Review

Androgen receptor co-regulation in prostate cancer

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Abstract Prostate cancer (PCa) progression relies on androgen receptor (AR) action. Preventing AR's ligand-activation is the frontline treatment for metastatic PCa. Androgen deprivation therapy (ADT) that inhibits AR ligand-binding initially induces remission but eventually fails, mainly because of adaptive PCa responses that restore AR action. The vast majority of castration-resistant PCa (CRPC) continues to rely on AR activity. Novel therapeutic strategies are being explored that involve targeting other critical AR domains such as those that mediate its constitutively active transactivation function, its DNA binding ability, or its interaction with co-operating transcriptional regulators. Considerable molecular and clinical variability has been found in AR's interaction with its ligands, DNA binding motifs, and its associated coregulators and transcription factors. Here, we review evidence that each of these levels of AR regulation can individually and differentially impact transcription by AR. In addition, we examine emerging insights suggesting that each can also impact the other, and that all three may collaborate to induce gene-specific AR target gene expression, likely via AR allosteric effects. For the purpose of this review, we refer to the modulating influence of these differential and/or interdependent contributions of ligands, cognate DNA-binding motifs and critical regulatory protein interactions on AR's transcriptional output, which may influence the efficiency of the novel PCa therapeutic approaches under consideration, as co-regulation of AR activity.
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Peer review under responsibility of Second Military Medical University.
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1. Introduction

In 2019, more than 31 000 men are expected to die from prostate cancer (PCa) in the United States alone [1]. With few exceptions, these deaths will be due to resistance to systemic treatments for metastatic disease. Ligand activation of the androgen receptor (AR) is the main target for therapy of non-organ-confined PCa [2–6]. The frontline therapy, androgen deprivation therapy (ADT), interferes with androgen production and/or utilizes antiandrogens that compete with androgen binding to AR. ADT initially induces remission, for which the duration is highly variable among patients, but inevitably fails and gives rise to castration-resistant PCa (CRPC). CRPC continues to rely on AR because of several adaptive tumor responses that facilitate ligand availability and ligand activation of AR or foster the emergence of AR forms that are ligand-independent or have acquired broader ligand sensitivity [2]. Novel more potent second generation ADT drugs such as abiraterone, enzalutamide and apalutamide can prolong CRPC survival by a few months, but none are curative [7,8]. Ultimately, resistance to these agents also develops while for the majority of patients PCa growth still depends on AR action. In a minority of patients, prolonged exposure to these potent ADT drugs induces poorly differentiated and more aggressive neuroendocrine PCa (NEPC) that is even more difficult to treat [9]. All PCa that fails ADT is essentially fatal.

In view of this sustained importance of AR action in CRPC, alternative approaches to block AR function have been sought. These include the development of drugs against AR domains that are not directly involved in its ligand binding. For instance, EPI compounds target the constitutively active ligand-independent transcription activation function in AR's N-terminal domain (NTD). The small molecule EPI analogues were isolated after screening a library of marine sponge Geodialindgreni extracts. This library was explored for compounds that inhibit both ligand-dependent and ligand-independent AR activation, specifically by interacting with and blocking transactivation of the AR NTD. EPI-001-related compounds structurally resemble bisphenol A diglycidic ether, suggesting that they may be of industrial origin and accumulate in the sponges via contaminated seawater. EPI compounds, which prevent important AR-coregulator interactions, were promising in reducing clinically relevant AR-dependent mechanisms of treatment resistance and restricted PCa growth in preclinical studies [10]. However, a clinical trial using EPI-506 in CRPC setting was terminated early (NCT02606123). Other approaches have involved the design of small molecules (such as Vancouver Prostate Center [VPC] compounds) to selectively target a surfaced exposed pocket on the AR DNA-binding domain (DBD) as an alternative drug-target site for AR inhibition. These molecules effectively blocked transcriptional activity of full-length and splice variant AR forms at low to sub-micromolar concentrations. This was achieved via interference with AR-DNA interactions and by preventing recruitment of AR to chromatin, resulting in decreased cell viability in multiple PCa cell line models [11]. VPC agents are currently undergoing clinical testing in men suffering from CRPC. Other approaches have been designed to disrupt the interaction between AR and AR-associated coregulators or secondary transcription factors, or to directly target the activity of such AR-collaborators that are essential to achieve its full transcriptional output. The latter efforts have yielded a multitude of peptides, peptidomimetics, small molecule inhibitors [12]. Most of these showed promise as PCa therapeutics when tested preclinically, and several are currently in or have completed clinical trials. Yet other strategies aim to target AR stability overall without focusing on a specific AR function(s) or domain(s). Some of these drugs, including niclosamide, or its more bioactive derivatives [13,14], decrease levels of full-length as well as ligand-independent AR variant (AR-V) forms that emerge under ADT, and are currently tested clinically in men. The success rate for clinical transition and ultimate patient benefit for a novel AR-targeting compound is unpredictable, but to our knowledge not substantially different from that for any other drugs under development.

Apart from attempts to decrease AR stability and expression, most alternative CRPC therapeutic approaches that are currently pursued target the three key determining events needed to achieve full AR-dependent transactivation at AR target genes: 1) Ligand-dependent transcriptional activation of AR mediated by its ligand-binding domain (LBD) or, relevant to CRPC, ligand-indifferent AR transcription function that is contained in its NTD; 2) binding of AR to its consensus binding sites at AR target genes via the AR DBD, and 3) context-dependent and gene-specific regulation of AR's transactivation function at target genes by coregulators or other transcription factors that interact with multiple AR domains [15]. A growing body of evidence, however, indicates molecular and clinical variability in these three regulating aspects of AR activation, which can occur independently but is not necessarily mutually exclusive, to influence AR action (Fig. 1). In view of the increasing interest in PCa treatments that target these aspects of AR function and AR domains, a review of their control over AR activity and the potential interplay among these determinants is timely. The goal of the current overview is to explore the reported variation, *in vitro* and *in vivo*, in the contribution of 1) AR ligands, 2) composition and extent of AR binding sites, and 3) AR-collaborating transcriptional regulators. We will consider also evidence for reciprocal effects of each of these aspects on the other(s) and, ultimately, the global output of AR that modulates PCa aggressiveness. For the purpose of this review, we will broadly define the impact of one or more of these determinants of AR's function on AR's transcriptional output at target genes as co-regulation of AR.

