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Review

Posttranslational regulation of androgen dependent and independent androgen receptor activities in prostate cancer

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Abstract Prostate cancer (PCa) is the most commonly diagnosed cancer among men in western countries. Androgen receptor (AR) signaling plays key roles in the development of PCa. Androgen deprivation therapy (ADT) remains the standard therapy for advanced PCa. In addition to its ligand androgen, accumulating evidence indicates that posttranscriptional modification is another important mechanism to regulate AR activities during the progression of PCa, especially in castration resistant prostate cancer (CRPC). To date, a number of posttranscriptional modifications of AR have been identified, including phosphorylation (*e.g.* by CDK1), acetylation (*e.g.* by p300 and recognized by BRD4), methylation (*e.g.* by EZH2), ubiquitination (*e.g.* by SPOP), and SUMOylation (*e.g.* by PIAS1). These modifications are essential for the maintenance of protein stability, nuclear localization and transcriptional activity of AR. This review summarizes posttranslational modifications that influence androgen-dependent and -independent activities of AR, PCa progression and therapy resistance. We further emphasize that in addition to androgen, posttranslational modification is another important way to regulate AR activity, suggesting that targeting AR posttranslational modifications, such as proteolysis targeting chimeras (PROTACs) of AR, represents a potential and promising alternate for effective treatment of CRPC. Potential areas to be investigated in the future in the field of AR posttranslational modifications are also discussed.

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1. Introduction

Prostate cancer (PCa) is the most commonly diagnosed malignancy and the second leading cause of cancer-related death among American men [1]. The prostate is an androgen dependent organ and AR signaling plays vital roles in controlling the development of benign prostate hyperplasia (BPH) and PCa [2—4]. Testosterone, the major male hormone and the cognate ligand of AR is produced by the teste, the adrenal cortex and PCa tissue. Testosterone can be converted by 5α-reductase to dihydrotestosterone (DHT), a more potent androgen that binds more strongly to the AR [5]. The AR is a member of the nuclear receptor superfamily. It is a 919-amino-acid protein encoded from a ~180-kb gene located at chromosome Xq11-12. AR is comprised of four major domains [6], including N-terminal domain (NTD), the DNA-binding domain (DBD), a hinge region, and the ligand-binding domain (LBD) (Fig. 1). The largest, comprising of over half of the length of the receptor, is NTD. NTD is highly disordered [7] and possesses an activation function (AF1), which contains transcriptional activation unit 1 (TAU1) and TAU5 [8]. The DBD contains two zinc fingers, which interact with the half portion of the androgen-response element (ARE) and facilitate dimerization of AR [9—11]. The hinge region connects the DBD and the LBD. The LBD contains the second transcriptional AF2 [12,13].

In the absence of androgen, AR is localized primarily in the cytoplasm and remains in an inactive state. AR interacts with heat shock proteins (HSPs) such as HSP90, HSP70, HSP56, and HSP27 in the cytoplasm [14] and such interaction prevents AR from entering the nucleus. Androgens bind to the LBD of AR and cause conformation changes in AR, which promotes the release of AR from HSPs. After dimerized, AR protein is transported into the nucleus to exert its function [15]. AR functions are also dependent on its interaction with various transcriptional co-regulators that are differentially expressed in different types of cells [16,17]. The androgen/AR/co—regulator complex eventually binds to AREs in the promoter/enhancer of various target genes and promotes development of the normal prostate and PCa progression by turning on or off the target genes.

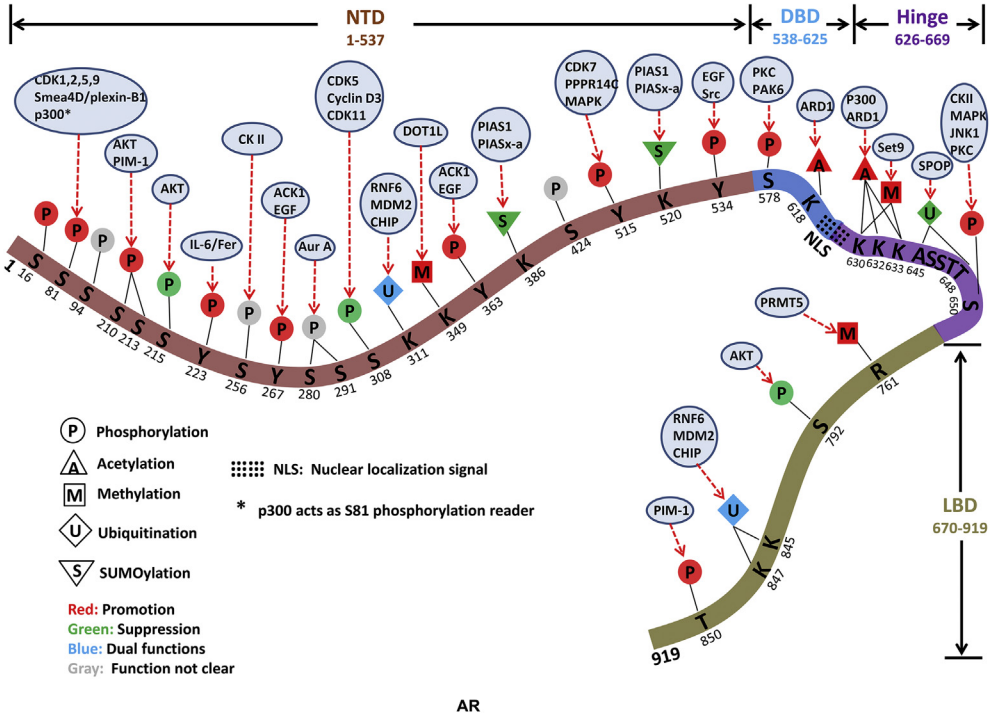

Figure 1 A diagram summarizing posttranslational modifications of the AR. Residues on AR that are known to be modulated by posttranslational modifications, including phosphorylation (P), acetylation (A), methylation (M), ubiquitination (U) and SUMOylation (S) are listed. Modifications of phosphorylation, acetylation and methylation with a positive effect on AR transcriptional activity are highlighted in red color whereas those with a negative effect were colored in green. Some modifications have dual functions, while the functions of some modifications remain unclear. An asterisk indicates p300 does not function as a kinase for S81 phosphorylation, but instead acts as a "reader" that can bind to this phosphorylated site. AR, androgen receptor; NTD, N-terminal domain; DBD, DNA-binding domain; LBD, ligand-binding domain.

In addition to regulation by androgen, the AR is also regulated by posttranslational modifications. AR is capable of undergoing a variety of posttranslational modifications, including phosphorylation, acetylation, methylation, ubiquitination, and SUMOylation. Most of these modifications can positively activate the transcriptional activity of the AR, while others repress its transcriptional activity. A few years ago we summarized AR posttranslational modifications in a review [18]. We also listed the proteins that were responsible for these modifications. Over the recent years, many new AR posttranslational modifications sites and new regulators have been discovered. In this review, we aim to provide an updated view of posttranslational modifications of AR (Fig. 1), and try to specify which modifications are androgen-dependent and which are androgen-independent. We have also discussed the areas that are related to the posttranslational modifications of AR in PCa, but are currently understudied.

