REVIEW

AR-dependent phosphorylation and phospho-proteome targets in prostate cancer

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Abstract

Prostate cancer (CaP) is the second leading cause of cancer-related deaths in Western men. Because androgens drive CaP by activating the androgen receptor (AR), blocking AR's ligand activation, known as androgen deprivation therapy (ADT), is the default treatment for metastatic CaP. Despite an initial remission, CaP eventually develops resistance to ADT and progresses to castration-recurrent CaP (CRPC). CRPC continues to rely on aberrantly activated AR that is no longer inhibited effectively by available therapeutics. Interference with signaling pathways downstream of activated AR that mediate aggressive CRPC behavior may lead to alternative CaP treatments. Developing such therapeutic strategies requires a thorough mechanistic understanding of the most clinically relevant and druggable AR-dependent signaling events. Recent proteomics analyses of CRPC clinical specimens indicate a shift in the phosphoproteome during CaP progression. Kinases and phosphatases represent druggable entities, for which clinically tested inhibitors are available, some of which are incorporated already in treatment plans for other human malignancies. Here, we reviewed the AR-associated transcriptome and translational regulon, and AR interactome involved in CaP phosphorylation events. Novel and for the most part mutually exclusive AR-dependent transcriptional and post-transcriptional control over kinase and phosphatase expression was found, with yet other phospho-regulators interacting with AR. The multiple mechanisms by which AR can shape and fine-tune the CaP phosphoproteome were reflected in diverse aspects of CaP biology such as cell cycle progression and cell migration. Furthermore, we examined the potential, limitations and challenges of interfering with AR-mediated phosphorylation events as alternative strategy to block AR function during CaP progression.

Key Words

- kinase
- phosphatase
- castration
- hormonal therapy
- treatment resistance
- coregulator

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Introduction

Prostate cancer (CaP) is expected to cause the death of 33,300 American men in 2020 and remains the second leading cause of cancer-related deaths in Western men (Siegel *et al.* 2020). It is well recognized that the androgen receptor (AR), a ligand-activated transcription factor, drives CaP progression (Huggins & Hodges 2002, Dai *et al.* 2017). Therefore, blocking AR activation, known

as androgen deprivation therapy (ADT), has been the default treatment for metastatic CaP for almost 80 years. Although ADT is successful in inducing an initial remission, CaP cells eventually adapt to acquire resistance to ADT, and castration-recurrent CaP (CRPC) develops. Adaptations that allow the emergence of CRPC include several mechanisms by which AR activation is restored,

such as activating somatic mutations, amplifications and rearrangements in the AR gene, intratumoral metabolism and synthesis of (precursor) androgens, compensatory expression of the AR-related glucocorticoid receptor that is able to take over parts of AR function, all of which have been the subject of excellent reviews before (Mills 2014, Watson *et al.* 2015, Narayanan *et al.* 2016, Dai *et al.* 2017).

Because AR is still active and continues to drive CaP progression after failure of ADT, alternative means of interfering with its activity are sought. In view of AR's transcription factor function such efforts have mostly focused on its ligand-independent constitutively active transcription activity, its ability to bind DNA, and to form active transcriptional complexes. Several compounds targeting these aspects of AR function have been developed and/or tested clinically, yet none have moved into clinic (Kumari *et al.* 2017, Senapati *et al.* 2019).

Recent genomic and proteomics analyses of CaP models and clinical specimens have provided substantial new evidence for critical roles for AR in various cellular events relevant to aggressive CaP behavior and CaP progression such as cell cycle regulation, epigenetic modifications, DNA repair, and translational regulation (Schiewer *et al.* 2012, Xu *et al.* 2012, Schiewer & Knudsen 2016, 2019, Liu *et al.* 2019). While delineating the involvement of AR in these cellular processes, it has become clear that AR controls several phosphorylation events in which specific kinases and phosphatases play important roles. In other

human malignancies, defining and understanding the phosphorylation-based signaling cascades that drive disease progression resulted in novel therapeutic strategies, some of which have shown exceptional success rates for therapeutic intervention and overcoming treatment resistance (Lynch *et al.* 2004, Chapman *et al.* 2011, Shaw *et al.* 2014). For instance, in non-small cell lung cancer, the tyrosine kinase inhibitors gefitinib or erlotinib are effective in targeting activating mutations in EGF receptor (EGFR), a driver of disease progression (Ciardiello & Tortora 2008). Upon emergence of somatic mutations, second- or even third-generation EGFR inhibitors are used to improve outcomes (Janjigian *et al.* 2014, Mok *et al.* 2017). The possibility of similar therapeutic successes targeting phosphorylation regulators in CaP is supported by the emerging phosphoproteomic characterizations of clinical CRPC specimens (Drake *et al.* 2016 Faltermeier *et al.* 2016) and testing of kinase inhibiting drugs, alone or in combination therapy, in CaP co-clinical trials (Munster *et al.* 2019, Sweeney *et al.* 2019).

Here, we review information on AR's control over kinase and phosphatase expression and its collaboration with such key regulators of phosphorylation events in CaP. We explore the biology that is impacted by AR-associated phosphorylation, its relevance during CaP progression, and the overall potential, limitations and challenges associated with exploiting AR-dependent phosphorylation as alternative strategy to overcome resistance to AR-targeting treatments in CaP (Fig. 1).

Figure 1

Differential AR action in CaP. Canonical AR signaling (left panel). Androgens bind to and activate AR. Activated AR homodimerizes, relocates to the nucleus and binds to androgen response elements (AREs) where it interacts with coregulators to transcribe target genes such as the gene encoding prostate-specific antigen (PSA). Androgen deprivation therapy, the first-line standard of care for metastatic CaP, blocks ligand-activation of AR. Treatment strategies to block AR's transcription function in an alternative manner include inhibiting AR-coregulator interactions, AR's constitutively active transcription by the N-terminal domain (NTD), and AR-DNA binding via the DNA-binding domain (DBD) of AR. Schematic representing AR's regulation of several cellular processes via its control over and interactions with kinases and phosphatases (right panel).