2. Key determinants of AR transcriptional activity

2.1. Ligands that bind AR

AR is a member of the steroid hormone-activated nuclear receptor family of transcription factors [15,16]. AR is preferentially activated by androgens, its cognate ligands. The most bioactive AR ligand, dihydrotestosterone (DHT), is
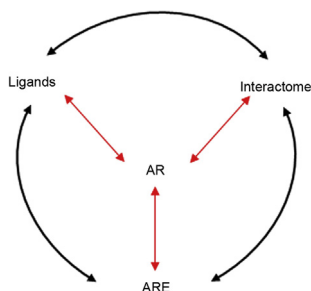
Figure 1   Key determinants of AR transcriptional activity. AR transcriptional output requires ligand activation, binding to cognate DNA binding motifs known as AREs, and interaction with coregulators and secondary transcription factors (interactome). Interactions between these three determinants may fine-tune AR transcription factor function at target genes. AR, androgen receptor; AREs, androgen response elements.

mainly derived from circulating testosterone that is intracellularly converted into its more active metabolite [2,15]. Both testosterone and DHT directly bind AR, but DHT does so with higher affinity, and its biological activity exceeds that of testosterone up to ten times. The latter effect has been attributed DHT's dissociation from the AR, which occurs more slowly than testosterone, and the observation that AR bound to DHT is more stable and persists in cells for greater lengths of time [17,18]. Other immature DHT precursors are synthesized by the adrenals and, after uptake in PCa cells, can be intracellularly metabolized to DHT via a number of biochemical pathways, including the canonical, 5alpha-dione, and backdoor pathways [2]. Other reports indicate the possibility of *de novo* androgen synthesis pathways that start from cholesterol synthesized by PCa cells [19]. Most precursor androgens can transcriptionally activate AR. Although their intracellular anabolism to DHT may be a likely explanation for their effect on AR, at least some of these DHT precursors and metabolites, such as androstenediol and androsterone, have been demonstrated to also directly bind AR [20]. In addition to androgens, other steroids, including for instance estrogens, progestins, glucocorticoids and mineralocorticoids can interact directly with AR and modulate its activity. The use of ADT drugs that inhibit different sites of androgen synthesis and act at distinct steps of androgen bioconversion pathways gives rise to build-up of androgen precursors that are then shunted into alternate steroid biosynthesis pathways. For instance, blocking CYP17A1 activity by abiraterone acetate, prevents pregnenolone's downstream maturation to testosterone and DHT; instead build-up of pregnenolone is used as substrate for progesterone synthesis. The latter results in alternative route of AR activation that may bypass the effect of ADT, particularly when PCa expresses AR with a point mutation in LBD that broadens ligand specificity [21]. Alternatively, glucocorticoids or mineralocorticoids that are administered to alleviate side effects induced by ADT drugs can also directly bind and activate AR [20], which may decrease the intended therapeutic benefit of the administered treatment. Apart from these steroids, a number of synthetic ligands and environmental agents can interact with AR [20]. It is not surprising that AR antagonists

such as enzalutamide [22] that were rationally designed to inhibit AR by competing with ligand binding interact with AR. More unexpectedly, several CYP17A1 inhibitors (including seviteronel, galeterone, abiraterone and ketoconazole), which were developed to interfere with a critical biochemical step in precursor androgen conversion, have also been found to directly bind AR [23]. In addition, at least one of these drugs, abiraterone, undergoes extensive intraPCa metabolism. At least seven abiraterone metabolites can be formed and each show varying extents of AR agonism and antagonism [24]. Androgens are not required for life, but are main mediators of the male phenotype and as such have effects also on many non-prostate-related tissues and functions, including muscle, bone, and cognition. These tissue-specific effects of androgens and the significant side effects that result from broad complete AR inhibition [25] have led to the development of selective androgen receptor modulators (SARMs) [26], which were designed to exert tissue-selective AR agonist/antagonist functions. These drugs bind AR directly and some, *e.g.* MK-4541, inhibit PCa-growth while showing anabolic effects towards skeletal muscle [27]. In addition to natural ligands and drugs that interact with AR, a number of environmental factor or so-called endodisruptors can bind AR with consequences for its activity [20]. As an example, bisphenol A is a chemical used as starting product synthesis of plastics, and a xeno-estrogen, which was recognized as a direct AR agonist [20].

As discussed in more detail below, binding to AR by compounds other than its preferred ligand DHT can alter AR's transcriptional activity at target genes *in vitro*. Differential ligand availability may have thus important implications for level of AR action in clinical PCa and the efficacy of AR-targeting treatments. Expression patterns of AR target genes are known to vary widely among PCa patients; this has been observed in PCa tissues from patients who did not yet receive ADT as well as in men suffering from CRPC [28,29]. Exposure to environmental factors that influence AR activity may explain such inter-patient variability. As potential alternative explanation, multiple groups have reported on germline mutations (*e.g. HSD3B1*) [30], or differences in the expression levels (*e.g.* SRD5A1), [31] or somatic alterations (*e.g.* HSD17B4), [32] that impact steroidogenic genes. It has been suggested that ADT may enhance such inter-patient variability, as it can induce the expression of steroidogenic enzymes such as AKR1C3 and HSD3B2 [31]. The frequency of point mutations affecting these genes has also been reported to increase in CRPC. Since most of these findings were derived from NextGen analyses on a relatively small number of tissues, we reviewed the incidence of somatic mutations in 55 genes involved in synthesis, metabolization, degradation, and transport of androgenic steroids [33] in several larger genomic datasets that are now available and that assessed tissues from ≥100 patients each via genome wide sequencing approaches. For only eight of 55 genes, no mutations had been reported in ADT-naïve PCa and CRPC tissues. The remaining 47 genes were somatically mutated at least one PCa stage. Consistent with known incidence of genomic alterations in PCa, all of these mutations occurred at low incidence and followed a "long tail" distribution, in which no mutation was present in more than 3% of cases.

Some did show enrichment in CRPC, such as ATP-binding cassette transporter ABCG5 and several solute carrier organic anion transporter family members, including SLCO2B1. Although the true significance of such low incidence findings is difficult to infer, these data do suggest enrichment for somatic alterations in genes that impact AR ligand availability. The continuing and steady increase in the number of PCa cases subjected to NextGen sequencing will facilitate defining the clinical implications of these mutations, or specific combinations of mutations, on AR ligand synthesis and AR activity in clinical PCa. In combination with environment, genotype and a PCa's specific genetic alterations, the choice for specific ADT drugs and sequencing or combination of treatments may lead to considerable inter-individual differences in intraPCa androgen levels and AR agonist and antagonist availability.