2. Phosphorylation

Phosphorylation accounts for the majority of the posttranslational modifications of AR (Fig. 1 and Table 1). At least 19 phosphorylation sites in AR protein have been reported up to now. Majority of AR phosphorylation occurs in the presence of androgens, while some is androgen-independent. Moreover, these phosphorylation sites are predominantly serine residues while some also occur on threonine and tyrosine residues, and most phosphorylation sites are in the NTD of AR.

2.1. AR phosphorylation sites with potential therapy implication

2.1.1. Serine 81 (S81) phosphorylation

S81 is located in the NTD and is the most highly studied phosphorylation site. S81 can be phosphorylated in both

Table 1 Phosphorylation residues of AR.

Residue	Location	Androgen	Regulator	Function	References
S16	NTD	D/I	—	Increase AR dimerization	21,57
S81	NTD	D/I	CDK1,2,5,9 Smea4D/plexin-B1	Increase stability, localization, transcriptional activity	19—39
S94	NTD	I	—	—	19,21,48,57,59,68,69
S210/213	NTD	D/I	AKT, PIM—1	Increase stability, localization, transcriptional activity	40—45
S256	NTD	I	CK II	—	48,57,64
S280/291	NTD	D/I	Aur A	—	70
S308	NTD	—	cyclin D3/CDK11, CDK5	inhibition of transcriptional activity	46—51
S424	NTD	—	—	Increase cell growth	48
S515	NTD	D	CDK7, MAPK kinase, PPP1R14C	Increase stability	33,52—54
S578	DBD	D	PKC, PAK6	localization	54—56
S650	Hinge	D/I	CK II kinase, MAPK, JNK	Increase localization, transcriptional activity	19,57—65
S215/S792	LBD	D	AKT	Inhibition of stability, localization, transcriptional activity	42,71
T850	LBD	D	PIM—1	Increase stability, transcriptional activity	43
Y223	NTD	—	IL6	Increase transcriptional activity, cell growth	72
Y267/363	NTD	D/I	ACK1, EGF	Increase localization, transcriptional activity	73—75
Y534	NTD	—	EGF, Src	Increase localization, transcriptional activity	73,76,77
Y307, Y46, Y357, Y362, Y393, Y551, and Y915	—	—	—	—	76,78

AR, androgen receptor; NTD, N-terminal domain; DBD, DNA-binding domain; LBD, ligand-binding domain; D, androgen dependent; I, androgen independent; CDK, cyclin dependent kinase; AKT, protein kinase B; MAPK, mitogen-activated protein kinase; PPP1R14C, protein phosphatase 1 regulatory inhibitor subunit 14C; PKC, protein kinase C; JNK, JUN N-terminal kinase; PIM-1, Pim-1 proto-oncogene, serine/threonine kinase; IL-6, interleukin 6; ACK1, activated Cdc42-associated kinase 1; EGF, epidermal growth factor; Src, SRC proto-oncogene, non-receptor tyrosine kinase. "—" means not clear.

androgen dependent (AD) [19,20] and androgen independent (AI) fashion [21]. It has been reported that S81 phosphorylation regulates AR protein stability, cellular localization and transactivation.

In LNCaP cells, S81 phosphorylation increased upon DHT treatment [19,20]. These results were confirmed in 22Rv1 and LACP-4 cell lines, suggesting that S81 phosphorylation can be regulated by androgens [81]. Moreover, S81 was also phosphorylated when the LBD-truncated AR was overexpressed in HEK293 cells [21], suggesting that the LBD is dispensable for S81 phosphorylation. S81 phosphorylation of AR was highly detected in primary PCa specimens and in the VCaP PCa xenograft model [22]. S81 phosphorylation level was decreased at 7 days post-castration in VCaP xenografts, and restored after tumors relapsed. These data indicate that AR reactivation in CRPC is associated with S81 phosphorylation, and S81 phosphorylation may be useful as a biomarker of AR activity in CRPC [22].

Many kinases and proteins have been reported to be responsible for S81 phosphorylation. Among these factors, members of the cyclin dependent kinase (CDK) family have been extensively studied, including CDK1, CDK2, CDK5, and CDK9 [20,23—30]. Activation of CDK1 by cyclin B resulted in increased S81 phosphorylation of AR [20]. Interestingly, expression of CDK1 also increased during the transition from androgen dependent PCa to CRPC cells [28]. Cell cycle analysis reveals that there is an increase in AR S81 phosphorylation during mitosis, which coincides with the increased CDK1 activity. Small interfering RNA (siRNA)-mediated depletion of CDK2 suppressed androgen-stimulated S81 phosphorylation of AR in C4-2 cells [24]. CDK5 enhances S81 phosphorylation along with its p35 activator and the p25 byproduct of p35 prevents AR phosphorylation at S81 [31]. PP1 is a serine/threonine specific protein phosphatase. AR recruits and binds to PP1, resulting in CDK9 mobilization and CDK9-mediated AR S81 phosphorylation [29]. CDK9 inhibitors, like 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole and flavopiridol, reduce S81 phosphorylation [30].

It has been reported that Sema4D/plexin-B1 increases the expression of androgen-responsive genes and activates the AR transcriptional activity by phosphorylating AR at S81 [32]. The NTD domain (activation function) of AR, which contains binding sites for transcription co-regulators, is not required for this response. Targeting Plexin-B1 suppresses AR activity, even in the low androgen situation condition [32]. In contrast, myosin light chain phosphatase (MLCP), a protein phosphatase 1 (PP1) holoenzyme, functions as a novel ligand-independent regulator of the AR by dephosphorylating AR at S81 [33]. Caveolin 1 can also mediate the AR S81 phosphorylation [34] and it is probably mediated by AKT signaling indirectly [35].

Phosphorylation at S81 regulates AR protein stability, cellular localization and transactivation. While the pan CDK inhibitors block the activity of CDK1, CDK5, and CDK9, they also inhibit AR phosphorylation at S81, consequently leading to AR protein degradation in PCa cells [36]. Another study demonstrated that docetaxel might have the adverse effect in increasing the AR protein stability via enhancing AR phosphorylation at S81, which might contribute to the chemotherapy resistance in PCa [37].

Cellular localization of AR is also linked to S81 phosphorylation. Overexpression of CDK5 in LNCaP cells increased AR nuclear localization while knockdown increased AR cytoplasmic localization [36]. Ectopic expression of the S81 phosphorylation-resistant mutant S81A failed to activate the ARE4-Luc reporter gene in androgen-treated HeLa and 293T cells [20]. S81 phosphorylation is associated with AR reactivation in CRPC [22] and important for AR transcriptional activity [29,32]. It has been reported that androgen-independent AR activation in CRPC cells is contributed by increased CDK1-mediated S81 phosphorylation. Additionally, activation of CDKs due to PTEN loss also results in S81 phosphorylation, which enhances AR binding with p300, increased AR acetylation, decreased AR ubiquitination and degradation, and therefore prevents complete loss of AR activity [38]. Indeed, it has been reported that AR activity is relatively low due to PTEN loss because there is a negative feedback between PI3K activation and AR signaling in PTEN-null PCa [39]. Furthermore, CDK1- and CDK9-mediated AR S81 phosphorylation can enhance p300 recruitment, histone acetylation and BRD4 binding that sustain transcription. In contrast, blocking S81 phosphorylation markedly suppresses AR activity [29].