AR dependence of kinase and phosphatase expression and activity

Control of AR over important phosphorylation events and AR's collaboration with kinases and phosphatases in CaP has been recognized before. A few well-known examples that immediately come to mind are AR's regulation of cyclin-dependent kinase (CDK) activity and its interaction with epigenetic modifiers such as TRIM24 (a kinase) and the tumor suppressor PTEN, a phosphatase (Knudsen *et al.* 1998, Li *et al.* 2001, Groner *et al.* 2016). Yet, despite the emerging insights in the important role and the clinical relevance of the phospho-proteome during CRPC progression (Drake *et al.* 2012, 2013, 2016, Faltermeier *et al.* 2016), the extent of AR's contribution to phosphorylation events in CaP is yet to be fully appreciated. Here, we review literature to define more comprehensively the transcriptional and translational control by AR over kinase and phosphatase expression and to examine alternative mechanisms by which AR impacts phosphorylation-dependent CaP cell biology.

Transcriptional control by AR over kinases and phosphatases

First, we explored the possibility that AR regulates the CaP phosphoproteome by directly controlling transcription of genes that encode kinases or phosphatases. To this end, members of a CaP gene signature representing hundreds of *bona fide* AR target genes ($n=452$, LNCaP cell dataset) (Liu *et al.* 2017), i.e., genes that contain at least one androgen response element (ARE) and whose androgen responsiveness has been experimentally verified were cross-matched with 536 kinases listed on KinHub (Eid *et al.* 2017), a resource that maintains and curates knowledge on the human kinome. This approach isolated 28 genes encoding kinases as direct AR target genes. Expression of 13 kinases was androgen-repressed, the remaining 15 were upregulated by AR stimulation. The majority ($n=23$) of these genes encoded for Ser/Thr kinases, while only five led to Tyr kinase expression (Table 1). Kinases controlled transcriptionally by AR include for instance CAMKK2, a member of the CAMK family of kinases,

Table 1 AR-associated kinases.

Gene symbol	AR dependence	Type	Family	Inhibitor	CaP phosphoproteome
AR regulation: transcriptional					
BMPR1A	Up	Ser/Thr	TKL	DMH-1	NA
BMPR1B	Up	Ser/Thr	TKL	LDN-212854	NA
CAMKK2	Up	Ser/Thr	CAMK	A 484954, NH125	pS495, pS511 ^c
CDK8	Down	Ser/Thr	CMGC	Senexin A	NA
CDKL5	Down	Ser/Thr	CMGC	None	NA
CLK2	Down	Ser/Thr	CMGC	TG003	NA
DDR1	Down	Tyr	TK	DDR1-IN-1, nilotinib	Y520, Y792, Y796 ^d
EPHA3	Up	Tyr	TK	KB004	Y596 ^d
ERBB2 ^a	Down	Tyr	TK	>90 inhibitors	pS1054, pS1078, pS1083 ^c
MAK1 ^a	Up	Ser/Thr	CMGC	None	NA
MAP2K4	Up	Ser/Thr	STE	MEK inhibitors	pS257 ^c
MAP4K1	Down	Ser/Thr	STE	None	NA
MAPK6 ^b	Up	Ser/Thr	CMGC	None	pS386, pT389 ^e
MAPK8	Down	Ser/Thr	CMGC	None	pT183 ^d
MAPKAPK3	Down	Ser/Thr	CAMK	None	NA
MERTK	Up	Tyr	TK	None	NA
MYLK	Down	Ser/Thr	CAMK	ML 9 Hydrochloride	pS911, pS1768 ^c
PAK2	Down	Ser/Thr	STE	FRAX597	pS2, pS141 ^c
PRKAA1	Up	Ser/Thr	CAMK	None	pS496 ^c
PRKCA	Up	Ser/Thr	AGC	None	pT638, pS657 ^f
PRKD1 ^a	Down	Ser/Thr	CAMK	CID 2011756	pS473 ^e
ROR1	Up	Tyr	TK	None	NA
RPS6KA1 ^a	Down	Ser/Thr	AGC	BRD 7389	pS232 ^e
RPS6KA3 ^a	Up	Ser/Thr	AGC	AT9283	pS221 ^e
SGK1 ^a	Up	Ser/Thr	AGC	None	NA
SNRK	Down	Ser/Thr	CAMK	None	NA
STK17B	Up	Ser/Thr	CAMK	None	NA
STK39	Up	Ser/Thr	STE	None	pS385, pS315, pT354 ^c

(Continued)

Table 1 (Continued)

Gene symbol	AR dependence	Type	Family	Inhibitor	CaP phosphoproteome
AR regulation: translational					
HIPK3 ^a	NA	Ser/Thr	CMGC	None	NA
MAPK6 ^b	NA	Ser/Thr	CMGC	None	pS386, pT389 ^e
MAPKAPK2	NA	Ser/Thr	CAMK	PF-3644022	NA
PINK1	NA	Ser/Thr	Other	None	NA
TRIM24 ^a	NA	Ser/Thr	Atypical	None	pS1028 ^c
AR regulation: interactor					
AKT1	NA	Ser/Thr	AGC	Nelfinavir, Everolimus, PHT 427	pT34 ^c
CDK11B	NA	Ser/Thr	CMGC	None	pS265, pS271, pS422 ^c
CDK6	NA	Ser/Thr	CMGC	LEE011 - CDK4/6	NA
CDK7	NA	Ser/Thr	CMGC	Alvocidib, seliciclib, BS-181	pS164 ^g
CDK9	NA	Ser/Thr	CMGC	Alvocidib, seliciclib, Dinaciclib, AZD 5438	NA
DAPK3	NA	Ser/Thr	CAMK	None	NA
DYRK1A	NA	Ser/Thr	CMGC	Harmine, INDY, ProINDY	pY321 ^f
EGFR	NA	Tyr	TK	>200 inhibitors	pS1039, pS1042 ^c
ERBB2 ^a	NA	Tyr	TK	>90 inhibitors	pS1054, pS1078, pS1083 ^c
GAK	NA	Ser/Thr	Other	None	pS16, pS73, pS826, pS829, pS1185 ^c
GSK3B	NA	Ser/Thr	CMGC	SB 216763, AZD1080	pY216 ^d
HIPK3 ^a	NA	Ser/Thr	CMGC	None	NA
LATS2	NA	Ser/Thr	AGC	None	NA
MAK1 ^a	NA	Ser/Thr	CMGC	None	NA
MAPK1	NA	Ser/Thr	CMGC	FR 180204	Y187 ^d
MAPK15	NA	Ser/Thr	CMGC	None	NA
NLK	NA	Ser/Thr	CMGC	None	NA
PAK6	NA	Ser/Thr	STE	None	pS560 ^d
PKN1	NA	Ser/Thr	AGC	lestaurtinib, tofaticinib	pS69, pS916 ^d
PRKD1 ^a	NA	Ser/Thr	CAMK	CID 2011756	pS473 ^e
PRKDC	NA	Ser/Thr	Atypical	AZD7648,	pT2609, pS2612, pS3205 ^d
RNASEL	NA	Ser/Thr	Other	None	NA
RPS6KA1 ^a	NA	Ser/Thr	AGC	BRD 7389	pS232 ^e
RPS6KA3 ^a	NA	Ser/Thr	AGC	AT9283	pS221 ^e
SGK1 ^a	NA	Ser/Thr	AGC	None	NA
SRC	NA	Tyr	TK	Herbimycin A, MNS, Dasatinib	pS104 ^e
TAF1	NA	Ser/Thr	Atypical	None	pS1152, pS1155, pS1669, pS1672 ^e
TNK2	NA	Tyr	TK	AIM-100, Dasatinib	pY827 ^e
TRIM24 ^a	NA	Ser/Thr	Atypical	None	pS1028 ^c