2.2. Genomic AR binding motifs and AR cistromes

Activated AR binds to cognate DNA motifs, which are known as androgen response elements (AREs). The generally accepted consensus motif is an inverted repeat of a 5′-TGTTCT-3′ hexamer sequence, which is separated by three base pairs. Thorough documentation and analyses of genome-wide AR binding peaks isolated via ChIP-chip, ChIP-Seq or ChIP-exo approaches over the last dozen or so years have demonstrated decreased stringency for ARE sequences. When interpreting results from these ChIP-based studies, it is important to keep in mind that each group analyzed the resulting AR binding peaks for AREs using their own software settings and cut-off criteria. Nonetheless, the presence of canonical AREs, usually defined as 15 bp full-length sequence, ranged from about 2.5% to 54% among studies [34—39]. These studies suggested also that AR binding to AREs allows for one to three bases degeneration in the consensus ARE motif, differences in orientation of the hexamer half sites, more variable spacing between these half sites, or in some cases just one ARE half site/hexamer, the latter possibly in combination with recognition motifs for other transcription factors such as FoxA1 [12]. In addition to liberty in the specific sequence of DNA motif to which AR binds, it is now clear that the scope and composition of the AR cistrome, i.e. the entirety of genomic AR binding sites, within PCa cells are highly variable. The number and specific location of AR binding sites differs between cell lines, between cell lines that represent different stages of PCa progression, especially before and after ADT, exposure to AR agonists and/or antagonists, and can be influenced by the specific AR form(s) expressed [34—36,39—42]. With respect to the latter, the AR cistromes for full length AR or AR variants (AR-Vs) such as AR-V7 and ARv567es may vary in overlap or exclusivity depending on cell lines and experimental conditions used. In clinical PCa, similar shifts in AR cistrome have been observed between ADT-naïve and ADT-recurrent PCa, in which ARE surrounding areas are selectively enriched also for other transcription factor motifs between PCa stages. Prior to ADT, motifs for HoxB13 are for instance enriched [43], whereas binding sites for STAT and NFκB are more frequently found adjacent to AREs in CRPC [44]. Striking diversity in the composition of AR cistromes can be seen even in clinical PCa from the same stage of disease, in which a relatively low overlap in genome-wide AR binding sites, for instance 25% in a study on ADT-naïve PCa [45], is considered relevant. In an AR-V7 cistrome analysis on CRPC biopsies, the overlap in binding sites was even more limited [38]. In clinical PCa, Genome-Wide Association Studies (GWAS) analyses and whole genome sequencing have revealed the presence of SNPs in AREs, which can affect the ability of AR to bind these sites, or similar function-altering mutations in the binding motifs for AR-associated transcription factors [46,47].

2.3. AR-associated coregulators and transcription factors

Like many transcription factors, AR relies on functional and structural interactions with other transcriptional regulators to ensure proper transcription of its target genes [12,15]. Some of these AR-associated proteins clearly display the characteristics of a coregulator, i.e. a protein that does not bind DNA butis recruited to DNA-bound transcription factors and modifies transcription of their target genes by influencing chromatin accessibility or transactivation function. Such proteins function, for instance, as chromatin modifiers (such as SMARCA4 [48]) or serve as protein scaffold (e.g.PELP1 [49]). Other proteins do exert AR coregulator functions but are better known for other roles, for instance in the regulation of endocytosis (e.g. HIP-1 [50]) or apoptosis (e.g.Par-4 [51]). To date, more than 280 proteins have been isolated as AR-interacting coregulators that can influence either positively (coactivators) or negatively (corepressors) [15,33] transcription of AR target genes. Our previous work indicates that, quite often, the direction of the coregulator effect on target gene expression, up or down, occurs in a context-dependent and gene-specific manner [52]. Collectively, AR-associated coregulators exhibit a large diversity in biological functions and pathways, underscoring the many aspects of cell biology that contribute to fine-tuning AR's activity [15]. Similarly, considerable functional variability has been noted also for the transcription factors that help regulate AR's transcriptional output. Some bind their own recognition motifs in close proximity (range: 0—1000 bp) of AR binding peaks, while others are recruited to AR as cofactor, or both [12,15]. In addition to transcription factors that are more broadly involved in regulation of gene expression such as AP1 or Sp1 [15], AR interacts also with other more specialized factors such as the stem cell transcription factor Nanog [53]. Although Nanog is not typically thought of as having major roles in the endocrine response of adenocarcinoma cells, the enrichment for Nanog binding sites close to some AREs has been independently confirmed [52]. Analyses of protein-protein and protein-DNA interactions and DNA sequences nearby AREs, support involvement of dozens of functionally diverse transcription factors in regulating the AR transcriptional complex [12,15]. In clinical PCa, it has long been recognized that multiple AR-associated coregulators are differentially expressed, and mostly overexpressed, compared to benign prostate tissue. In several cases, such deregulated expression correlates with worse progression and outcome, and is increased further in CRPC

[54]. Somatic alterations and mutations in coregulator genes such as *NCoA2* (amplified [55]) and *NCoR1* (point mutation [32]) have been reported in clinical PCa also. Previous work has suggested that somatic mutations (*e.g.FoxA1* [32,56]), copy number alterations (*e.g. GATA2* [32]), and gene rearrangements (*e.g. ERG* [56]) in AR-associated transcription factors can be more prevalent in CRPC than in ADT-naïve PCa. We reviewed the same whole exome sequencing studies on more than 100 patient specimens that were analyzed above (Table 1) for evidence of somatic mutations in AR-associated *NCoA* and *NCoR* coregulators [33]. For each of the coregulator genes studied, such alterations were present in at least one ADT-naïve PCa study and at least one CRPC study. Mutations in these genes occurred also at low incidence and were present in less than 3% of cases. Some, such as those impacting NCoR2 did show enrichment in CRPC (Table 2) compared to ADT-naïve PCa, suggesting increasing prevalence with PCa progression.