2.1.2. Serine 210/213 (S210/213) phosphorylation

Serine 210/213 (S210/S213) phosphorylation has been studied extensively in both androgen dependent (LNCaP and LAPC4) and independent cells (22Rv1) [40]. Treatment with the antiandrogen 2-hydroxyflutamide (HF) results in the reduction of the level of AR phosphorylated at S210/213 [41].

AKT [41,42] and PIM1 [43,44] have been shown to be responsible for S210/213/215 phosphorylation. Activation of AKT by PI3K increases S213 phosphorylation and the PI3K inhibitor LY294002 suppresses its phosphorylation [42]. PIM1 has distinct long (L) and short (S) isoforms, both of which are able to phosphorylate S213 as determined in LNCaP and COS cells [43]. Each isoform has a unique role in regulation of AR [45].

S210/213 phosphorylation is responsible for AR protein stability, nuclear translocation and transcriptional activity. Mutation of S213 to alanine results in a decrease in overall phosphorylation of AR. Inhibition of S213 phosphorylation by isosilybin B (a flavonolignan purified from silymarin) suppressed AR transcriptional activity, prevented androgen-induced AR nuclear localization and inhibited cell growth [40]. AR protein half-life was reduced approximately by half upon treatment with isosilybin B, suggesting that phosphorylation of AR at S213 is important for protein stability [40]. Additionally, expression of hematological and neurological expressed 1 (HN1), also known as jupiter microtubule associated homolog 1 (JPT1), decreases AR S210/213 phosphorylation, resulting in the degradation of AR and downregulation of AR target genes, including *KLK3*, *KLK4*, *NKX3.1* and *STAMP2*. In the absence of androgen, HN1 knockdown increased AKT (S473) phosphorylation and subsequent AR phosphorylation and stabilization in LNCaP cells [45].

2.1.3. Serine 308 (S308) phosphorylation

S308 exists in the TAU1 region of the NTD. This residue is identified to negatively regulate the transcriptional activity of AR. S308 can be phosphorylated by Cyclin D3/CDK11p58.

S308 phosphorylation was shown to decrease the transcriptional activity of AR [46,47]. Mutation of S308 to alanine decreases AR phosphorylation [48]. Upon the treatment of androgen, COS1 and PC-3 cells transfected with AR and CDK11p58 and/or Cyclin D3 decreased the luciferase reporter activity compared with control vector-transfected cells. A similar result was observed in LNCaP cells. An increase in Cyclin D3/CDK11p58 activity was observed upon DHT treatment of LNCaP cells [46]. DHT can induce the activation of Cyclin D3/CDK11p58, resulting in the phosphorylation of S308 and down-regulation of AR activity. However, androgen/Cyclin D3-induced S308 phosphorylation can be surpassed by other mechanisms, resulting in net activation of the AR following DHT stimulation.

Another study showed sLZIP regulated the transcription of Cyclin D3 by directly binding to the AP-1 site in the *Cyclin D3* gene promoter to regulate S308 phosphorylation [47]. sLZIP represses AR transcriptional activity by interaction with AR that is phosphorylated by Cyclin D3/CDK11p58, leading to the suppression of androgen dependent proliferation of PCa cells [47]. A study with clinical specimens also showed that high S308 phosphorylation is significantly associated with longer time to disease-specific death in patients with hormone naive PCa and longer time to death from disease recurrence in patients with CRPC [49]. CDK5 is another kinase shown to be responsible for S308 phosphorylation [50]. However, the transcription-inhibitory effect of S308 phosphorylation might be surpassed by CDK5-mediated AKT activation [50]. CDK1 was also able to phosphorylate the AR on S308, resulting in AR exclusion from condensed chromatin in mitotic cells [51]. Accordingly, CDK1 inhibitor could decrease S308 phosphorylation in PCa cells.

2.1.4. Serine 515 (S515) phosphorylation

S515 lies in the NTD of AR. S515 phosphorylation is able to regulate AR protein stability and transcriptional activity. S515 is phosphorylated by CDK7 and the MAP kinase (MAPK). The kinase CDK7 is part of the TFIIH transcription complex that regulates the function of two E3 ubiquitin ligases: One is carboxyl-terminus of HSP70-interacting protein (CHIP) and the other is mouse homologue of double minute 2 (Mdm2). The phosphorylation-resistant mutation S515A was reported to bind to CHIP but not Mdm2. Both CHIP and Mdm2 can bind to AR and are able to promote AR polyubiquitination [52]. A phosphomimetic mutant S515E can increase the transcriptional activity of AR as measured in a PSA-luciferase reporter assay. Also, the phosphorylation state of S515 alters the half-life of AR protein. S515-phosphorylated AR or phosphomimetic mutant S515E had a shorter half-life compared to WT AR, presumably due to the recruitment of Mdm2 to promote AR for protein degradation since Mdm2 had a lower affinity for binding to the S515A mutant [52]. Triptolide (TPL), an anti-cancer compound extracted from the Chinese herb Tripterygium wilfordii, inhibits phosphorylation of full length AR and AR-V7 variant at Ser515 through XPB/CDK7. TPL significantly suppressed androgen-induced AR binding to the ARE in an enhancer of the *PSA/KLK3* gene. The binding of XPB and CDK7 to the promoter of the *PSA/KLK3* gene was also decreased. TPL also significantly inhibited the recruitment of these proteins to the *PSA/KLK3* gene promoter in the absence of androgen. CDK7 inhibitor BS-181 has a similar effect to decrease S515 phosphorylation of both AR-full length (AR-FL) and AR-V7 variant [53].

In agreement with the finding that S515 is present within a known MAPK consensus sequence, MAPK inhibitor U0126 decreases S515 phosphorylation. As demonstrated in CWR-R1 cells derived from the CRPC cell line CWR22, expression of the phosphorylation-resistant mutant S515A resulted in decreased AR transcriptional activity as measured by PSA-Enh-Luc assay [54]. PPP1R14C, an inhibitory subunit of the PP1 phosphatase is another factor that regulates AR S515 phosphorylation. A reduction in S515 phosphorylation is observed following PPP1R14C depletion [33].

2.1.5. Serine 578 (S578) phosphorylation

S578 is the only residue that is known to be phosphorylated in the DBD of AR. Given that S578 is located within a PKC consensus phosphorylation motif, it is predicted that PKC might be responsible for phosphorylation at this site. Indeed, nuclear PKC expression is strongly correlated with S578 phosphorylation. S578 phosphorylation level is associated with poor outcome and is a potential independent prognostic marker in hormone-naïve PCa. PKC-mediated AR phosphorylation may promote PCa progression [55].

Treatment of CWR-R1 cells with epidermal growth factor (EGF) resulted in an increase in AR transcriptional activity, which coincided with an increase in phosphorylation of S578. Intriguingly, when a phosphomimetic mutant (S578D) and a phosphorylation-resistant mutant (S578A) were transfected into COS cells, the phosphomimetic mutant was equally distributed between the cytoplasm and the nucleus, while the alanine mutant exclusively resided in the nucleus [54]. These findings indicate that S578A can increase nuclear retention of AR while decrease AR nuclear-cytoplasmic shuttling and transcriptional recycling. These findings suggest that S578 phosphorylation may play an important role in regulation of AR cellular localization and activity.