Kinases that are transcriptionally (top part) or translationally (middle part) regulated by AR and kinases that interact with AR (bottom part). Up/down; kinase expression induced/repressed by AR. Inhibitors; commercially available kinase inhibitors, information obtained from [genecards.org](#). CaP phosphoproteome; site of kinase phosphorylation in CRPC samples obtained from [Drake et al. \(2016\)](#) and [Faltermeier et al. \(2016\)](#).

^aEntry occurs in top and bottom part of table; ^bentry occurs in top and middle part of table; ^cphosphorylation site is significantly enriched in CaP but functional consequence of phosphorylation is unknown; ^dphosphorylation site is significantly enriched in CaP and associated with kinase activation; ^ephosphorylation site is found but not significantly enriched in CaP and functional consequence of phosphorylation is unknown; ^fphosphorylation site not significantly enriched in CaP but activates the kinase; ^gphosphorylation site is significantly enriched in CaP and represses kinase activity.

AGC, members of PKA, PKG, and PKC family of kinases; CAMK, calcium/calmodulin-dependent kinase; CMGC, members of cyclin-dependent kinase, mitogen-activated protein kinase, glycogen synthase kinase and CDC-like kinase family; NA, not applicable; Ser/thr, serine/threonine kinase; STE, 'sterile' serine/threonine kinases; TK, tyrosine kinase; TKL, tyrosine kinase-like; Tyr, tyrosine kinase.

which is well known for its role in lipid metabolism, and has been proposed by several groups as viable therapeutic target in CaP ([Massie et al. 2011](#), [Karacosta et al. 2012](#), [Penfold et al. 2018](#), [White et al. 2018](#)). Other more neglected kinases include the discoidin domain receptor family member 1, DDR1, which has been implicated in epithelial-mesenchymal transition (EMT) and metastasis

in human cancers ([Maeyama et al. 2008](#), [Yeh et al. 2011](#), [Koh et al. 2015](#)), has not yet been extensively studied in CaP, but can be inhibited by nilotinib, a drug that is being tested in clinical trials for leukemia ([Jeitany et al. 2018](#)). Comparison with recent phospho-proteome data obtained via mass spectrometry on clinical CRPC cases ([Drake et al. 2016](#)) revealed several kinases, for instance

MERTK, to be over-activated in CaP compared to benign prostate, suggesting important roles in late-stage CaP and supporting their therapeutic potential. For several of these kinases, inhibitors have been developed, some of which have been tested or are being tested in clinical CaP trials, or have been used to treat other malignancies (Table 1).

These data demonstrate that a significant number of kinases is controlled by AR directly binding to their regulatory gene regions. We performed a similar examination of the AR target gene list for genes encoding any of the 226 phosphatases classified by the Human Gene Nomenclature Committee (HGNC). This analysis returned 13 AR-dependent and transcriptionally regulated phosphatases, consisting of 9 phosphatases that are androgen-stimulated and 4 that are downregulated upon AR activation, verifying that this class of proteins can be subject to similar AR regulation (Table 2). Among the 13 phosphatases were PTEN and INPP4B, which have been implicated in CaP progression before (Li *et al.* 1997, Hodgson *et al.* 2011, 2014), whereas others such as SYNJ1 have lesser recognized functions in CaP. Pharmacologic modulation of phosphatase activity is possible, as evidenced by availability of several phosphatase inhibitors; however, only a handful of them are in clinical trials (Bollu *et al.* 2017).

AR regulation of its target genes can be context dependent and may be influenced by, for instance, the genomic make-up or the AR expression levels of CaP cells. We therefore examined the extent to which the findings of AR dependence of genes encoding kinases and phosphatases derived from LNCaP cells can be extrapolated to other AR-positive cell lines, such as VCaP. VCaP cells express the TMPRSS2-ERG gene fusion and show AR gene amplification, which can each impact the composition of the AR cistrome (Waltering *et al.* 2009, Yu *et al.* 2010, Makkonen *et al.* 2011, Cai *et al.* 2013, Wasmuth *et al.* 2020). Analyses of two publically available and independent AR ChIP-Seq data sets derived from VCaP cells (Massie *et al.* 2011, Asangani *et al.* 2014) employing the same selection criteria used on LNCaP cell data (i.e. AR binding peaks present within 300 kb of transcriptional start sites (Liu *et al.* 2017)) revealed androgen-induced AR binding peaks in 27/28 kinase-encoding genes and all, or 13/13, phosphatase-encoding genes (Supplementary Table 1, see section on [supplementary materials](#) given at the end of this article). Moreover, except for the gene encoding CLK2 (two thirds of replicates positive), AR-binding sites were present in all biological replicates in androgen-stimulated conditions, indicating that AR regulation of these kinases and phosphatases occurs in multiple AR-positive CaP models.