3. Determinants of AR activation impact AR target gene transcription

3.1. Regulation of AR-dependent transcription by different ligands

Androgens such as testosterone and DHT are known to exert different effects during male development [57]. Since the isolation of point mutations in the AR LBD that emerge mainly under the selective pressure of ADT [58] and may impact on AR's ligand sensitivity, there has been a lot of interest in the contribution of different ligands to intraPCa AR activity. A number of approaches have been undertaken to define variability in ability of different ligands to activate AR. Some included determining the conformational changes within AR that can be indicative of its transcriptional activation, more specifically ligand-induced interaction between its NTD and LBD, which generates a platform for coregulator interaction [59]. These domain interactions can be queried using techniques such as AR mammalian-2-hybrid assays. More direct approaches involve measuring expression levels of AR target genes via promoter reporter assays or a variety of more sophisticated candidate gene or genome-wide gene expression profiling. Because of the above mentioned intracellular androgen metabolism, it can be difficult to determine the impact of androgen precursors on transcriptional activity of AR. However, the extensive number of studies done so far, do support differences in regulation of AR-dependent gene expression by different mature and precursor androgens. In line with expectation, opposite effects by AR agonists and antagonists have also been observed. In the most elaborate of these studies, up to 95 different AR-binding compounds were tested in of a mammalian-2-hybrid assay measuring NTD-LBD interactions, the activity of a promoter-reporter construct derived from the Mouse Mammary Tumor Virus (*MMTV*) gene and a small signature of AR target genes [60]. The compounds tested represented a variety of both natural and synthetic AR agonists, antagonists, and SARMs. Marked differences were noted in all three assays, even among ligands of the same class, *i.e.* (partial) agonists. Another study by the same

group noted overlap in the identity of genes whose expression was affected after treatment with mechanistically different AR ligands, but found marked differences in the kinetics of altered gene expression for distinct compounds. Importantly, these differences in kinetics were associated with differential regulation of distinct physiological pathways in PCa cells [61]. Along the same lines, the seven metabolites of abiraterone that are generated via intraPCa metabolism showed varying degrees of AR antagonism or agonism. Notably, the majority of genes impacted by the most active metabolite, 5alpha-abiraterone (5α-abi), were regulated also by DHT [24]. These findings indicate that different AR ligands, even those belonging to same class (agonist or antagonist) can have markedly differential effects on the transcriptional output by AR.

3.2. Regulation of AR-dependent transcription by AREs

Soon after the identification of the first AREs, it was recognized that even when cells were stimulated with the same AR agonist, AR could bind to distinct classes of AREs. Initially, distinctions were made for instance between AREs that were also responsive to GR ("classical" AREs) whereas others were selective for AR only ("selective" AREs), or based on differential responses to testosterone and DHT [62—64]. Variation in the number of AREs per gene were also noted, and it was suggested that differential organization of AR binding sites among genes may explain, for example, the variations in the magnitude of androgen regulation or the kinetics of androgen response among AR target gene transcripts [63]. That such different kinetics reflect variation in the molecular underpinnings of AR transactivation was supported in more recent studies integrating AR ChIP-Seq and gene expression profiling, which revealed distinct patterns of "early" and "late" androgen regulation of ARE-containing genes [39]. The exact molecular machinery that controls kinetics of resulting AR-dependent transcriptomes remains to be determined. However, notable differences were found in the pattern of co-recruitment of AR and components of transcriptional machinery between these gene sets, in which for instance overlap between AR and serine 5-phosphorylated RNA polymerase II binding was enriched at androgen-induced transcripts. Other evidence for functionally diverse classes of AREs was derived from the systemic isolation of the AR cistrome in mitotically active cells that were stimulated with the same ligand, and arrested at different stage of the cell cycle. This work revealed the existence of cell cycle stage-selective as well as overlapping AR cistromes and AR-dependent transcriptomes, and showed differential and selectively enrichment for other transcription factor binding motifs in each cell cycle stage-specific set of AR binding sites [41]. The impact of different agonists on the PCa AR cistrome remains to be fully determined. Most AR-dependent cistromes have been derived using just one agonist, mostly DHT or the synthetic androgen R1881. While some overlap in AR cistromes between studies has been reported, the full extent of the overlap, or mutual exclusivity, is not always easy to compare due to the use of different protocols and

Table 1 Somatic mutations in steroidogenic genes in clinical PCa tissues. COSMIC and cBIO databases were queried for somatic mutations in genes that encode protein involved in the synthesis, metabolism, and transport of androgens [28]. The COSMIC database was queried via the Cancer browser, selecting for "prostate cancer" and "carcinoma" histology, and retrieving only data from genome-wide sequencing efforts. For cBIO analysis, datasets analyses are marked by first author of study that was examined.