It has been reported that p21-activated kinase 6 (PAK6) reduced the level of wild-type AR following DHT stimulation while AR-S578A mutant was unaffected [56]. In normal prostate epithelium, AR co-localizes with PAK6 in the cytoplasm and translocates into the nucleus in PCa. Furthermore, AR phosphorylation at S578 by PAK6 promotes the association of AR with its E3 ubiquitin ligase Mdm2, causing AR degradation upon androgen stimuli. PAK6-mediated AR phosphorylation promotes its ubiquitin-mediated degradation. PAK6 knockdown promotes PCa growth *in vivo*. Interestingly, there is a strong inverse correlation between PAK6 and AR expression in the cytoplasm of PCa cells. These observations indicate that PAK6 may be important for the maintenance of androgen-induced AR signaling homeostasis, PCa oncogenesis, and being a possible new therapeutic target for AR-positive and hormone-sensitive PCa [56].

2.1.6. Serine 650 (S650) phosphorylation

S650 is the only residue located within the hinge region that can be phosphorylated. S650 occurs both in androgen

dependent and independent manners [57] and regulates AR localization and transcriptional activity [19,57—59].

Although S650 is present within the CK II kinase phosphorylation consensus motif, the CK II inhibitor DRB was unable to prevent the phosphorylation of S650 in LNCaP cells. Stimulation of LNCaP cells with phorbol-12-myristate-13-acetate (PMA), an upstream activator of PKC, resulted in an increase in S650 phosphorylation. However, the PKC inhibitor bisindolylmaleimide was unable to prevent such phosphorylation event [57]. These observations suggest that the bisindolylmaleimide-insensitive isoforms of PKC (ζ, ι, and μ) may be responsible for S650 phosphorylation. It is also possible that PMA affects AR S650 phosphorylation, but this phosphorylation could be induced indirectly by PKC. Treatment of LNCaP cells with the PKA activator (forskolin) resulted in increased phosphorylation of S650 in the absence of androgen. These data suggest that S650 is potentially regulated in a cAMP dependent mechanism [57].

In COS-1 cells, but not other cells, S650 was constitutively phosphorylated [59]. Expression of MAPK kinase (MKK) 4/c-Jun N-terminal kinase 1 (JNK1) or MKK6/p38 increased S650 phosphorylation in COS7 cells. Inhibition of either p38 (SB203580) or JNK1 (SP600125) by their small molecule inhibitors significantly decreased the level of S650 phosphorylation [58]. The DBD/hinge region contains a bipartite nuclear localization signal (NLS) [60]. The bipartite sequence ^{617}RKCYEAGMTLGARKLKK^{633} has a role in regulation of the localization of AR in the nucleus [61—63]. Given that S650 is in close proximity to NLS, it may prevent AR from localizing/exporting out of the nucleus to the cytoplasm. S650 phosphorylation is also responsible for the regulation of transport of the AR between the nucleus and the cytoplasm. Accordingly, when p38 and/or JNK1 activity was inhibited, there was a reduction in the amount of AR in the nucleus [58]. Additionally, mutation at this phosphorylation site (S650A) decreased AR transcriptional activity by 30% [19], indicating a role of S650 in regulating AR transcriptional activity. As a serine/threonine phosphatase, PP1 directly regulates AR protein stability and nuclear localization through dephosphorylation of S650 [64]. Meanwhile, PP1 indirectly regulates AR degradation through dephosphorylation of SKP2 and MDM2, which are ubiquitin ligases of AR [65].

2.2. Additional AR phosphorylation sites

2.2.1. Serine 16 (S16) phosphorylation

S16 lies within the NTD and its phosphorylation is responsible for the N-/C-terminus interaction of AR upon dimerization. Deletion of residues 14—150 disrupts the dimerization of AR [66,67]. S16 can be phosphorylated in androgen dependent manner [21,57]. Although S16 is a consensus site for both PKA and calcium calmodulin II, PKA in LNCaP cells failed to increase S16 phosphorylation as determined by ^{32}P-phosphopeptide mapping, suggesting that PKA is not responsible for AR S16 phosphorylation. When the LBD of AR was deleted, it was found that S16 in AR was phosphorylated in the absence of androgens [21]. Thus, S16 can also be phosphorylated in an androgen independent manner.

2.2.2. Serine 94 (S94) phosphorylation

S94 is also located in the NTD of AR. S94 is constitutively phosphorylated and androgen insensitive [19,48,57,59]. S94 was phosphorylated even when the LBD was truncated [21]. The function of S94 phosphorylation remains unclear. It has been shown that the transcriptional activity of AR was not altered when S94 was mutated to alanine (A) [19,68]. It is also reported that low expression of AR phosphorylation S94 in cytoplasm was associated with high Ki67 score [69], but further investigation of the underlying mechanism is warranted.

2.2.3. Serine 256 (S256) phosphorylation

S256 lies within the NTD of AR and can also be phosphorylated [48,57,64]. S256 phosphorylation was observed only in the presence of androgens. Casein kinase II (CKII) is predicted to be responsible for this phosphorylation due to the presence of the CKII consensus phosphorylation sequence at this site [57]. The function of S256 phosphorylation has yet to be determined.

2.2.4. Serine 424 (S424) phosphorylation

S424 is located in the TAU5 region of the NTD. The role of S424 phosphorylation in regulation of AR activity has yet to be identified. In conjunction with mutation of six other residues (S16, S81, S94, S256, S308, and S650) there was a decrease in AR transcriptional activity [48]. However, no study has identified the kinase responsible for S424 phosphorylation. Since S424 is located in the TAU5 region in NTD, phosphorylation of this site may play a role in androgen independent activation of the AR and development of CRPC, but this warrants further investigation.

2.2.5. Serine 215/792 (S215/S792) phosphorylation

S215 locates in the NTD and S792 is the only serine residue that can be phosphorylated in the LBD. Both S215 and S792 match to the AKT phosphorylation consensus sequence (RXRXXS/T*), and these sites are conserved in mammals, suggesting an important role in AR function. Phosphorylation of S215 and S792 is dependent on AKT signal [42,70]. Activation of PI3K by insulin growth factor 1 (IGF-1) leads to activation of downstream kinases AKT or p70S6K. Indirect inhibition of p70S6K with rapamycin was unable to prevent S215 and S792 phosphorylation, while the indirect inhibition of AKT by the PI3K inhibitor LY294002 negated this site-specific phosphorylation. Phosphor-mimetic mutations, in which serine residues were mutated to aspartate (S215D, S792D) to mimic constitutive phosphorylation, were used to investigate the function of AKT phosphorylation at these two sites. It has been shown that phosphorylation at S215 and S792 might suppress nuclear translocation of the AR in response to DHT, decrease protein stabilization, and decrease AR transcriptional activity [70].

