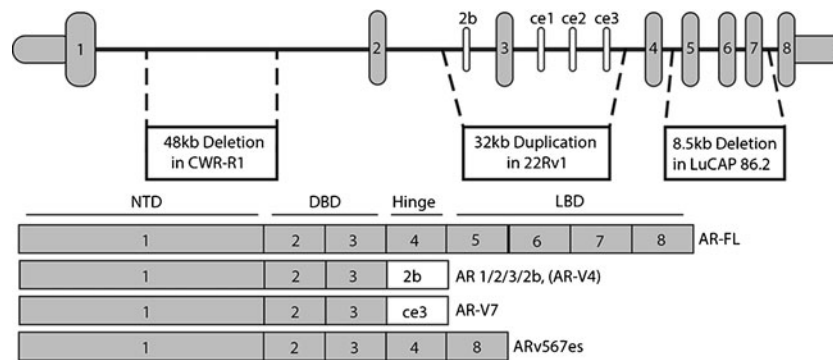
Epigenetic marks impart an additional layer of regulatory information on top of the information embedded in the PCA genome. Therefore, it is not surprising that many genomic studies have demonstrated that dysregulated epigenetic control accompanies the progression of PCA. Indeed, CRPC genomes display a general overall hypermethylation of CpG dinucleotides when compared with benign prostate [8]. Intriguingly, DNA methylation and genomic changes appear to coordinately alter the regulation of the cell cycle and testosterone metabolism, providing possible specific mechanisms through which DNA methylation promotes PCA progression [8]. Changes in the epigenetic regulatory environment can potentially change the physiology of the cancer cell and perhaps change the context in which existing and subsequent genomic events are manifest. One example of this is coordinate binding activity between AR and ERG. ERG represses AR transcriptional activity at loci involved in differentiation by recruiting the polycomb group (PcG) protein EZH2, which gives PCA cells more stem cell-like characteristics [17]. PcG proteins are responsible for maintaining bivalent repression domains to prevent the expression of lineage genes in embryonic stem cells [18]. Another recent study associated shared AR and ERG binding sites with transcriptional programs relating to movement and cell proliferation [19]. This study further showed that HDAC1, HDAC2, and HDAC3 and EZH2 bound to these AR/ERG

binding sites [19]. This may have clinical relevance, as HDAC 1, HDAC 2, and HDAC 3 display high expression levels in a majority of PCA and HDAC2 levels in PCA tissues predict shorter PSA-free survival [20]. Mixed lineage leukemia 2 (MLL2), a H3K4-specific histone methyltransferase is mutated in 8.6 % of PCAs [7]. MLL2 is also commonly mutated in other cancers and is involved in the regulation of many signaling pathways including nuclear hormone signaling [21]. Indeed, MLL2 complex members could be immunoprecipitated with an AR antibody along with ERG and FOXA1 [7]. FOXA1 is mutated in 4 % of PCAs and is an important pioneer factor for the AR transcriptional program [22]. Interestingly, FOXA1 is preferentially recruited to binding sites enriched in H3K4me1/2 marks, which in turn influences AR binding to nearby androgen response elements [23, 24]. It is tempting to speculate that alterations in MLL2 activity could lead to global alterations in H3K4me1/2 marks, which could in turn affect FOXA1 and AR binding and downstream regulatory processes in PCA cells.