Translational control by AR over kinases and phosphatases

The impact of AR-dependent transcriptional control over its target genes is readily measured via oligoarray, RNA-Seq and similar gene expression assays, and can be validated using AR ChIP-chip, ChIP-Seq or ChIP-exo approaches. The AR-controlled transcriptome has therefore been well-documented and characterized in an array of CaP model systems as well as clinical specimens (Mills 2014, Kumari *et al.* 2017, Senapati *et al.* 2019). The extent to which AR-dependent transcription is translated into CaP proteomes or to which CaP translational events are AR-dependent is poorly understood. This is an important question since recent studies have shown discordance between mRNA levels and protein abundance (Sinha *et al.* 2019). The lack of our knowledge in this regard, particularly as it regards AR's control over CaP translation (rates), relates to the well-recognized, and not CaP-specific, limitations in the technical assessment of translational rates. The results from techniques such as polysome profiling, Ribo-Seq and ribosome profiling can serve as surrogate for translation rates, but until very recently these had not been used on CaP models, let alone in the context of CaP's AR dependency. A recent study found that 4EBP1, an inhibitor of the translation initiation complex, contains an ARE in its first intron, and upon androgen stimulation of CaP cells, 4EBP1 mRNA levels are induced (Liu *et al.* 2019). These findings point to an indirect mechanism for AR to shape the CaP proteome. Subsequently, ribosome profiling was done on CaP tissues from a transgenic mouse CaP model driven by prostate-specific PTEN deletion, which mimics aggressive CRPC progression. Tissues from intact versus castrated animals were analyzed, which allowed to verify AR dependence. The authors report a subset of mRNAs that are differentially regulated at the post-transcriptional level and are subject to AR signaling, which encode five kinases and three phosphatases (Tables 1 and 2). Remarkable, only one entry (i.e. MAPK6) overlapped between transcriptionally and post-transcriptionally AR-dependent genes, indicating distinct levels of AR control over the expression of phosphoregulators.

Alternative AR involvement in kinase and phosphatase action

Direct transcriptional or post-transcriptional control over kinase and phosphatase expression may not capture the full extent of AR's ability to regulate phosphorylation status of CaP cells.

Table 2 AR-associated phosphatases.

Gene symbol	AR dependence	Group	Inhibitor	CaP phosphoproteome
AR regulation:	Transcriptional			
INPP4B	Up	Phosphoinositide phosphatases	None	NA
INPP5A	Down	Phosphoinositide phosphatases	None	NA
INPP5D	Down	Phosphoinositide phosphatases	None	pS971 ^a
PMM2	Up	HAD Asp-based non-protein phosphatases	None	NA
PPM1K	Up	Protein phosphatases, Mg ²⁺ /Mn ²⁺ dependent	None	NA
PPP1CB	Up	Protein phosphatase catalytic subunits	FK 506	NA
PPP2CB	Up	Protein phosphatase catalytic subunits	FK 506	NA
PTEN ^b	Down	Phosphoinositide phosphatases	SF1670	NA
PTPN11	Up	Protein tyrosine phosphatases non-receptor type	BVT 948, TCS 401	pY584 ^a
PTPN21	Up	Protein tyrosine phosphatases non-receptor type	None	pS658 ^c
PTPRM	Up	Protein tyrosine phosphatases receptor type	None	NA
PTPRR	Down	Protein tyrosine phosphatases receptor type	None	NA
SYNJ1	Up	Phosphoinositide phosphatases	None	pS830 ^c
AR regulation:	Translational			
MINPP1	NA	Phosphoinositide phosphatases	None	NA
PHLPP1	NA	Protein phosphatases, Mg ²⁺ /Mn ²⁺ dependent	None	NA
PPP4C	NA	Protein phosphatase catalytic subunits	None	NA
AR regulation:	Interactor			
CDC25A	NA	Class III Cys-based CDC25 phosphatases	NSC 663284, NSC 95397	NA
CDC25B	NA	Class III Cys-based CDC25 phosphatases	NSC 663284, NSC 95397	NA
CTDSP2	NA	CTD family phosphatases	None	NA
PGAM5	NA	Serine/Threonine protein phosphatases	None	pS80 ^a
PPP1CA	NA	Protein phosphatase catalytic subunits	FK 506	NA
PPP1CC	NA	Protein phosphatase catalytic subunits	FK 506	NA
PTEN ^b	NA	Phosphoinositide phosphatases	SF1670	NA

Phosphatases that are transcriptionally (top part) or translationally (middle part) regulated by AR and phosphatase that interact with AR (bottom part).

Up/down; phosphatase expression induced/repressed by AR. Inhibitors; commercially available kinase inhibitors, information obtained from genecards.org. CaP phosphoproteome; site of phosphorylation in CRPC samples obtained from Drake *et al.* (2016) and Faltermeier *et al.* (2016).

^aPhosphorylation site is significantly enriched in CaP samples but consequence of phosphorylation is not defined; ^bEntry occurs in top and bottom part of table; ^cPhosphorylation site is found but not significantly enriched in CaP samples and consequence of phosphorylation is not defined.

NA, not applicable.

Mechanisms that involve genomic AR function

It has been long recognized that the (de)phosphorylation activity of these regulators depends on activating ligands, interactions with adaptors, scaffold proteins and binding patterns that ensure their proper spatial and temporal activation and complex formation within the cell (Pawson & Scott 1997) or E3 ligases that modulate their action. In CaP, several aspects of such 'indirect' modulation of kinase and phosphatase action may involve transcriptional or translational regulation by AR. For instance, AR stimulates the enzymatic pathways that govern synthesis of fatty acids (Heemers *et al.* 2006), which are critical activators of protein kinase C family members (Bell & Burns 1991). Similarly, several adaptor proteins (e.g. GRB10) and scaffold proteins (e.g. AKAP12)

have been identified as ARE-driven genes (Liu *et al.* 2017). Kinase and phosphatase-associated E3 ligases such as MID1 and TRIM36 are *bona fide* AR target genes. MID1 interacts with the kinase PDPK1 and the phosphatase PP2A (Aranda-Orgilles *et al.* 2011), and thus, potentially providing indirect AR control over their respective phospho-proteomes. TRIM36, on the other hand, is an androgen-induced gene whose overexpression in CaP blocks MEK/ERK signaling, while its decreased activity following ADT increases MEK/ERK kinase transduction pathways (Liang *et al.* 2018).

Literature indicates that AR can employ also yet other mechanisms to influence the CaP proteome. One well-known example is AR-dependent activity of several CDKs, which was not captured by the above analyses,

consistent with its regulation by AR-responsive cyclin expression (Knudsen *et al.* 1998). It is also conceivable that AR controls enzymatic activity of phosphorylation modulators without altering their expression levels. One such mechanism by which AR may steer kinase activity to specific CaP functions is its signaling, via activation of RhoA, to the Ser/Thr kinase PKN1, which then mediates androgen control to the secondary transcription factor serum response factor (SRF) (Venkadakrishnan *et al.* 2019). SRF controls the immediate early response, cell cycle regulation, and organization of cytoskeleton. AR-RhoA-PKN1-mediated activation of SRF signaling is linked with aggressive CaP behavior and poor outcome (Heemers *et al.* 2011, Lundon *et al.* 2017, Prencipe *et al.* 2018). Interestingly, PKN1 serves also as an AR-associated coregulator, which points toward bidirectional regulation in which AR transactivation is modulated by kinases and phosphatases (Metzger *et al.* 2008). Review of the RAAR database that contains more than 280 AR-associated coregulators that we compiled before (DePriest *et al.* 2016) shows that 29 and 6 genes possess kinase and phosphatase moieties, respectively (Tables 1 and 2) (DePriest *et al.* 2016). Examples include the kinase PRKDC and the phosphatase, CDC25B. Such proteins are part of AR transcriptional complexes at ARE in target genes that are formed in a context-dependent manner, preferentially control subsets of AR target genes and thus aspects of CaP biology (Liu *et al.* 2017). Differential phosphorylation of transcriptional complex components may underlie some of this specificity (Rasool *et al.* 2019). Other means of cross-talk between AR and phosphorylation machinery are conceivable, such as AR control of non-coding RNAs (miRNA, eRNA, circRNA) that impact kinase or phosphatase expression.