2.2.6. Threonine 850 (T850) phosphorylation

T850 is also present in the LBD of AR. T850 phosphorylation is responsible for the AR protein stability and low androgen transcriptional activity. The phosphorylation of T850 is cell cycle dependent, and is increased during the G2 and M

phase, which coincides with increased PIM-1 expression. AR mutation at T850 resulted in lower AR protein levels during the M phase, suggesting that T850 phosphorylation stabilizes AR protein during the M phase of the cell cycle [43].

2.2.7. Tyrosine 223 (Y223) phosphorylation

Y223 is located in the TAU1 region in the NTD of AR. IL6 treatment can lead to Y223 phosphorylation, which results in increased AR transcriptional activity and PSA expression. Further mechanism study demonstrated that Fer tyrosine kinase mediates IL-6-induced AR activation by phosphorylating Y223 on AR via its SH2 domain. Fer, AR and phosphorylated STAT3 co-localize in the nucleus in PCa cells from CRPC patients. Fer can also control IL-6-induced growth response and PSA expression in PCa cells [71].

2.2.8. Tyrosine 267 and 363 (Y267, Y363) phosphorylation

Y267 and Y363 are two closely related tyrosine residues in AR. Both sites are present in the NTD of AR. Ack1 is responsible for the phosphorylation of Y267 and Y363 in the absence of androgens. Y267F and Y267F/Y363F mutations abolished Ack1-mediated AR tyrosine phosphorylation. Y267 and Y363 phosphorylation can promote androgen-independent growth of LNCaP and LAPC4 xenografts in castrated mice. This androgen-independent growth may be resulted from increased recruitment of AR to AREs in chromatin. The expression of an inactive Ack1 (dAck1) had limited effect on tumor growth. When dAck1-expressing cells were exposed to DHT, AR failed to induce PSA expression. Expression of the constitutively active mutant of Ack1 (ca-Ack1) in LNCaP cells increased AR binding to the *KLK3* gene promoter in the absence of androgen. After ca-Ack1-expressing LNCaP cells were treated with low doses of androgen, there was also an increase in PSA expression. This suggests that Ack1 sensitizes PCa cells to low levels of androgens and that Ack1 may facilitate progression from AD to AI phenotype [72,73]. All these data suggest that Ack1 plays an important role in the activation of AR as a transcription factor in both AD and AI PCa cells. These findings were confirmed by another report [74].

2.2.9. Tyrosine 534 (Y534) phosphorylation

Y534 is present in the NTD of AR. Y534 is a highly conserved residue in AR among species [72,75]. Similar to the altered phosphorylation state of Y267 and Y363, EGF was able to induce phosphorylation of AR at Y534. EGF treatment of LNCaP cells induces Y534 phosphorylation and results in the increased AR transcriptional activity. These results were similar to the observed role of EGF in phosphorylation of Y267 and Y363 [75]. Chromatin immunoprecipitation (ChIP) analysis revealed that EGF-stimulated Y534 phosphorylation resulted in the recruitment of AR to the *KLK3* gene promoter. Y534 phosphorylation also promotes the nuclear localization of AR [75]. Another study demonstrated that LNCaP cells cultured in androgen deprived conditions might result in the induction of Src activation as well as an increase in AR Y534 phosphorylation level [76].

AR tyrosine phosphorylation on other residues, including Y307, Y46, Y357, Y362, Y393, Y551, and Y915, was identified by mass spectroscopy [75]. These tyrosine residues are predominantly located within exon 1 of the AR, which is conserved between AR full-length and variants. They might play pivotal roles in the transition from a hormone-sensitive to a castration-resistant tumor [77]. However, the detailed function and mechanism of these phosphorylation events remain to be studied.

3. Acetylation

Several acetylation sites have also been identified over the years and acetylation of these sites influences AR transcriptional activity, cell growth and survival (Fig. 1 and Table 2).

3.1. Lysine 630/632/633 (K630/K632/K633) acetylation

Acetylation was detected at three adjacent residues within the hinge region of the AR, including K630, K632, and K633. Mutation of these residues to alanine resulted in decreased overall level of AR acetylation, suggesting that these three residues are the major sites of acetylation [78]. Acetylation of these residues is androgen dependent. DHT treatment increases AR acetylation.

The K630/K632/K633 acetylation is mediated by p300 and p300/cAMP-response element binding protein association factor (p/CAF). K630/K632/K633 acetylation augments p300 binding to AR *in vitro*. Mutations mimicking neutral polar substitution for acetylation (*e.g.* AR-K630Q) enhance p300 binding and reduce N-CoR/HDAC/Smad3 corepressor binding with AR, whereas mutations with charged residue substitution (*e.g.* AR-K630R) reduced p300 binding and enhanced corepressor binding of AR [79].

Mass spectrometry analysis revealed that long non-coding RNAs (lncRNAs) PRNCR1 and PCGEM1 can also bind to AR and regulate AR posttranslational modifications, including K630/K633 acetylation. Consistent with the proposed importance of acetylation of AR in activation of an AR target gene, a K630R/K633R mutation inhibited AR interaction with lncRNAs PRNCR1 and PCGEM1 and DHT-induced expression of AR target genes, suggesting that PRNCR1 and PCGEM1 interact with AR in a K630/K633 acetylation-dependent manner [80]. Notably, an independent study reported that PRNCR1 and PCGEM1 lncRNAs are not implicated in CRPC [81].

Arrest-defective protein 1 (ARD1) is another key factor that regulates K630/K632/K633 acetylation. Silencing of ARD1 in LNCaP cells significantly inhibited AR acetylation and acetylation-mediated AR transactivation [82]. The acetylation of the three residues K630, K632, and K633 is important for the ligand-dependent activation of AR. AR acetylation promotes cell survival and growth in PCa cells in culture and in immune-deficient mice and augments transcription of a subset of growth control target genes [79,80,82].

3.2. Lysine 618 (K618) acetylation

Similar to K630, K632, and K633, ARD1 can also acetylate AR at K618 in the DBD *in vitro* and *in vivo* and increases AR transcriptional activity and nuclear translocation [83]. Mutation of the substitution of charged lysine residue by

Table 2 Acetylation residues of AR.

Residue	Location	Androgen	Regulator	Function	References
K618	DBD	—	ARD1	Promote transcriptional activity	79—83
K630/632/633	Hinge	D	p300 lncRNA ARD1	Promote transcriptional activity	84

DBD, DNA-binding domain; D, androgen dependent; ARD1, arrest-defective protein 1; p300, E1A binding protein P300; AR, androgen receptor. "—" means not clear.

arginine (AR-618R) reduces K618 acetylation, RNA Pol II binding of AR, AR transcriptional activity, PCa cell growth, and xenograft tumor formation. In contrast, a neutral polar mimicking substitution of acetylation at K618 by glutamine (AR-618Q) enhances these effects. Mechanistic study revealed that expression of ARD1 increased level of AR acetylation and AR-HSP90 dissociation in a dose dependent manner. The AR acetylation-defective mutant K618R was unable to dissociate from HSP90 while the HSP90-dissociated AR is acetylated following ligand exposure [83].