Genes	COSMIC		cBIO													
	#	ADT-naïve n=1 976 %	Barbieri #	ADT-naïve n=112 %	Fraser #	ADT-naïve n=477 %	TCGA #	ADT-naïve n=333 %	Armenia #	+ CRPC n=1 013 %	Abida #	CRPC n=444 %	Beltran #	CRPC n=114 %	ADT-naïve n=922 average%	CRPC n=558 average%
ABCG5	9	0.46	0	0.00	0	0.00	0	0.00	5	0.49	6	1.35	1	0.88	0.00	1.11
ABCG8	5	0.25	0	0.00	0	0.00	1	0.30	5	0.49	4	0.90	0	0.00	0.10	0.45
AKR1C1	5	0.25	0	0.00	0	0.00	1	0.30	3	0.30	1	0.23	0	0.00	0.10	0.11
AKR1C2	1	0.05	0	0.00	0	0.00	0	0.00	1	0.10	2	0.45	0	0.00	0.00	0.23
AKR1C3	1	0.05	0	0.00	0	0.00	0	0.00	3	0.30	1	0.23	1	0.88	0.00	0.55
AKR1C4	1	0.05	0	0.00	0	0.00	0	0.00	3	0.30	2	0.45	1	0.88	0.00	0.66
CYP11A1	8	0.40	0	0.00	0	0.00	0	0.00	2	0.20	1	0.23	0	0.00	0.00	0.11
CYP11B1	8	0.40	1	0.89	0	0.00	0	0.00	10	0.99	8	1.80	2	1.75	0.30	1.78
CYP11B2	7	0.35	0	0.00	0	0.00	1	0.30	8	0.79	6	1.35	0	0.00	0.10	0.68
CYP17A1	1	0.05	0	0.00	0	0.00	0	0.00	2	0.20	1	0.23	0	0.00	0.00	0.11
CYP19A1	7	0.35	0	0.00	2	0.42	1	0.30	3	0.30	1	0.23	0	0.00	0.24	0.11
CYP1A1	5	0.25	1	0.89	0	0.00	1	0.30	5	0.49	2	0.45	1	0.88	0.40	0.66
CYP1B1	4	0.20	0	0.00	0	0.00	0	0.00	3	0.30	4	0.90	0	0.00	0.00	0.45
CYP21A2	8	0.40	0	0.00	0	0.00	1	0.30	3	0.30	2	0.45	0	0.00	0.10	0.23
CYP3A4	1	0.05	0	0.00	0	0.00	0	0.00	3	0.30	1	0.23	0	0.00	0.00	0.11
CYP3A43	2	0.10	0	0.00	0	0.00	2	0.60	3	0.30	2	0.45	0	0.00	0.20	0.23
CYP3A5	2	0.10	1	0.89	1	0.21	0	0.00	4	0.39	1	0.23	1	0.88	0.37	0.55
CYP3A7	3	0.15	0	0.00	0	0.00	0	0.00	2	0.20	1	0.23	0	0.00	0.00	0.11
HSD17B1	0	0.00	0	0.00	0	0.00	0	0.00	1	0.10	0	0.00	1	0.88	0.00	0.44
HSD17B10	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0.00	0.00
HSD17B11	1	0.05	0	0.00	0	0.00	2	0.60	0	0.00	0	0.00	0	0.00	0.20	0.00
HSD17B12	2	0.10	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0.00	0.00
HSD17B13	1	0.05	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0.00	0.00
HSD17B14	2	0.10	0	0.00	0	0.00	0	0.00	2	0.20	2	0.45	1	0.88	0.00	0.66
HSD17B2	1	0.05	0	0.00	0	0.00	0	0.00	3	0.30	3	0.68	0	0.00	0.00	0.34
HSD17B3	14	0.71	0	0.00	0	0.00	0	0.00	4	0.39	2	0.45	0	0.00	0.00	0.23
HSD17B4	4	0.20	0	0.00	0	0.00	1	0.30	5	0.49	3	0.68	0	0.00	0.10	0.34
HSD17B6	2	0.10	0	0.00	1	0.21	0	0.00	2	0.20	1	0.23	1	0.88	0.07	0.55
HSD17B7	2	0.10	0	0.00	0	0.00	0	0.00	0	0.00	1	0.23	0	0.00	0.00	0.11
HSD17B8	1	0.05	0	0.00	0	0.00	0	0.00	1	0.10	0	0.00	0	0.00	0.00	0.00
HSD3B1	5	0.25	0	0.00	0	0.00	1	0.30	2	0.20	0	0.00	0	0.00	0.10	0.00
HSD3B2	1	0.05	0	0.00	0	0.00	1	0.30	3	0.30	1	0.23	0	0.00	0.10	0.11
HSD3B7	6	0.30	1	0.89	0	0.00	0	0.00	4	0.39	2	0.45	0	0.00	0.30	0.23
RDH15	0	0.00	0	0.00	0	0.00	0	0.00	1	0.10	1	0.23	0	0.00	0.00	0.11
RDH16	0	0.00	0	0.00	0	0.00	0	0.00	3	0.30	2	0.45	0	0.00	0.00	0.23
RDH5	1	0.05	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0.00	0.00

Gene	#	%	#	%	#	%	#	%	#	%	#	%	#	%	Avg %	Avg %
SHBG	2	0.10	0	0.00	0	0.00	1	0.30	1	0.10	1	0.23	0	0.00	0.10	0.11
SLCO1A2	5	0.25	0	0.00	1	0.21	0	0.00	6	0.59	2	0.45	0	0.00	0.07	0.23
SLCO1B1	6	0.30	0	0.00	1	0.21	1	0.30	4	0.39	0	0.00	0	0.00	0.17	0.00
SLCO1B3	5	0.25	0	0.00	0	0.00	0	0.00	2	0.20	1	0.23	0	0.00	0.00	0.11
SLCO1C1	5	0.25	0	0.00	0	0.00	1	0.30	4	0.39	1	0.23	0	0.00	0.10	0.11
SLCO2A1	5	0.25	1	0.89	0	0.00	1	0.30	4	0.39	5	1.13	0	0.00	0.40	0.56
SLCO2B1	7	0.35	0	0.00	0	0.00	1	0.30	8	0.79	3	0.68	6	5.26	0.10	2.97
SLCO3A1	18	0.91	0	0.00	0	0.00	0	0.00	9	0.89	3	0.68	1	0.88	0.00	0.78
SLCO4A1	9	0.46	0	0.00	0	0.00	0	0.00	5	0.49	3	0.68	2	1.75	0.00	1.22
SLCO4C1	8	0.40	4	3.57	1	0.21	0	0.00	12	1.18	5	1.13	0	0.00	1.26	0.56
SLCO5A1	11	0.56	0	0.00	0	0.00	1	0.30	5	0.49	5	1.13	0	0.00	0.10	0.56
SLCO6A1	12	0.61	1	0.89	0	0.00	1	0.30	14	1.38	9	2.03	0	0.00	0.40	1.01
SRD5A1	1	0.05	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0.00	0.00
SRD5A2	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0.00	0.00
SRD5A3	3	0.15	0	0.00	1	0.21	0	0.00	3	0.30	3	0.68	0	0.00	0.07	0.34
STAR	3	0.15	0	0.00	0	0.00	0	0.00	2	0.20	2	0.45	1	0.88	0.00	0.66
UGT2B15	2	0.10	0	0.00	0	0.00	3	0.90	3	0.30	0	0.00	0	0.00	0.30	0.00
UGT2B17	3	0.15	0	0.00	0	0.00	0	0.00	4	0.39	0	0.00	0	0.00	0.00	0.00
UGT2B7	2	0.10	0	0.00	2	0.42	1	0.30	7	0.69	1	0.23	1	0.88	0.24	0.55

Corresponding cBIO annotations is as follows: Barbieri, Prostate Adenocarcinoma (Broad/Cornell, Nature Genetics 2012); Fraser, Prostate Adenocarcinoma (CPC-Gene, Nature 2017); Armenia, Prostate Adenocarcinoma (MSKCC/DFCI, Nature Genetics 2018); Abida, Metastatic Prostate Adenocarcimoma, SU2C/PCF Dream Team, PNAS 2019; Beltran, Neuroendocrine Prostate Cancer (Multi-institute, Nature Medicine 2016). Note that the Armenia et al. study includes data from 680 ADT-naïve and 333 CRPC cases; data from this study have not been taken into consideration in determining average %.