#### Truncated AR Splice Variants

Overall, these genomic studies have demonstrated that the AR can be the instigator and the accomplice in effecting cascading genomic and epigenetic changes via chromatin interacting complex members. Interestingly, a pervasive role for the AR in driving deterministic events in PCA is further evidenced by the high rate of mutation and gene amplification of the AR gene in CRPC. These AR gene aberrations have been shown to alter AR signaling and regulation as well as facilitate AR transcriptional activity despite administration of targeted therapies designed to inhibit the AR [25–27]. For example, AR gene amplification leads to overexpression of AR protein, which sensitizes PCA cells to castrate levels of androgens, and can also elicit inappropriate agonist responses to antiandrogens such as bicalutamide [28]. Similarly, a myriad of point mutations have been described in the AR, many of which occur in the ligand binding domain (LBD) and broaden the repertoire of potential agonists [27, 29]. More recently, rearrangements that alter AR gene structure and splicing patterns have been described in PCA cell lines and xenografts [30, 31]. These AR splicing alterations underlie high expression levels of truncated AR variants that lack the AR LBD, which is the target of AR-centered therapies for PCA (Fig. 1). The role of AR splice variants in CRPC has been gaining importance in recent years due to the discovery of their prevalence in CRPC tissues [32–34], and their association with PCA progression and resistance to AR-targeted therapy [35–37].

Truncated AR splice variants were initially discovered in CRPC cell lines derived from the CWR22 xenograft model



**Fig. 1** A schematic of the AR gene structure is shown with cryptic exons (not to scale). Genomic regions involved in AR gene rearrangements associated with enhanced expression of truncated AR variants are indicated with *dashed lines* (*above*). The domain structure of full-

length AR and truncated AR variants with included exons are indicated (*below*). Regular AR exons are in *gray*, cryptic exons in *white*. NH<sub>2</sub>-terminal domain (*NTD*), DNA-binding domain (*DBD*), ligand binding domain (*LBD*)

of PCa progression [32, 33, 38, 39]. They are produced by aberrant splicing and premature translation termination, which results in synthesis of LBD-truncated, constitutively active forms of AR. To date, well over a dozen discrete AR splice variants have been described [40]. Identification and validation of protein translation of these AR variants in PCa cell lines has traditionally involved cloning the mRNA and designing AR variant sequence-specific siRNAs in order to achieve selective knockdown of that truncated AR variant [32, 39]. This selective knockdown strategy has also facilitated functional characterization in PCa cell lines that naturally express high levels of truncated AR variants, such as the 22Rv1, CWR-R1, and VCaP cell lines. In these models, these strategies have revealed that truncated AR variants support androgen-independent expression of AR target genes and drive androgen-independent growth in a manner that is resistant to antiandrogens [32, 33, 35, 37, 41]. These key findings have highlighted the importance of understanding the role and origin of AR variants in clinical disease progression.

#### AR Variant Splicing Mechanisms

Recent investigation of the mechanisms underlying the synthesis of truncated AR splice variants in CRPC has highlighted genomic rearrangements as a previously unrecognized class of alterations affecting the AR gene [30, 31]. For example, our group reported that the 22Rv1 cell harbored a 32-kb intragenic duplication of a segment encompassing AR exon 3, which harbors cryptic exons 2b, CE1, CE2, and CE3 (Fig. 1). All of these cryptic exons have been shown to splice with high efficiency downstream of AR exon 3 in this cell line; therefore, this rearrangement is an attractive explanation for these altered AR splicing patterns. Interestingly, the CWR22Pc cell line is an androgen-dependent subline derived from the same parental CWR22 xenograft as 22Rv1 cells. When CWR22Pc cells were cultured under castrate conditions, rare cells

harboring this intragenic duplication emerged and continued to display enrichment over several weeks of culture in vitro. Importantly, outgrowth of these duplication-positive cells coincided with increasing expression of truncated AR variant mRNA and protein, indicating that expression of these species was restricted to the cell population harboring this underlying rearrangement in the AR gene [30]. These data provided the first clues that alterations in AR splicing may be due to underlying alterations in the structure of the AR gene.

An additional CRPC cell line in which truncated AR variants were identified and functionally characterized is the CWR-R1 cell line. In these cells, expression of the AR-V7 variant encoded by contiguously spliced AR exons 1, 2, 3 and CE3 was shown to be enriched in CWR-R1 xenografts grown in castrated vs. intact mice [32]. We recently developed a strategy for paired-end massively parallel sequencing following hybridization-based enrichment of the AR locus, which revealed a 48-kb deletion in AR intron 1 in these cells [31]. Interestingly, quantitative analysis of this deletion indicated that the CWR-R1 cell line is heterogeneous, with a variable proportion of cells harboring this deletion. However, long-term culture under castrate conditions resulted in enrichment for this deletion-positive population to near-uniformity. As with the 22RV1 model, the expression level of the AR-V7 variant in the CWR-R1 cell line was directly proportional to the percentage of cells harboring this deletion [31]. Similarly, derivation of discrete subclones from this heterogeneous parental population revealed that high levels of truncated AR variant expression was restricted to the deletion-positive cell population [37].