3.3. Deacetylation

There are mainly two factors that mediate AR deacetylation. Firstly, deacetylation of AR can be mediated by HDAC1. Inhibition of HDAC1 results in increased AR acetylation, which further increases AR activity in response to low concentrations of androgens [84]. The second AR deacetylase that suppresses its transcriptional activity is SIRT1, a nuclear NAD^+-dependent deacetylase [78,85,86]. Deacetylation of AR by SIRT1 was identified by mass spectroscopy upon treatment with androgen [78]. Overexpression of SIRT1 in LNCaP cells resulted in a 3-fold reduction in AR activity in DHT stimulated cells. Depletion of SIRT1 by RNAi resulted in a 2.5-fold increase in AR activity upon DHT treatment. Similar results were obtained upon inhibition of SIRT1 by nicotinamide (NAM), a form of vitamin B3, consistent with the role of acetylation in AR activation.

3.4. BRD4 binding of AR

Bromodomain (BD) containing 4 (BRD4) is a member of the bromodomain and extra-terminal domain (BET) protein family. As a histone "reader", BRD4 binds to acetylated residues on histone proteins. This function of BRD4 is mediated by BD1 and BD2 domains. It has been shown that BRD4 can bind to non-histone proteins such as AR [87,88]. Specifically, the BD1 domain in BRD4 physically interacts with a region of amino acids 121—159 in the NTD of AR. The bromodomain inhibitors of BRD4 block nuclear translocation and transcriptional activity of both full-length AR and variants including AR-V7. Notably, it remains unclear whether BRD4—AR interaction requires AR acetylation in a residue-specific manner and further investigation is warranted.

4. Methylation

A few lysine and arginine residues in AR have been shown to be methylated, and methylation of these sites affects AR transcriptional activity and PCa progression (Fig. 1 and Table 3).

4.1. Lysine 630/632 (K630/K632) methylation

Methylation of AR occurs at two residues within the hinge region, K630 and K632, which overlap with the residues for acetylation. Methylation at K630/632 is responsible for AR N-/C-terminal interaction, AR nuclear localization and transcriptional activity. AR K630 methylation is mediated by Set9, which was originally identified as a histone H3K4 monomethyltransferase. Alanine substitution of K630 prevented AR methylation *in vitro* and *in vivo*. Set9 methylated both nuclear and cytoplasmic AR. Set9 overexpression potentiated AR-mediated transactivation of the target gene promoter, whereas Set9 depletion inhibited AR activity and target gene expression. K630A mutation reduced amino- and carboxy-terminal (N—C) interaction in Set9-intact cells, whereas the N—C interaction in WT AR was reduced upon Set9 depletion. In contrast, either Set9 silencing or overexpression failed to affect the transcriptional activity of the K630A mutant of AR [89].

4.2. Arginine 761 (R761) methylation

AR can also be methylated at R761 residue in the LBD by the arginine methyltransferase PRMT5. The transcription factor ERG is a potential "pioneer factor" that can regulate AR

Table 3 Methylation residues of AR.

Residue	Location	Androgen	Regulator	Function	References
K349	NTD	—	lncRNA/DOT1L	Promote transcriptional activity	81
K630/632	Hinge	—	Set9	Promote transcriptional activity	90
R761	LBD	—	PRMT5	Promote transcriptional activity	91,92

NTD, N-terminal domain; LBD, ligand-binding domain; DOT1L, DOT1 like histone lysine methyltransferase; Set9, SET domain containing lysine methyltransferase 9; PRMT5, protein arginine methyltransferase 5; AR, androgen receptor. "—" means not clear.

engagement with chromatin [90]. It can also recruit PRMT5 to AR target genes where PRMT5 methylates AR on R761 [91]. The R761 methylation attenuates AR recruitment and transcription of genes in differentiated prostate epithelium. The AR inhibitory function of PRMT5 is restricted to TMPRSS2-ERG fusion-positive PCa cells. R791A mutation results in a transcriptionally hyperactive AR, suggesting that the proliferation-promoting effects of ERG and PRMT5 are mediated, at least in part through attenuating AR ability to induce expression of genes normally involved in lineage differentiation.

4.3. Lysine 349 (K349) methylation

Similar to K630/633 acetylation, K349 methylation can also be regulated by lncRNAs PRNCR1 and PCGEM1. The lncRNA-regulated K349 methylation is mediated by DOT1L. K349R point mutation significantly inhibited AR methylation. DOT1L-specific knockdown impaired the interaction between AR and PCGEM1, but not that with PRNCR1, suggesting that AR K349 methylation mediated by PRNCR1-bound DOT1L is critical for the recruitment of PCGEM1 to AR. Indeed, overexpression of AR K349R mutant significantly reduced DHT-induced gene activation in LNCaP cells [80].

4.4. AR methylation by enhancer of zeste homolog 2 (EZH2)

EZH2 is methyltransferase, an only enzymatic subunit of the Polycomb repressive complex 2 (PRC2). It primarily functions to repress gene expression via its histone methyltransferase activity. It has been reported that EZH2 can also act in "solo" to exert a gene transactivation function by increasing AR overall methylation without altering AR mRNA and protein level [92]. However, it remains to be determined which residue of AR is methylated by EZH2. Further study confirmed that EZH2 could directly interact with AR in PCa cells and positively regulate the AR pathway [93]. EZH2 also increases AR activity indirectly by repressing CCN3/NOV, which reduces AR nuclear translocation, chromatin binding and transactivation [94]. Notably, some other studies demonstrated that EZH2 suppressed AR activity and promoted neuroendocrine PCa (NEPC) transdifferentiation [95—97].

5. Ubiquitination

Ubiquitination, a modification of covalent linkage of the small ubiquitin (Ub) protein to target proteins, is one of the most widely studied AR posttranslational modifications in recent years. Ubiquitination is mediated by sequential activating enzymes (E1), conjugating enzymes (E2) and ligase enzymes (E3). Ubiquitination is a reversible covalent process and ubiquitin can be removed from target proteins by deubiquitinating enzymes (DUBs) (Fig. 2). So far, accumulating evidence shows that most sites ubiquitination plays important roles in AR protein degradation, while a few sites ubiquitination enhances AR transcriptional activity. Both AR-FL and AR-V7 proteins are known to be regulated by ubiquitin-proteasome degradation pathway. Blocking of AR or AR variant protein degradation might be the potential mechanisms of CRPC (Fig. 1 and Table 4) [98—100].

5.1. Lysine 845/847 (K845/K847)

K845/K847 located in the LBD of AR are found ubiquitinated by mass spectrometry. These modifications are mediated by RNF6, an E3 ubiquitin ligase. Intriguingly, RNF6 enhances AR transcriptional activity by promoting AR polyubiquitination at these two sites. In CWR-R1 and LNCaP cells treated with androgens, there was an increase in global ubiquitination of the AR [101]. Upon knockdown of RNF6 by siRNA, there was a decrease in AR transcriptional activity measured by luciferase assay [101].