#, number of somatic mutations/study that affect the gene for which the symbol is listed.

%, percentage of cases/study that harbor somatic mutations that affect the gene for which the symbol is listed.

Average %, average of percentage of mutations from studies on either ADT-naïve PCa or CRPC specimens; ADT, androgen deprivation therapy; CRPC, castration-resistant PCa; PCa, prostate cancer.

Table 2 Somatic mutations in AR-associated NCoA and NCoR coregulators in clinical PCa. COSMIC and cBio databases were queried for somatic mutations in genes that encode AR-associated NCoAs and NCoRs (28). The COSMIC database was queried via the Cancer browser, selecting for "prostate cancer" and "carcinoma" histology, and retrieving only data from genome-wide sequencing efforts. For cBIO analysis, datasets analyses are marked by first author of study that was examined.

Genes	COSMIC		cBIO													
	#	ADT-naïve n=1 976 %	Barbieri #	ADT-naïve n=112 %	Fraser #	ADT-naïve n=477 %	TCGA #	ADT-naïve n=333 %	ADT-naïve Armenia #	+CRPC n=1 013 %	Abida #	CRPC n=444 %	Beltran #	CRPC n=114 %	ADT-naïve n=922 average%	CRPC n=558 average%
NCOA1	10	0.51	0	0.00	0	0.00	2	0.60	6	0.59	2	0.45	0	0.00	0.20	0.23
NCOA2	9	0.46	2	1.79	1	0.21	1	0.30	8	0.79	4	0.90	0	0.00	0.77	0.45
NCOA3	11	0.56	1	0.89	3	0.63	2	0.60	6	0.59	2	0.45	1	0.88	0.71	0.66
NCOA4	0	0.00	0	0.00	0	0.00	2	0.60	3	0.30	1	0.23	0	0.00	0.20	0.11
NCOA6	17	0.86	1	0.89	1	0.21	2	0.60	10	0.99	4	0.90	0	0.00	0.57	0.45
NCOR1	19	0.96	2	1.79	4	0.84	6	1.80	26	2.57	13	2.93	2	1.75	1.48	2.34
NCOR2	28	1.42	1	0.89	1	0.21	0	0.00	23	2.27	14	3.15	1	0.88	0.37	2.02

Corresponding cBIO annotations is as follows: Barbieri, Prostate Adenocarcinoma (Broad/Cornell, Nature Genetics 2012); Fraser, Prostate Adenocarcinoma (CPC-Gene, Nature 2017); Armenia, Prostate Adenocarcinoma (MSKCC/DFCI, Nature Genetics 2018); Abida, Metastatic Prostate Adenocarcimoma, SU2C/PCF Dream Team, PNAS 2019; Beltran, Neuroendocrine Prostate Cancer (Multi-institute, Nature Medicine 2016). Note that the study of Armenia et al. includes data from 680 ADT-naïve and 333 CRPC cases; data from this study have not been taken into consideration in determining average %.

#, number of somatic mutations/study that affects the gene for which the symbol is listed.

%, percentage of cases/study that harbor somatic mutation that affects the gene for which the symbol is listed.

Average %, average of percentage of mutations from studies on either ADT-naïve PCa or CRPC specimens; ADT, androgen deprivation therapy; CRPC, castration-resistant PCa; PCa, prostate cancer.

platforms, and the possibility that different AR forms expressed between cell line models confounds results. A recent ChIP-exo study did directly compare the effect of agonist and antagonist stimulation, and reported that antagonist-bound AR can also bind AREs, and that nature and composition of these sequences is different from agonist and differs between antagonist depending on ADT drug (bicalutamide or enzalutamide) used [37].

3.3. Regulation of AR-dependent transcription by AR-associated coregulators and transcription factors

To date, several hundreds of AR interactors have been isolated [15,33,65]. In this respect, it is important to note that the majority of AR-associated coregulators do not have an exclusive relationship with AR, but interact also with other transcription factors. It has been clear for a while that different coregulators differentially impact on the androgen response of AR target genes. Whether competition between AR and other transcription factors for a limited cellular pool of a particular coregulator contributes to these findings remains to be resolved. However, silencing or overexpression of the same coregulator was found to have distinct effects on androgen induction of promoter-reporter constructs derived from different ARE-driven genes. These observations were confirmed in studies on small numbers of direct AR target genes. Genome-wide gene expression profiling has shown also that individual loss of a given coregulator generally impacts on a fraction of androgen-regulated genes, but not all [12,52]. A recent study from our group revealed context-dependent and gene-selective modulation of androgen response of direct AR target genes by 18 coregulators, which impacted differentially also on associated biology and PCa stage [52]. The 18 coregulators were selected from more than 180 because their overexpression in PCa, compared to benign prostate, correlated with aggressive PCa features such as larger tumor volumes at the time of radical prostatectomy and higher PCa recurrence after such surgery. These characteristics suggested that these 18 coregulators may be most relevant to clinical PCa. The behavior of the resulting coregulator-dependent AR target gene sets during PCa progression varied widely. As an example, expression of WDR77- and AR-dependent transcriptome remained equally high in CRPC as in localized ADT-naïve PCa, in contract to the HTATIP2-dependent AR target gene signature whose expression was markedly lower in CRPC. The data indicate that WDR77-dependent AR target gene sets are more relevant to CRPC progression. Our work indicated also that the same coregulator can act as coactivator or corepressor for different AR target genes [52]. Although the number of AR-associated coregulators for which a PCa cistrome has been defined is limited, the available data do support some selectivity for distinct sets of AR target genes. For instance, rather limited overlap is seen between in the cistromes for the coregulator GRHL2 and the AR cistrome [66]. For some coregulators, such as EZH2 and TRIM24 [67,68], the overlap with their cistrome and AR genome-wide binding pattern becomes more pronounced in CRPC models. Comparison of transcriptomes and cistromes that depend on AR-interacting transcription factors, also shows they impact on fractions of AR target genes only. For instance, several ETS transcription factors (ERG, ETV1, GABPα) impact androgen regulation of some but not all androgen-responsive genes, and their cistromes overlap only partially with each other's and that of AR [69]. Similar findings have been reported for other transcription factors (e.g. HoxB13, GATA2) [12]. The extent of overlap between cistromes may change depending on PCa stage and AR form(s) expressed. Partial overlap between HoxB13 and AR cistromes was found in ADT-naïve PCa models [43], but in CRPC cells that express full length AR and AR-V7, HoxB13 DNA-binding preferable and exclusively overlaps with AR-V7 only [38].