In addition to these CRPC cell lines, there is also strong evidence to support a role for AR gene rearrangements in clinical CRPC metastases and xenograft tissues derived from clinical CRPC metastases. For example, deletions and duplications involving the AR gene directly alter gene copy number, and imbalances in copy number have been observed across the length of the AR gene in CRPC, but not

primary prostatectomy specimens [30, 31]. The LuCaP 86.2 xenograft was derived following surgical resection of a CRPC bladder metastasis [41], and this tumor displayed reduction in copy number for AR exons 5–7 compared with flanking exons [31]. High-resolution analysis revealed deletion of an 8.5-kb segment encompassing AR exons 5–7 in approximately 50 % of the cells within this tumor. Importantly, LuCaP 86.2 is the tumor model in which the truncated AR v567es variant was originally identified (es refers to exon skipping, whereby the splicing machinery skips AR exons 5–7, see Fig. 1) [41]. Although a clear cause–effect has not been demonstrated, it would be a logical to predict that the cells in this CRPC tumor harboring deletion of AR exons 5–7 are those cells expressing the AR v567es variant. The discovery of AR gene rearrangements may also explain why certain tumors favor expression of specific species of truncated AR splice variants. For example, it is tempting to hypothesize that ARv567es expression occurs in tumors with genomic alterations that impair proper splicing of exons 5–7, whereas AR-V7 overexpression arises through genomic rearrangements that promote more efficient splicing of AR exon CE3.

An alternative, non-mutually exclusive mechanism underlying synthesis of truncated AR splice variants could be acute, ADT-induced changes in regulation of AR splicing. Such a mechanism was revealed when blockade of full-length AR expression or activity in late-passage LNCaP cells or VCaP cells resulted in acute increases in truncated AR-V7 protein levels [36, 42]. These acute splicing changes were rapidly reversed when expression or activity of full-length AR was restored [42]. However, although AR-V7 levels were clearly increased under these conditions, total levels relative to full-length AR remained low and these cells remained dependent on full-length androgen signaling [42]. Nevertheless, given the importance of truncated AR variants in CRPC, it will be important to elucidate the mechanisms that regulate this plasticity in AR splicing and understand the interplay between these mechanisms and AR gene rearrangements in CRPC.

### Clinical Significance

An important role for truncated AR variants in CRPC progression is supported by several studies demonstrating that metastatic CRPC displays high mRNA and protein expression of truncated AR variants compared with hormone-naïve prostatectomy specimens [32–34, 43]. Importantly, western blots of CRPC bone metastases demonstrated that a subgroup of CRPC tumors expressed nearly equivalent levels of full-length and truncated AR variants [34]. Moreover, these tissues displayed a higher AR immunostaining score, a higher proliferative index, and were associated with shorter survival after surgery (2 vs. 8 months) compared

with CRPC tissues that expressed predominantly full-length AR [34]. This finding is in line with another study reporting that prostatectomy specimens displaying above-median AR-V7 expression had a decreased probability of PSA progression-free survival [33].