Mechanisms that involve non-genomic AR function

It should be noted that all of the scenarios above consider only AR's genomic function, that is, AR exerting its transcription factor function in the cell nucleus. However, non-genomic AR action has been recognized, which involves signal transduction via a membrane-bound or cytoplasmic androgen-activated AR (Zarif & Miranti 2016, Leung & Sadar 2017). Non-genomic AR signaling occurs within seconds to minutes, thus much more rapidly than the canonical genomic AR function, and has been shown to activate, for example, Src and MAPK signaling pathways in the cytoplasm (Migliaccio *et al.* 2000, 2007). These signaling axes have been reported to be constitutively active in CRPC and could thus impact significantly the CaP phosphoproteome during CaP progression.

Delineating the full scope of the mechanisms by which AR intersects with CaP phosphorylation events will be important. Even taking into account this limitation and considering only the information provided in Tables 1 and 2, it is obvious that AR can exert major impact on CaP's phosphorylation status: a total of 53 kinases and 22 phosphatases are either controlled by AR or intersect with its signaling. The kinome tree for the impacted kinases shows distribution over seven kinase families, with most enrichment for the CMGC kinase family (Fig. 2) (Metz *et al.* 2018). AR-associated phosphatases belong to the classes of lipid phosphatases, protein phosphatases and HAD Asp-based phosphatases, while no acid phosphatases, alkaline phosphatases, sugar phosphatases or 5'-nucleotidases are enriched (Fig. 3).

Clinically relevant CaP biology controlled by AR-dependent kinases and phosphatases

Phosphorylation events control and fine-tune most, if not all, cellular processes. The overview provided in the previous section indicates that AR action can regulate and involve the activity of dozens of kinases and phosphatases, but the impact of subsequent alterations in downstream phosphorylations on CaP cell behavior and clinical CaP progression has not been explored. Even a superficial glance at the proteins listed in Tables 1 and 2, especially when considering the signaling cascades and substrates that are known to be modified by these at the post-translational level, already predicts that a diverse array of cellular functions is affected. For the purpose of this review, we will focus on those aspects of CaP biology that are most relevant to disease progression and/or are considered for therapeutic intervention.

Cell proliferation and cell cycle regulation

The ability of cells to proliferate and to progress through the cell cycle is a hallmark of all cancers (Hanahan & Weinberg 2011). Cell cycle progression is exquisitely regulated by phosphorylation events at critical transition steps, which are tightly controlled by cyclin-CDK interactions. In CaP, one of the best characterized consequences of AR action is control over cell proliferation: lower doses of androgens induce CaP cell proliferation whereas high doses lead to CaP cell differentiation and/or cell death (Damassa *et al.* 1991, Zhau *et al.* 1996, Chuu *et al.* 2005, Sedelaar & Isaacs 2009, Denmeade & Isaacs 2010). Although the molecular mechanism(s) by which AR controls CaP cell proliferation

Figure 2

Kinome tree for AR-associated kinases. Kinases transcriptionally regulated by AR are marked in dark blue, kinases translationally regulated by AR in sky blue, and AR-interacting partners in pink. Figure generated using online tool, CORAL (Mets *et al.* 2018).

remains incompletely understood (Balk & Knudsen 2008, Schiewer *et al.* 2012, Wen *et al.* 2014), biphasic expression patterns and post-translational modifications of regulators of cell cycle checkpoints, particularly at the G1-S phase transition, have long been recognized. Progression from G1 to S phase of the cell cycle following low dose androgen stimulation involves the AR-dependent action of the kinase CDK2, 4, and 6. CDK6 is listed in Table 1 as AR interactor, and activity of CDK6 as well as these two other CDKs is indirectly regulated by AR via control over cyclin availability (Knudsen *et al.* 1998). The substrate for the action of CDK2, 4 and 6 in AR-controlled

cells cycle progression is Rb, with Cdk-dependent Rb phosphorylation leading to E2F-controlled cell cycle progression (Markey *et al.* 2002). In addition to these components of the cell cycle machinery, several other kinases or phosphatases that are either transcriptionally or translationally regulated by AR or are part of the AR interactome at target genes can influence CaP cell proliferation (Table 1). These include multiple kinases that act at different levels of MAP kinase signaling cascades, for which substrates are not as well known, and may mediate resistance to CDK4/6 inhibition (de Leeuw *et al.* 2018), as well as regulators of MAPK activity, such as RPS6KA1

Figure 3

Classification of AR-associated phosphatases.

and RPS6KA3. In addition, ERBB2, AKT/PI3K, SGK3 signaling have all also been described to impact CaP cell proliferation (Shanmugam *et al.* 2007, Sherk *et al.* 2008, Edlind & Hsieh 2014, Gao *et al.* 2016). Changes in activity and expression of the tumor suppressor and phosphatase PTEN, both transcriptionally regulated by AR and an AR interactor, are well known to alter CaP cell proliferation and aggressiveness (Wang *et al.* 2003).