4. Cooperation between the three determinants of AR transactivation function

4.1. Do ligands impact the composition of the AR cistrome?

A complete answer to this question will require systematic side-by-side comparisons via AR ChIP-Seq or ChIP-exo approaches after stimulation with several different ligands in multiple cell lines. As indicated above, such technically challenging and expensive studies have yet to be done, but ChIP-exo data comparing the AR agonist DHT, and the AR antagonists bicalutamide and enzalutamide have shown agonist-specific and antagonist-specific AR recruitment sites [37]. In addition, some of the 3 292 antagonist-specific sites are preferentially bound by bicalutamide-liganded AR ($n=596$), others by enzalutamide-liganded AR ($n=1364$), and at other sites AR binding is induced by both AR antagonists ($n=1332$). Together, these data do suggest that unique AR ligands may induce a switch in the DNA motifs recognized by liganded AR.

4.2. Do ligands impact interactions between AR and its associated proteins?

It has long been recognized that agonist-bound AR and antagonist-bound AR interact with different classes of associated proteins. With regards to coregulator binding, the generally accepted view is that agonist treatment will induce preferential interaction with coactivators that stimulate transcription whereas exposure to AR antagonists will induce a switch to interaction with corepressors that limit transcription [70,71]. In cells treated with SARMs, however, specific signals or manipulations, such as overexpression of AR, can induce a switch in the AR interacting coregularome, in which corepressors are replaced by coactivators [72]. Overall, less attention has been paid to preferential AR-transcription factor interactions after stimulation with different ligands. Yet the above mentioned AR ChIP-exo studies showed stronger binding of FoXA1 at sites of antagonist-bound AR than for agonist-liganded AR [37], suggesting changes in relative strength of transcription factor interactions. These distinct interaction patterns are not entirely unexpected since it has long been known that agonist treatment induces a

conformational change in AR that fosters an intramolecular NTD-LBD terminal interaction and conformational changes that are the basis for the formation of a coactivator groove on the AR. Induction of this binding pocket does not occur after exposure to antagonists [59,73]. However, the extent, number, sites, and subtleties of ligand-induced conformational changes in AR and their potential impact on AR protein interactome and, ultimately, AR-dependent transcription were not known. The same study [60] that used up to 95 agonists, antagonists or SARMs mentioned above also studied the impact of these same ligands on the interaction between AR and 162 proteins/polypeptides that interact with AR in a T7 phage display. Using mammalian-2-hybrid assays, the authors distinguished between AR ligands based on their ability to present different protein-protein interaction surfaces on the receptor. Specifically, eight different classes of AR-interacting proteins grouped based on their separation by 10 ligand clusters. Importantly, protein-protein interactions induced by ligands correlated well with activity of an ARE-containing promoter-reporter construct and the expression patterns of AR target genes. Moreover, the pharmacologic activity of some previously uncharacterized ligands could be predicted by the AR-protein interactions they induced, confirming that different ligands impact variably on AR-protein interactions.
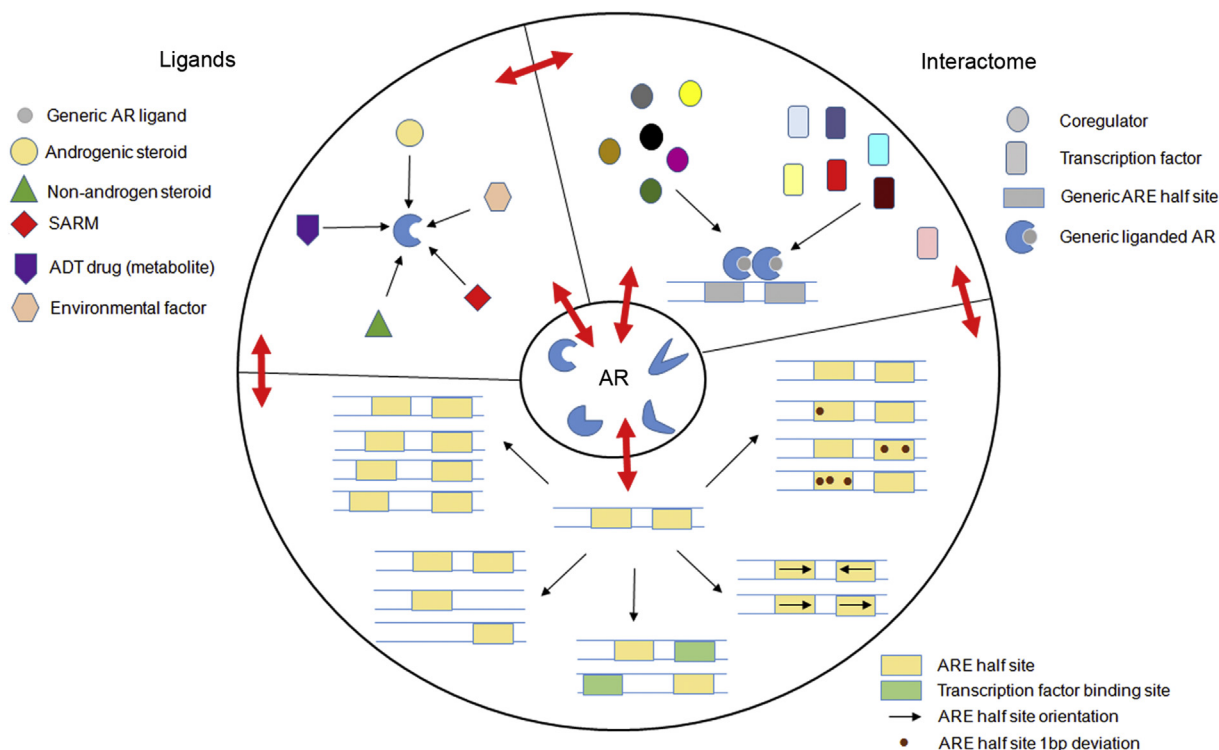
4.3. Do AR-associated coregulators and transcription factors impact the AR cistrome?