The expression of truncated AR variants in a subset of CRPC may be especially relevant in light of new second line AR-targeted therapies that have recently been approved for patients with CRPC. Abiraterone and enzalutamide both increase overall survival of patients with CRPC, but resistance that exists *de novo*, or resistance that develops during therapy, is a major limitation for many patients. In mouse xenograft models, abiraterone slowed disease progression, but expression of the truncated AR v567es variant was associated with development of resistance [35]. Similarly, enzalutamide resistance is driven by truncated AR variants in the 22Rv1 and CWR-R1 cell lines, both of which harbor underlying AR gene rearrangements [37], and treatment of late-passage LNCaP cells or VCaP cells with enzalutamide leads to rapid increases in AR-V7 protein expression [36]. A complete understanding of the role of truncated AR variants in driving resistance to abiraterone and enzalutamide in patients will require a detailed and integrated analysis of AR gene structure and mRNA/protein expression patterns in tissues that display progression during treatment with these new agents.

### Biochemical Properties of Truncated AR Variants

Nuclear localization of full-length AR is tightly regulated by ligand binding, and AR exists predominantly in the cytoplasm in the absence of androgens. Classical studies have demonstrated that AR nuclear import is supported by a bipartite nuclear localization signal located in the hinge domain encoded by AR exons 3 and 4 [44]. Therefore, truncated AR variants that do not harbor exon 4-derived sequence would be predicted to remain in the cytoplasm. However, loss of the AR LBD also eliminates the AR nuclear export signal. Recently, it has been shown that the net effect of losing both the canonical AR nuclear import and export signals results in constitutive nuclear localization of truncated AR variants in the absence of ligand [45]. In addition, this constitutive nuclear localization of truncated AR variants does not require full-length AR [45]. However, it has been demonstrated that the AR v567es variant can facilitate ligand-independent nuclear localization of full-length AR through unclear mechanisms [41].

Ectopic expression of truncated AR variants in the androgen-dependent LNCaP cell line drives androgen-independent AR target expression [39, 41, 45] and growth under androgen-depleted conditions [32, 41, 42, 45]. Moreover, androgen-independent growth and AR target gene expression in AR splice variant-driven 22Rv1 and CWR-

R1 cell lines are blocked when truncated AR variants are selectively knocked down. These data indicate that truncated AR variants drive androgen-independent growth by supporting ongoing AR transcriptional activity in CRPC cells. However, it has also been shown that truncated AR variants may have unique target gene specificities, perhaps due to the influence of variant-derived protein sequence located immediately at COOH-terminal to the AR DBD. For example, unique targets have been ascribed to AR-V7, including AKT1 [32] as well as a set of M-phase genes including UBE2C, which is associated with CRPC progression [36]. However, some of these apparent differences may be due to differences in AR signaling output as opposed to true differences in target gene specificity. Indeed, UBE2C and other M-phase-associated target genes appear to be transcriptional targets of both full-length AR as well as truncated AR variants, but these genes display a biphasic pattern with induction at proliferative levels of AR signaling and repression at higher, anti-proliferative levels of AR signaling [37]. More recently, gene expression profiling experiments in CWR-R1 cells were designed to compare genes responsive to truncated AR variants or proliferative doses of DHT. In this set of experiments, the gene expression profile supported by truncated AR variants appeared to be a subset of the broader androgen/AR transcriptional program [37]. In future experiments, it will be important to elucidate the sets of genes that are regulated by truncated AR variants in clinical tissues, as this may reveal new therapeutic targets downstream of the AR signaling axis.

### Exploiting Genome Alterations to Define PCa Subtypes

A major goal of cancer genomics is to understand the genomic changes that underlie the development and progression of PCa. This is expected to improve prognostic and predictive accuracy for PCa patients. In particular, classification of clinically relevant subtypes is likely to prove invaluable in a clinical setting by informing treatment decisions. However, the exact criteria of what constitutes a subtype may be difficult to define. Separating tumors based on mutually exclusive genomic aberrations or a coordinated set of aberrations that accompany distinguishing physiologies and also have bearing on prognostic outcomes and treatment decisions would be ideal. However, even if tumors could be categorized into informative subgroups, PCa is a heterogeneous disease and it is possible that more than one subgroup may be present in a given individual. This is especially relevant in light of the observation that multifocal PCas often have different genomic rearrangements occurring between foci [46]. Additionally, metastatic PCa also appears to have clonal subgroups that respond differently to treatments [47]. Even more daunting is the possibility that mutually exclusive genetic events

defined as subtypes in the development of PCa may not inform treatment decisions or have bearing on prognosis. Furthermore, genetic events that occur as an adaptive response to ADT may be the eminent factors to consider when choosing a therapy; this suggests it may be helpful to group cancers based on subtypes that result from acquired genomic changes causative of ADT resistance.