Cytoskeleton organization and function

Successful completion of cell division, the final step of cell proliferation, involves spindle formation and reorganization of microtubules, which rely on tightly regulated phosphorylation events. The potential role for AR control over these modifications in CaP cells have yet to be examined. None-the-less, changes in organization and remodeling of the (actin) cytoskeleton are among the most common alterations observed in CaP compared to benign prostate tissues (Purnell *et al.* 1987, Hemstreet *et al.* 2000, Haffner *et al.* 2017). Such alterations also underlie each step of the invasion-metastatic cancer cascade, another key hallmark of cancer (Hanahan & Weinberg 2011), that starts from local invasion, intravasation and extravasation of cancer cells leading to micrometastases, which develop into macroscopic metastatic cancers. Several of the proteins listed in Tables 1 and 2 play role in these key steps. For instance, DDR1, discoidin

domain receptor tyrosine kinase 1, is a collagen-induced receptor that activates signal transduction pathways involved in cell adhesion, proliferation, and extracellular matrix remodeling (Lee *et al.* 2019). Its family member DDR2 was shown to play an essential role in CaP bone metastasis and its silencing decreased CaP cell motility and invasiveness (Yan *et al.* 2014). Consistent with such a role, inhibition of DDR1 reduced EMT, linked to invasive/metastatic properties, in CaP (Maeyama *et al.* 2008, Yeh *et al.* 2011, Koh *et al.* 2015). Another AR controlled kinase, MERTK is well known to promote phagocytosis of apoptotic cells and reduce cytokine response (Graham *et al.* 2014) and to drive invasion and metastasis in melanoma (Schlegel *et al.* 2013), glioblastoma (Wang *et al.* 2013) as well as CaP (Faltermeier *et al.* 2016). Although the kinase MYLK is poorly studied in CaP, because of its involvement in phosphorylating myosin light chains and therefore, cytoskeletal reorganization (Zhi *et al.* 2005), it is anticipated to similarly impact CaP biology. PAK2 is known to phosphorylate MAPK4, MAPK6 and activate MAPKAPK5 to influence F-actin polymerization, induce cytoskeleton reorganization and cell migration (De la Mota-Peynado *et al.* 2011, Li *et al.* 2011, Wang *et al.* 2018). Other examples include PRKD1 (Luef *et al.* 2016), which negatively regulates cell migration, and MAPKAPK2, which along with HSP27 increased matrix metalloprotease type 2 (MMP2) activity, leading increased cell invasion (Xu *et al.* 2006).

Transcriptional control

Progression from normal prostate to treatment-naïve localized CaP that is responsive to ADT, and then to CRPC is marked by differential gene expression patterns, which involve also considerable shifts in the transcriptomes and cistromes that are controlled by AR, the major driver of CaP progression (Massie *et al.* 2011, Sharma *et al.* 2013, Stelloo *et al.* 2015). These alterations in gene expression profiles result from epigenetic and transcriptional events in which several of the AR-associated kinases and phosphatases listed above are known to fulfill critical roles. In addition to roles in cell cycle progression, several CDKs that interact with AR have been implicated also in transcription of AR target genes. CDK6 fulfills such dual roles and has been reported to coactivate also strongly transcription by the gain-of-function AR T878A mutant that occurs under selective pressure of ADT and is present in CRPC (Lim *et al.* 2005). Other CDKS, such as CDK7 and 9 have been mainly implicated in AR-dependent transcription (Lee *et al.* 2000, Pawar *et al.* 2018, Itkonen *et al.* 2019, Rasool *et al.* 2019). Whether kinase activity is relevant for this is not always clear, as it is necessary for some CDKs and dispensable for others. The target of CDK kinase action can differ also. CDK7 interacts with the N-terminal of AR, but has not reported to directly phosphorylate AR (Lee *et al.* 2000). Instead, CDK7 phosphorylates the MED1 component of Mediator complex, which enhances MED1 interaction with AR. Inhibition of CDK7 using a specific inhibitor (TZH1) blocks MED1 recruitment to chromatin (Rasool *et al.* 2019). On the other hand, CDK9 phosphorylates AR at serine 81 (Gordon *et al.* 2010), and a kinase-dead version of CDK9 impairs AR transactivation (Lee *et al.* 2001). Consistent with roles for several CDKs in regulating AR transactivation, also CDC25A and CDC25B, phosphatases that control CDK activity, can modulate transcription of AR target genes (Balk & Knudsen 2008, Chiu *et al.* 2009). Whether this entails CDC's phosphatase activity or alterations in phosphorylation of AR or associated transcriptional regulators is not known. Other classes of kinases and phosphatases have been implicated in AR transcriptional control. Functionally diverse coregulators such as HIPK3 aka ANPK (androgen receptor-interacting nuclear protein) or DAPK3 interact with AR and activate its transcriptional function but AR has not been shown to be a direct substrate of HIPK3 or DAPK3 (Moilanen *et al.* 1998, Leister *et al.* 2008, Felten *et al.* 2013). AKT1, whose activation and activity is a major issue during endocrine therapy in CaP (Carver *et al.* 2011), does phosphorylate AR at S210 and S790

(Lin *et al.* 2001), which leads to suppression of AR-target genes such as p21. PAK6-AR interaction (Yang *et al.* 2001, Lee *et al.* 2002) also impacts AR target gene transcription and relies on PAK6's kinase domain and PAK6-dependent phosphorylation of AR's DNA-binding domain (DBD) domain (Schrantz *et al.* 2004). Direct phosphorylation and activation of AR by SRC sensitizes AR to intracrine androgen levels, resulting in the engagement of canonical and non-canonical AR-dependent gene signatures (Guo *et al.* 2006, Chattopadhyay *et al.* 2017). We identified the histone phosphatase PGAM5 as integral and essential part of AR transcriptional complexes that contain also p53 and the AR-associated coregulator WDR77 and preferentially control CaP cell proliferation and survival (Liu *et al.* 2017). Whether this involves PGAM5's phosphatase function was not addressed. Other contributions of the AR-controlled phospho-regulators to the CaP transcriptome are conceivable. As an example, CLK2 is a transcriptionally AR-dependent kinase (Jin *et al.* 2013) that regulates the spliceosomal complex and alternative splicing in breast cancer and endothelial cells (Eisenreich *et al.* 2009, Yoshida *et al.* 2015), suggesting it may influence the spectrum of splice forms expressed.

The clinically relevant AR-dependent CaP phosphoproteome

The development of kinase inhibitors and their subsequent therapeutic success in other human malignancies such as non-small-cell lung cancer or melanoma (Lynch *et al.* 2004, Chapman *et al.* 2011, Shaw *et al.* 2014) was triggered by observations of activating kinase mutations and/or amplifications. These alterations rendered the kinase function oncogenic and turned it into a driver of cancer progression. In CaP, such somatic alterations in genes encoding kinases or for that matter phosphatases (except PTEN) are rare: only 0.9 to 8 percent (5226 specimens examined via cBioportal) of the AR-dependent phosphorylation regulators listed in Tables 1 and 2 are affected, with the exception of PTEN deletion, which occurs in 18% of cases/specimens (Fig. 4). The absence of comprehensive phospho-proteomics data from clinical CRPC cases further impeded the evaluation of wild-type kinases as valid therapeutic targets and kinase inhibitors as potential novel therapeutics in CaP. Some smaller scale phospho-proteome studies had been conducted using CaP cell lines (Giorgianni *et al.* 2007) and xenograft models under conditions that represent ADT-naïve CaP and CRPC (Ramroop *et al.* 2018). Results suggested active signaling by several kinases, including for instance YAP1 and the

Figure 4

Somatic alterations in AR-associated kinases and phosphatases. Percentage of clinical CaP cases with somatic alterations in AR-associated kinases (left panel) and phosphatases (right panel). Data reflect results for cBioportal analyses on 5226 CaP specimens.