Ligand binding of AR induces recruitment of coregulators and secondary transcription factors to AREs [12]. Studies in which impact of silencing or overexpression of such proteins on composition and scope of AR cistrome was explored, have uncovered that AR-associated transcriptional regulators may dictate also the specific sites to which AR binds in the genome, and, thus, impact on the organization and scope of the AR cistrome. For instance, silencing of AR-associated transcription factors such as FoxA1 causes a major change in the AR cistrome, in which loss of FoXA1 induces AR binding to AREs that were not AR-bound in the presence of FoxA1, and loss of AR recruitment to sites bound by AR in control conditions [74]. Moreover, the DNA sequence surrounding AREs to which AR bound in absence of FoxA1 showed different composition, including half-sites enriched in binding motifs for FoxA1. On the other hand, following overexpression of HoxB13, for which binding sites are enriched close to AREs in localized ADT-naïve clinical PCa, in benign prostate epithelial cells, a shift in AR cistrome/transcriptome to more malignant phenotype was noted [43]. Whether coregulators can play a similar regulatory role in determining the specific DNA binding patterns for AR, has not yet been fully addressed. Studies using forms of the AR-associated coregulator BAG-1L in which the domain needed for interaction with AR was mutated, however, did shown recruitment of AR to a subsets of AR target genes that were not AR-bound when wild-type BAG-1L was overexpressed [75]. These findings suggest that BAG-1L can similarly restrict AR genome binding patterns. Previous work from our group has indicated ternary transcription codes exist that consist of ARE-bound AR, which

interacts with specific combinations of secondary transcription factors and coregulators at subsets of AR target genes. These transcription factors appear recruited to their own binding sites in close proximity to AREs by the coregulator [52]. For one such code, namely AR-WDR77-p53, p53 was recruited specifically to AR target genes whose androgen regulation is mediated by the coregulator WDR77, and this androgen-dependent p53-DNA binding relied on WDR77. Particularly in view of other evidence that loss of p53 leads to reorganization of the AR cistrome [76], it is tempting to speculate that coregulators such as WDR77 may contribute to proper genome-wide AR DNA binding patterns.

4.4. Interplay between determinants of AR activity: Further evidence for allosteric regulation of AR?

Taken together, evidence that each of the three key determining aspects of AR function (Fig. 2) may impact on the other is consistent with the previously proposed concept of allosteric regulation of AR activity [59,77−79]. Allostery is routinely defined either as a mechanism by which the catalytic function of an enzyme is modified by interaction with small molecules, not only at the active site but also at a spatially distinct (allosteric) site of different specificity, or as an interaction of two or more functional sites on a protein, resulting in altered affinity of ligand binding. The latter definition is more applicable here, and may extend to altered affinity for DNA response elements. Previously, allosteric communication between the different domains of several nuclear receptors with important consequences for their transcription factor function has been recognized [59,77−79]. In the glucocorticoid receptor, the DBD and the DNA response element communicate towards the LBD, and binding of the ligand to LBD differentially influences receptor binding to structurally diverse DNA response elements. Strikingly, the latter influence also impacts on preferential coregulator interactions with the glucocorticoid receptor [80]. For the estrogen receptor, interplay between the DNA sequence of response element and ligand binding has been found to regulate its transcriptional output [81,82]. In AR, recognition of allosteric regulation has thus far been mostly based on the already mentioned ligand-mediated interaction between its NTD and LBD, which impact on coregulator recruitment [59,77]. The literature reviewed here suggests that instead both AR's ligand-, interactome-, and DNA-binding moieties may be involved. Findings that different classes of AREs differentially impact on AR target gene expression [62−64], observations of different degradation patterns of AR bound to selective or classical AREs [83], and reports of differential AR SUMOylation on cooperative control of selective versus classical AREs [84], strengthen this evidence. Allosteric regulation of AR action by the three determinants discussed here may provide a mechanistic explanation for finding that AR activity varies widely between primary PCa [28,85], and duration of response to ADT is notoriously variable. Indeed, as discussed above, clinical samples harbor heterogeneity and genomic alterations that can impact on these three levels of AR regulation. In addition, recent reports show difference in AR activity in PCa subclasses characterized by

Ligands

- Generic AR ligand
- Androgenic steroid
- Non-androgen steroid
- SARM
- ADT drug (metabolite)
- Environmental factor

Interactome

- Coregulator
- Transcription factor
- Generic ARE half site
- Generic liganded AR

AR

- ARE half site
- Transcription factor binding site
- → ARE half site orientation
- ARE half site 1bp deviation

Figure 2 Cooperativity and interdependence between key determinants of AR transcriptional activity are consistent with allosteric regulation of AR action. Schematic representation of variability in AR ligands, AR interactome, and ARE(s) and their influence on one another (outer circle) and their convergence on allosteric regulation of AR, which may exist as different forms (inner circle). ADT, androgen deprivation therapy; AR, androgen receptor; ARE, androgen response element; SARM, selective androgen receptor modulators.

specific genetic modifications. PCa subclasses for instance express SPOP mutants that impact on stability of AR-associated coregulators NCoA3 and TRIM24 [67,86]. Others harbor *TMPRSS2-ERG* fusions, which increase expression of AR-interacting transcription factor ERG, that are associated with subclusters of ARE-driven genes [45]. In view of the renewed interest in targeting AR domains other than its LBD, a better appreciation and understanding of allosteric events impacting AR activity, may lead to more effective PCa therapies.

5. Future directions

Disrupting the functional interplay between the three determinants of AR action that is most relevant to CRPC progression relies on relevant molecular insights from clinical PCa. To date, most such information reflects steady-state, non-interventional levels of AR action in patient specimens. The increasing access to fresh PCa tissue for use in explant and organoid models and patient-derived xenografting that can be manipulated pharmacologically and genetically is likely to facilitate more faithful modeling of AR action that occurs in course of a patient's treatment plan. From a technical perspective, advances in CRISPR technologies (modulation of DNA sequences), rapid immunoprecipitation mass spectrometry of endogenous proteins aka RIME, (biotin-based) proximity ligation assay and Chia-PET assays coupled with mass spectrometry (protein-protein and protein-DNA interactions) are expected to provide critical novel molecular insights. The latter techniques may be helpful in defining and implementing appropriate biomarkers of response to such treatments, which impact on apoptosis, proliferation, hormone refractiveness and immunological response, biological processes shown to be impacted most significantly by neodadjuvant ADT in clinical PCa [87].

Author contributions

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Data analysis: Dhirodatta Senapati, Sangeeta Kumari, Hannelore V. Heemers.
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Critical revision of the manuscript: Dhirodatta Senapati, Sangeeta Kumari, Hannelore V. Heemers.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgement

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