Polyubiquitination of the AR can also be catalyzed by the E3 ubiquitin ligases MDM2 and CHIP. MDM2 and CHIP-mediated polyubiquitination of the AR promotes AR proteasome degradation [101—104]. Moon et al. [105] demonstrated that CHIP E3 ligase-mediated ubiquitination could degrade AR-V7 in CRPC. Deleted in breast cancer (DBC1), also known as CCAR2, is a coactivator of some transcription factors (*e.g.*, AR). It also functions as an inhibitor of some epigenetic regulators (*e.g.*, SUV39H1, HDAC3 and MDM2). DBC1 functions as a coactivator for AR-V7 and is required for the expression of AR-V7 target genes. DBC1 enhances DNA-binding activity of AR-V7 by direct interaction and inhibits AR-V7 ubiquitination by competing with CHIP for AR-V7 binding, thereby stabilizing and activating AR-V7. BMI1, a Polycomb group (PcG) protein, can

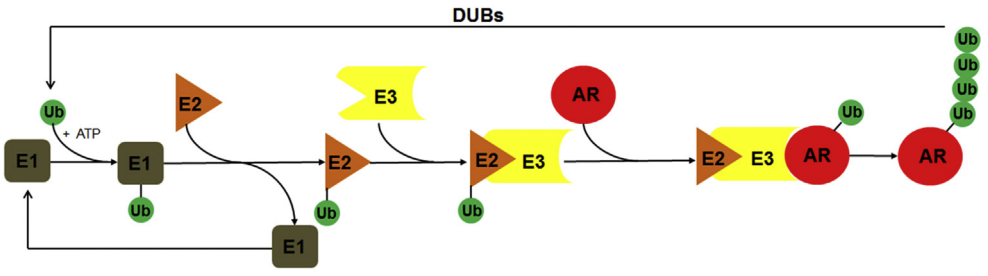

Figure 2 Scheme of AR Ub. Ub is catalyzed by several enzymes, including the E1, E2, and E3. Once the protein substrate is tagged with ubiquitin through the lysine48-linked polyubiquitination, it is recognized by the proteasome for degradation. AR, androgen receptor; Ub, ubiquitin; ATP, adenosine-triphosphate; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzymes; E3, ubiquitin ligases; DBD, DNA-binding domain.

Table 4 Ubiquitination residues of AR.

Residue	Location	Androgen	Regulator	Function	References
K311	NTD	—	RNF6, MDM2, CHIP	Stability Transcriptional activity Chromatin retention	108
^{645}ASSTT^{648}	Hinge	—	SPOP	Promote degradation	109—119
K845/847	LBD	—	RNF6	Transcriptional activity	101—107
			MDM2,CHIP	Degradation	

NTD, N-terminal domain; LBD, ligand-binding domain; RNF6, ring finger protein 6; MDM2, MDM2 proto-oncogene; CHIP, carboxy terminus of HSP70-interacting protein; SPOP, speckle type BTB/POZ protein; AR, androgen receptor. "—" means not clear.

also bind the AR and prevents MDM2-mediated AR protein degradation, resulting in sustained AR signaling in PCa cells [99]. Auranofin (Aur) inhibits USP14 and UCHL5 mediated AR deubiquitination, facilitates the ubiquitination and degradation of AR, and suppresses PCa cell growth [106].

Another AR-targeting E3 ubiquitin ligase is STUB1. STUB1 significantly induced AR-FL/AR-V7 ubiquitination, suggesting that STUB1-mediated AR-V7 protein degradation is through the induction of AR-V7 ubiquitination. In addition, HSP70 formed complexes with AR-V7/AR-FL, while STUB1 disassociated AR-V7/AR-FL from HSP70 binding, leading to AR/AR-V7 ubiquitination and degradation. Inhibition of HSP70 significantly inhibits prostate tumor growth and improves the efficacy of enzalutamide/abiraterone treatment through AR/AR-V7 suppression. These results indicate that the STUB1/HSP70 complex regulates AR-V7 and AR-FL protein expression through the ubiquitin-dependent proteasome pathway. However, such activity is largely suppressed in enzalutamide/abiraterone-resistant PCa [100].

5.2. Lysine 311(K311)

K311 is present in the NTD of AR and was firstly reported by McClurg et al. [107] to be ubiquitinated. K311 ubiquitination plays a role in AR stability. Inactivated mutation K311R (lysine to arginine) of this site increases AR protein stability. It is worth noting that K311 also plays a critical role in regulating transcriptional activity and chromatin retention of AR. AR protein lacking the K311 ubiquitination site is transcriptionally inactive due to its chromatin retention. K311 ubiquitination-resistant mutation of AR dramatically reduced transcriptional activity of AR and AR target gene expression. Even though this site is present in clinically relevant AR-variants, it can only be ubiquitinated in cells when AR retains LBD, suggesting that AR C-terminus is needed for binding of AR by the unidentified E3 ubiquitin ligases responsible for K311 ubiquitination [107].

5.3. SBC (^{645}ASSTT^{649}) motif-dependent AR ubiquitination

Speckle type BTB/POZ protein (SPOP) is a substrate-binding adaptor of the Cullin3-RING E3 ubiquitin ligase (CRL) complex. SPOP is one of the most frequently mutated genes in primary PCa, with mutation rate ranging from 10% to 15% depending on the cohorts studied [108]. An SPOP-binding consensus (SBC) motif ^{645}*ASSTT*^{649} is identified in the hinge region of AR [109]. SPOP functions as an E3 ubiquitin ligase that promotes AR poly-ubiquitination and degradation. In addition to gene mutations, SPOP expression is also often downregulated in PCa in patients [110]. It has been demonstrated that SPOP induced degradation of full-length AR. However, given that most AR variants lack the functional hinge region, they often evade SPOP-mediated degradation, thereby contributing to AR-mediated gene transcription and PCa cell growth [109]. Moreover, PCa-associated mutants of SPOP also fail to bind to and cause AR ubiquitination and degradation. Androgen treatment antagonizes SPOP-mediated degradation of AR, whereas antiandrogens promote this process, implying that this pathway might play important roles in resistance to anti-androgen therapy. Similar findings were obtained by an independent study [111]. In addition to AR itself, transcriptional regulators such as SRC-3 and BET proteins (BRD2, BRD3, and BRD4) are also the bona fide substrates of SPOP, and prostate cancer-associated SPOP mutations cause upregulation of these proteins in PCa cells in culture and in patient specimens [112—116]. These findings provide a plausible explanation for the finding in the TCGA patient samples that SPOP mutated subtype of PCa has the highest AR activity among different subtypes of PCa examined [108]. Intriguingly, the finding from a clinical trial shows that PCa patients with both SPOP mutation and CHD1 deletion, two genetic alterations co-occurring in PCa patients, are highly sensitive to the next-generation AR inhibitory drug abiraterone [117]. These findings suggest that SPOP mutated PCa are addicted to AR signaling, providing further support to the notion of "oncogene addiction". In agreement of these findings, the SPOP mutation rate was found relatively lower in advanced PCa in patients who have gone through anti-AR therapies such as enzalutamide and abiraterone than that in primary hormone-native PCa [108,118].

6. Proteolysis targeting chimeras (PROTACs) of AR: Targeting AR ubiquitination and degradation for PCa therapy

Proteolysis targeting chimeras (PROTACs) are chimeric bifunctional small molecules that recruit an E3 ubiquitin ligase to force the destruction of a target protein. PROTACs

contain a ligand for the target protein connected via a linker to an E3 ubiquitin ligase, which allows ubiquitination and subsequent degradation of the target protein by the proteasome [119—122].