AR-dependent PAK2, which were put forward as novel viable therapeutic targets (Jiang *et al.* 2015). Other studies had also recognized that AR can repress major proliferative kinase signaling pathways in CaP, and conversely that ADT can, depending on one's perspective, either cause unwanted activation of these pathways in CRPC or render these kinases as targets for novel CRPC treatments. The most prominent example is ADT-induced activation of PI3K/AKT signaling, may impact downstream kinase signaling such as that mediated by SGK1 (Carver *et al.* 2011, Toska *et al.* 2019). Similar observation have been made for the tyrosine kinase BMX. BMX expression in CaP is suppressed by AR and rapidly increased in response to ADT. BMX contributed to CRPC development *in vitro* and *in vivo* by positively regulating the activities of multiple receptor tyrosine kinases, such as MET, FGFR1 (Chen

et al. 2013). Inhibition of BMX kinase activity markedly enhanced the response to castration in CaP models (Chen *et al.* 2018). These studies put forward the concept that modulation of AR activity during CRPC progression may alter the kinase signaling pathways and associated biology that are active in a patient's CaP.

None-the-less, it was not until the concept of personalized medicine gained traction in the CaP field that more in-depth proteomic analysis on clinical CaP specimens were undertaken and activity of some wild-type kinases started to emerge as valid targets for new CaP treatment schemes. A 2012 paper from the Witte group laid the foundation by performing immunohistochemistry for tyrosine phosphorylation marks in patient samples representing progression from benign prostate to CRPC and tissues from a mouse CaP model driven by oncogenes such as AKT, ERG, AR, or K-RASG12V, which showed increased tyrosine phosphorylation marks during both patient and mouse cancer progression (Drake *et al.* 2012). Subsequent use of phospho-antibodies and mass spectrometry on phospho-tyrosine peptides indicated differential activation of the EGFR, EPHA2, JAK2, SRC and ABL1 tyrosine kinases between these models. These results were the first to suggest that, based on the proteomics footprint of an individual patient's CaP specimen, inhibition of tyrosine kinase, include the AR-dependent EGFR and SRC, could be administered as personalized therapeutics (Drake *et al.* 2012). Follow-up studies suggesting that kinase activity patterns were similar among the different CaP metastases within the same patient, but differed between patients, increased the attractiveness of this possibility- although the authors indicate some caution since the relatively small number of specimens studied ($n=16$) may have led this particular study to be somewhat underpowered (Drake *et al.* 2016). None-the-less, a more comprehensive analysis of clinical samples that involved mass spectrometry on phospho-tyrosine peptides as well as phospho-serine/ phospho-threonine-peptides, in combination with genomic and transcriptome analyses and sophisticated bioinformatics tools again confirmed differentially activated kinases in CRPC progression. In the same study, an online portal 'pCHIPS' was developed and made publically available as a tool that can define the druggable kinase pathways for drug prioritization in individual patients. Several analyses in the latter study pointed to cell cycle progression, cell migration and AR function as associated with phospho-proteome alterations and point to wild-type not somatically altered kinases, some of which AR-dependent, being the major determinants of CRPC progression (Drake *et al.* 2016).

Table 3 Overview of clinical trials targeting kinases in metastatic CaP.

Kinase functional class	Number of trials	Trial acronym	Drug targets	Clinical trial phase
Growth factor receptors	99	INV342, ARS, KHLAD, NRR, GeniPro, QUERGEN, GCP, P1, AZD2171IL/0003, ARCAP, PROSUT, SUN 1120, SMART	VEGFR, PDGFR, FGFR, EGFR , BCR-ABL, KIT,	Phase 1, phase 2
Tyrosine kinases	14	BrUOG PR255, READY, PROACT	ALK, ROS1, BCR-ABL, SRC , MEK1, MEK2	Phase 1, phase 2
Cell cycle regulators	11		ATR, AURKA, CDK4, CDK6 , CDK9 , CHEK1, CHEK2	Phase 1, phase 2
Akt/mTOR pathway	9		AKT1, FKBP12, MNK1, MNK2, MTOR, CRTC2, PIK3CA, PI3KCB, PRKDC	Phase 1, phase 2
Other	2		FAK, PYK2, PIM1	Phase 1, phase 2

Columns indicate class of kinases, number of clinical trials, acronyms of clinical trials (if any), drug targets, and phases of clinical trials. Kinases marked in bold and italics are present in Table 1. Note that 2 clinical trials relating to precision medicine (MATCH and SMMART) were left out; these target several kinases based on the patient's genotype. An elaborate version of the table can be found in Supplementary Table 1.

The possibility that wild-type kinase action can induce aggressive CaP behavior was consistent with the results from a functional screen in which the impact of 125 kinases that show either increased mRNA expression, gene amplification or increased protein levels in human CaP on experimental metastasis was examined (Faltermeyer *et al.* 2016). In an *in vivo* screen, these kinases were overexpressed in murine prostate cells that were tail-vein injected into mice that were subsequently screened for lung metastasis. Twenty of these kinases, namely MAP3K8, NTRK2, ARAF, BRAF, CRAF, MAP2K15, MERTK, FGFR3, FLT3, LYN, MAP3K2, NTRK3, PI3KCa, EGFR, EPHA2, HER2, PDGFRa, FGFR1, SRC, BMX (note again some AR-dependent kinases) (Tables 1 and 2) were able to induce such metastasis. Further validation efforts using human prostate cells validated ARAF, BRAF, CRAF, MERTK and NTRK2 as key drivers of bone and visceral tissue metastasis although overexpressing the kinases might not directly correlate to activation of the kinase, and the focus on bone metastasis as endpoint limits implication of kinases involved in treatment resistance in CaP.