### PCa Developmental Subtypes

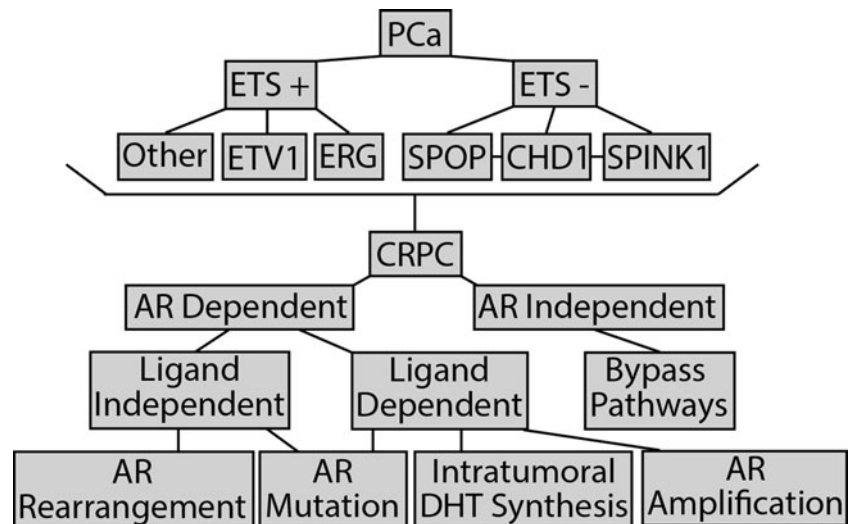
TMPRSS2–ERG fusions occur in roughly half of all PCa. Therefore, one obvious PCa subtype is TMPRSS–ERG+/- or, more generally, the presence or absence of any AR-regulated or otherwise highly active gene promoter fused to any ETS family protein [5]. Recently, genomic studies have provided insights to mutations that appear to be important drivers in ETS– tumors. For example, SPOP mutations appear to be restricted to primary PCa and CRPC that do not harbor TMPRSS2–ERG fusions [7, 22]. Similarly, copy number loss or mutation of CHD1 was found to occur in 5.2 % of tumors but most frequently in ETS– tumors [7]. Of note, CHD1 binds to H3K4me histone marks and is commonly deleted in PCa contributing to an invasive cell phenotype [48]. ETS2, a putative tumor suppressor, is also deleted in tumors with the deletion of the 3 Mb region between TMPRSS2 and ERG; however, this locus is also mutated at some frequency in TMPRSS2–ERG fusion-negative cancers [7].

SPINK1 overexpression is another PCa alteration that appears to be mutually exclusive with ETS+ cancers [49] and constitutes a subtype that occurs in 10 % of all PCas. Notably, SPINK1 shares sequence homology with EGFR suggesting that it could act as an EGFR agonist. Moreover, overexpression studies have demonstrated that SPINK1 mediates increased invasiveness *in vitro* [49], and knock-down of SPINK1 expression with shRNA or antibodies that neutralize SPINK1 have been shown to suppress cellular invasiveness [50]. Additionally, EGFR and SPINK1 suppression has an additive effect in inhibiting cellular invasion in SPINK+/ETS– cell line 22RV1 [50].