Early study showed that a DHT-based PROTAC introduced into cells promoted the rapid disappearance of AR in a proteasome-dependent manner [121]. The PROTACs comprised of DHT linked to the IκBα phosphopeptide recruited AR to Skp1-Cullin-F-box protein β-transducin repeat-containing protein (SCFβ-TRCP) for ubiquitination and degradation. PROTAC-mediated AR degradation specifically inhibited the proliferation of hormone-dependent PCa cells [123]. The same group confirmed the DHT-based PROTAC also works under *in vivo* conditions [124]. Except DHT-based PROTAC, another compound (ARD-69) was invented as a highly potent AR PROTAC degrader, which is capable of reducing the AR protein level by >95% in LNCaP, VCaP, and 22Rv1 cell lines. Along with AR degradation, AR target genes and cell growth were suppressed, indicating that this PROTAC might be a potential therapeutic option for AR-positive CRPC [93].

7. SUMOylation

SUMOylation is the attachment of approximately 100 amino acids of small ubiquitin-like modifier (SUMO) to lysine residues [125]. SUMOylation alters the regulation of transcription, cell cycle, nucleocytoplasmic transport, DNA replication and repair, and apoptosis. Unlike ubiquitination, SUMO does not depend upon the linkage of SUMO residues to be functional. SUMO is a reversible covalent isopeptide linkage modification that can regulate or alter the cellular targets of proteins [126]. Similar to the ubiquitin pathway, SUMOylation involves three different catalytic enzymes (E1, E2, and E3). PIAS1 and PIASx-alpha are the E3 SUMOylation ligases responsible for AR SUMOylation (Fig. 1 and Table 5) [127,128].

7.1. Lysine 386/520 (K386/K520)

Two SUMOylation residues K386 and K520 are defined in the NTD of AR [129]. The SUMO-1-conjugating enzyme Ubc9 interacts with AR. AR SUMOylation is in an androgen-enhanced fashion. Although only a small percentage of AR is SUMOylated at the steady state, AR SUMOylation sites have an impact on the protein stability, nuclear localization, and chromatin interactions, and expression of its target genes. The E3 ligases responsible for AR SUMOylation include PIAS1 and PIASx-alpha. It is reported that short-term proteotoxic cell stress, such as hyperthermia, induces detachment of the AR from the chromatin and triggers accumulation of the SUMO-2/3-modified AR pool which concentrates into the nuclear matrix compartment. Alleviation of the stress allows rapid reversal of the SUMO-2/3 modifications and force the AR to return to chromatin [130]. AR SUMOylation plays important roles in regulation of its transcriptional activity. K386R mutation alone or in combination with K520R resulted in a 2 to 3-fold enhancement of androgen-dependent transcription in ARE-positive promoters [131,132]. A study indicated that SUMOylated AR had lower transcriptional activity [133]. However, further study revealed that the SUMOylation modulates the AR function in a target-gene- or pathway-selective manner. SUMOylation does not simply repress the AR activity, but it regulates AR interaction with the chromatin and selection of genes targeted by AR. ChIP-sequencing (ChIP-seq) analyses showed that the SUMOylation can modulate the chromatin occupancy of AR on many loci in a fashion that parallels differential expression of androgen-regulated genes. FOXA1, C/EBP and AP-1 DNA binding elements are differentially enriched at the AR-WT and the AR-K386R, K520R mutant-preferred genomic binding sites [134].

8. Conclusion

In addition to be regulated by its cognate ligand androgen, AR is also regulated by a variety of posttranslational modifications. In this review, we summarize a number of posttranslational modifications of AR that have been extensively studied, including phosphorylation, acetylation, methylation, ubiquitination, and SUMOylation. Previously, most of the studies of AR posttranslational modifications mainly focused on phosphorylation, which account for the vast majority of AR posttranslational modifications. In recent years, accumulating studies have shown that ubiquitination-mediated degradation of AR plays an important role in the progression of PCa.

The functions of AR posttranslational modification are multifaceted, which include regulation of AR protein stability, intracellular localization of AR, transcriptional activity of AR, and regulation expression of AR target genes. There are at least 19 phosphorylation sites identified in AR protein, and most of them occur on the serine residue and mainly located in the NTD. Most of the AR phosphorylation sites play a positive role in regulating AR transcriptional activity, and only a handful of phosphorylation sites (such as S308) inhibit the transcriptional activity of AR. The primary role of acetylation and methylation also tends to promote the transcriptional activity of AR. Researchers are

Table 5 SUMOylation residues of AR.

Residue	Location	Androgen	Regulator	Function	References
K386/K520	NTD	—	PIAS1, PIASx-a	Decrease stability localization chromatin interaction transcriptional activity	130—135

NTD, N-terminal domain; PIS1, protein inhibitor of activated STAT 1; PIASx-a, protein inhibitor of activated STAT X-a; AR, androgen receptor. "—" means not clear.

paying more and more attention to the study of ubiquitin-mediated degradation of AR, especially AR variants. AR variants are believed to be an important factor that may lead to the resistance to next-generation anti-androgen therapies such as enzalutamide and abiraterone. Intriguingly, the newly discovered ubiquitination of AR on K311 or mediated by the $^{645}ASSTT^{649}$ SBC motif fails to cause degradation of AR-V7 variant, which is considered to be an important cause of antiandrogen resistance in PCa.

There are many hypotheses for the development of CRPC. One of the most important reasons is the constitutively high activity of AR in the presence of the castration level of androgens. The proposed mechanisms that induce high activity of AR include 1) AR becomes more sensitive to low concentrations of androgens due to enhanced post-translational modifications; 2) non-canonical activation of AR by non-androgenic factors; 3) constitutively activated AR variants. It is generally accepted that the role of AR in PCa is indispensable. In addition to androgen, post-translational modification is another important way to regulate AR activity. When antiandrogen treatment fails to block the AR signaling pathway, targeting AR post-translational modification may represent a potential and promising alternate for effective treatment of CRPC.

9. Perspective

Although AR posttranslational modifications have been studied to a certain extent, a number of outstanding issues remain to be addressed. For example, how many AR post-translational modification sites are mutated in primary PCa, CRPC, metastatic PCa and/or NEPC patient samples? What roles may different AR posttranslational modifications play during the progression of ADPC to CRPC? Does AR post-translational modification play any role in regulating PCa cell lineage plasticity? Which AR posttranslational modification site(s) can be effectively targeted to overcome ADT or chemotherapy resistance in PCa? Particularly, several p300 specific inhibitors have been developed and some of them are currently in clinical trials [135]. Because CDKs are often aberrantly activated during PCa progression and S81 phosphorylation of AR plays important roles in disease evolution, specific targeting of p300 interaction with S81-phosphorylated AR may offer a new opportunity for development of new treatments for CRPC, especially those expressing high level of AR N-terminal variants such as AR-V7 and AR-V9. Development of more useful PROTACs of AR is another promising direction to target AR PTM for treatment of PCa. Additionally, with more efforts undertaken to identify small molecules that can specifically bind to ARN, more AR PROTACs could be developed for PCa treatment by targeting full-length AR or AR variants for protein degradation.

Author contributions

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Conflicts of interest

The authors declare no conflict of interest.

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