Targeting AR-dependent phosphorylation as CaP treatment strategy

The therapeutic successes obtained with kinase inhibition in other human malignancies have led to clinical testing of several kinase inhibitors for CaP treatment. A search of the clinicaltrial.gov site, a database of privately and publicly funded clinical studies, using the terms 'prostate cancer' and 'kinase inhibitor' returned 137 clinical trials which are listed in Supplementary Table 2. The kinase

targets for the inhibitors tested fall into 5 broad classes, namely growth factor receptors, tyrosine kinases, cell cycle regulators, Akt/mTOR pathway-related kinases and others (Table 3). These include several kinases that are AR-associated, such as EGFR, SRC, CDK6, CDK9, and PRKDC.

To date, the success of completed trials testing kinase inhibitors as CaP therapeutics has been modest and none of the inhibitors tested have moved into routine clinical practice. Several factors may have contributed to the clinical failure of inhibitors that were successful in preclinical therapeutics studies. Notably, recent studies combining phospho-peptide enrichment with mass spectrometry have shown that CaP cell lines and xenografts poorly model the phospho-proteome present in clinical CaP specimens (Drake *et al.* 2016). These findings imply that the experimental models used to testing the inhibitor do not fully capture the clinical setting they were used in. In addition, these trials also recruited patients without stratifying them based on the proteomic make-up of their CaP, and thus without knowing the activation status of the kinase being targeted. This approach still leaves the possibility of therapeutic success in a subset of patients with the right target activation (i.e. the outliers, or exceptional responders) that is not reflected in the overall negative trial results obtained for entire patient cohort. Another well-known limitation of most kinase inhibitors is their lack of selectivity aka polypharmacology: that is the inhibitor is able to inhibit more than one kinase target often at similar IC50s (Davis *et al.* 2011, Klaeger *et al.* 2017). This can be especially problematic to the success of a treatment when the activity of the target kinase(s) in the patient is not known and when affected kinases have

opposite effects on the same CaP pathways or biology. Another important and often underappreciated limitation is that the endpoint used to measure therapeutic success is not adequate for the compound under investigation, leaving investigators essentially unable to judge the efficacy of drugs tested and to misinterpret trial results. An example of this relates to our and others' testing of the multi-kinase inhibitor, lestaurtinib, as CaP therapeutic (Venkadakrishnan *et al.* 2019). Lestaurtinib also inhibits the kinase activity of PKN1, which drives CaP progression, and is highly expressed and activated in CRPC. Lestaurtinib had been well tolerated in phase I clinical trials for advanced carcinomas (Marshall *et al.* 2005) and was studied clinically for treatment of myelofibrosis and acute myeloid leukemia (Knapper *et al.* 2006, Shabbir & Stuart 2010). To date, two phase II clinical trials using lestaurtinib have been initiated in CRPC patients. Patients were considered responders if they had >50% decrease in serum PSA, a surrogate marker of CaP progression, compared to baseline. No patients met these response criteria. Instead, serum PSA tended to increase during treatment with lestaurtinib and declined when treatment was stopped. The decision not to move lestaurtinib forward clinically in CRPC was made solely based on the failure to meet the endpoint of PSA response. However, studies *in vivo* and *in vitro* CaP models have shown increased PSA production by cells that were growth-arrested by lestaurtinib (Collins *et al.* 2007, Venkadakrishnan *et al.* 2019). The implication is that a potentially effective agent to target AR action downstream of activated AR that has a different mechanism of action than traditional ADT and is tolerated by patients was abandoned mainly because of the use of an inappropriate biomarker to evaluate treatment response.

Appreciating the issues that prevented therapeutic successes using kinase inhibitors may help resolve them and increase the likelihood of future therapeutic successes. Targeting the right kinase in the right patient may be achieved via proteomics analysis on one or more of his CaP tissues. Similar genomic CaP characterizations are the premise for personalized medicine approaches, and the basis for patient stratifications into for instance the NCI MATCH trials (Coyne *et al.* 2017). Improving the specificity of kinase inhibitors relies in part on a better understanding of the kinase activity. Indeed, for most kinases, the full spectrum of substrates has not yet been described, the possibility of tissue or cancer type substrate specificity is under-appreciated or the extent of substrate overlap among kinases is not known. Technical advances may help resolve these limitations. Several approaches

including SILAC, TiO2 enrichment of phosphoproteins for a label-free quantification, phospho-specific-immunoprecipitation followed by mass spectrometry, motif analyses using phospho-peptide arrays are extensively used to identify substrates and substrates binding motifs (Montoya *et al.* 2011, Wu *et al.* 2015, de Oliveira *et al.* 2016). Integration of such assays with computational modeling and bioinformatics analyses to better define linear and non-linear substrate motifs, and proper *in vitro* and *in vivo* validation of substrate phosphorylation sites using not only kinase domains but full-length versions of kinases under investigation, are all likely to expand the *bona fide* spectrum of kinase substrates. Better knowledge of a kinases substrates or the kinase-substrate interactions will facilitate monitoring kinase-targeting treatment responses. This could be achieved using either relevant phospho-motif-specific antibodies or proximity ligation assays on circulating tumor cells obtained from non-invasive liquid biopsies. A second way to improve inhibitor specificity will rely on our enhanced understanding of kinase inhibitor binding pockets or molecular details on substrate-kinase interactions (de Oliveira *et al.* 2016), which have been proposed as alternative targets to inhibit kinase activity using peptides or peptidomimetics. For many kinases, reliable 3D structural models are not available and crystallization efforts with inhibitors have not yet been undertaken. Ongoing advances in cryoEM are expected to provide this much-needed information.

Conclusions

The majority of the more than 30,000 CaP deaths annually in the United States are due to failure of ADT, which prevents ligand-activation of AR. Despite an initial remission, CaP progresses while continuing to rely on AR action, emphasizing the need for novel druggable targets downstream of activated AR. Our literature review uncovered that AR associates with dozens of functionally diverse kinases and phosphatases, and that the ensuing phosphorylation events control CaP biology relevant to aggressive CaP progression. Several of these phosphorylation events were reflected in recently recognized alterations in phospho-proteome in late stage CaP. Kinase inhibitors targeting some of these have been clinically tested and could serve as novel therapies that can overcome CaP's resistance to ADT, provided some challenges and limitations related to therapeutic

efficiency, target specificity and monitoring of treatment responses are addressed adequately.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/ERC-20-0048>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

V B V and H V H wrote the manuscript. V B V, S B S and H V H performed literature searches, selected data to be discussed and critically reviewed the manuscript. All authors reviewed and approved the final manuscript.

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