### PCa Treatment Subtypes

Genomic changes that occur as a result of ADT may be useful for defining treatment-specific PCa subtypes. Because these would occur later in the natural history of PCa progression, these may be constrained or promoted by preceding genomic alterations that characterize specific PCa developmental subtypes (Fig. 2). For instance, it is unclear whether certain truncated AR variants would be able to interact and assemble into a functional complex with the same co-regulator proteins that interact with full-length AR. An example of this is FOXA1, which interacts with full-length AR via the DNA-binding domain and hinge region

**Fig. 2** The relationship between PCa developmental subtypes is depicted on *top*. The relationship between developmental subtypes and treatment subtypes is largely unknown but subsequent changes to AR in response to ADT can be organized into the hierarchical categories depicted on the *bottom*



[51]. Because the AR hinge region is missing from most truncated AR variants (with the exception of ARv567es), it is possible that FOXA1 pioneer activity may not have the same impact on truncated AR variants as full-length AR. Similarly, the ETS factor ETV1 interacts with the AR ligand binding domain [52], which is absent in all truncated AR variants. Interestingly, many of the studies involving ectopic expression of truncated AR variants have been performed in the LNCaP cell line, which displays overexpression of ETV1, including a study which found that full-length AR was required for androgen-independent function of truncated AR variants [42]. In contrast, DAXX, which is involved in cellular differentiation and proliferation, regulates AR activity by binding to the AR NTD [53]. Therefore, DAXX may be able to interact with and regulate activity of truncated AR variants in a manner similar to full-length AR. Interestingly, SPOP has been shown to bind to and regulate DAXX [54], suggesting a potential interplay between SPOP and the AR in PCa. In addition, SPOP has also been shown to regulate SRC-3, an important AR co-regulator overexpressed in CRPC [55, 56].

Changes to the necessity or nature of AR signaling inductive to CRPC such as bypass pathways or changes to the AR itself such as mutations to change ligand specificity, amplifications to sensitize AR to ligand, and rearrangements that induce truncated AR splice variants may occur regardless of developmental subtypes and potentially be the dominant factor with regard to ADT response [25, 57]. For example, AR amplification can occur regardless of TMPRSS2–ERG fusion or other developmental subtype status [58]. However, it may be significant that AR gene amplification is associated with copy number gain of NCOA2 and a higher overall incidence of genomic copy number alterations [8]. AR gene amplification can also increase AR levels and sensitivity to residual DHT levels and lead to bicalutamide resistance [28]. Alternatively,

inappropriately expressed biosynthetic enzymes that enable intratumoral synthesis of androgens, reviewed extensively elsewhere [59], are likely to drive ongoing AR signaling without structural changes to the AR gene locus. In these cases, it is anticipated that tumors harboring AR gene amplifications or altered expression of DHT biosynthetic enzymes would respond to therapy with abiraterone or enzalutamide. Indeed, enzalutamide was developed with the goal of addressing the challenge of treating CRPC driven by AR protein overexpression.

In contrast to amplification- or mutation-based alterations in full-length AR activity, evidence suggest that cancers driven by truncated AR variants may not require full-length AR and would thus be resistant to treatments targeting the LBD or androgen synthesis [22, 36, 37]. This highlights the need to develop drugs that target the AR NTD or DBD, which are encoded by the first three exons of the AR gene. One such drug is EPI-001, which has been shown to bind the AR NTD and prevent transcriptional activity of full-length AR by blocking interactions with important AR coactivators [60]. Additionally, changes in growth factor signaling axes such as EGFR or IGF1, reviewed elsewhere [25], may activate AR signaling in the absence of ligand or bypass AR signaling altogether by activating mitogenic signaling pathways and render tumors resistant to antiandrogen therapies. Importantly, AR gene amplification events and AR gene rearrangement events may not be mutually exclusive, as AR gene copy number imbalances have been observed in CRPC xenografts and clinical metastases displaying overall increases in AR copy number [30, 31].

### Future Perspectives

Recent advances in technology and increased sophistication have illuminated complex changes that occur in PCa and

possible patterns that can be exploited therapeutically. However, AR alterations that drive ADT resistance, such as point mutations, copy number increases, and intragenic rearrangements have not been linked to other contextual alterations in the PCa genome. Whole-genome sequencing on CRPC metastases is anticipated to yield insights into the context and patterns of resistance to the expanding repertoire of drug options for ADT.

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