Published in final edited form as:
*Mol Cell Endocrinol*. 2012 April 16; 352(0): 34–45. doi:10.1016/j.mce.2011.06.033.

# The AR dependent cell cycle: Mechanisms and cancer relevance

**Matthew J. Schiewer**[a,b,1], **Michael A. Augello**[a,b,1], and **Karen E. Knudsen**[a,b,c,d,*]

[a]Kimmel Cancer Center, Thomas Jefferson University, 233 S 10th St., Philadelphia, PA 19107, USA

[b]Department of Cancer Biology, Thomas Jefferson University, 233 S 10th St., Philadelphia, PA 19107, USA

[c]Department of Urology, Thomas Jefferson University, 233 S 10th St., Philadelphia, PA 19107, USA

[d]Department of Radiation Oncology, Thomas Jefferson University, 233 S 10th St., Philadelphia, PA 19107, USA

## Abstract

Prostate cancer cells are exquisitely dependent on androgen receptor (AR) activity for proliferation and survival. As these functions are critical targets of therapeutic intervention for human disease, it is imperative to delineate the mechanisms by which AR engages the cell cycle engine. More than a decade of research has revealed that elegant intercommunication between AR and the cell cycle machinery governs receptor-dependent cellular proliferation, and that perturbations in this process occur frequently in human disease. Here, AR–cell cycle interplay and associated cancer relevance will be reviewed.

### Keywords

Androgen receptor; Cell cycle; Prostate cancer

## 1. Androgen receptor function and prostate cancer progression

The androgen receptor (AR) is a ligand-dependent transcription factor that elicits context specific effects in the prostate. In the developing gland, active AR acts as a differentiation factor that is requisite for prostate function and maintenance. However, in prostate cancer (PCa), AR acquires a cell-autonomous function in actively promoting cancer cell growth and survival, mediated in part through exquisite dependence of this tumor type on AR function to induce cell cycle progression (Balk, 2002; Balk and Knudsen, 2008; Culig and Bartsch, 2006; Evans, 1988; Klotz, 2000; Knudsen and Penning, 2010; Knudsen and Scher, 2009; Shand and Gelmann, 2006). Prior to ligand (testosterone or dihydrotestosterone, DHT) binding, the receptor is present diffusely throughout the cytoplasm and is held inactive through association with heat shock proteins. Ligand binding induces rapid nuclear translocation and accumulation, chromatin association at multiple sites that govern gene expression (including those that contain canonical androgen response elements, AREs), recruitment of cofactors that influence downstream gene expression events, and initiation of a gene expression program that promotes tumor phenotypes (Gelmann, 2002;



Corresponding author at: Kimmel Cancer Center, Thomas Jefferson University, 233 S 10th St., BLSB 1008, Philadelphia, PA 19107, USA. Tel.: +1 215 503 8574 (office)/+1 215 503 8573 (lab). karen.knudsen@kimmelcancercenter.org (K.E. Knudsen).
[1]These authors contributed equally to this work.

Gnanapragasam et al., 2000; Heinlein and Chang, 2002, 2004; Marivoet et al., 1992; Trapman and Brinkmann, 1996). In addition, the product of a prostate-specific AR target gene (KLK3/PSA, prostate specific antigen), is used clinically to monitor prostate cancer development and progression (Nash and Melezinek, 2000; Riegman et al., 1991; Ryan et al., 2006). As PSA is secreted into and detected in human serum, quantification of serum PSA provides a clinical means to assess prostate cancer tumor burden. Prostatic adenocarcinomas respond poorly to standard chemotherapy (including both cytostatic and cytotoxic agents); therefore, AR-directed therapeutics are utilized as the first line of intervention for non-organ confined disease (Chen et al., 2008; Knudsen and Penning, 2010; Knudsen and Scher, 2009). At the biochemical level, suppression of AR function is readily achieved through pharmacological methods that ablate testicular androgen synthesis (androgen deprivation therapy, ADT) and therefore deprive AR of circulating ligand. Such modalities are frequently accompanied by adjuvant use of direct AR antagonists (*e.g.* bicalutamide), which not only compete with androgens for AR binding, but also induce recruitment of corepressors to the bicalutamide-bound AR complexes on chromatin(Chodak, 2005; Klotz, 2006; Shang et al., 2002). Efficacy is monitored biochemically through marked reduction of serum PSA levels, and clinically through radiographic evidence of tumor regression. Although the vast majority of patients respond to ADT and AR-directed therapeutics, these responses are transient – within a median time of 2–3 years (Chen et al., 2008; Knudsen and Penning, 2010; Knudsen and Scher, 2009), recurrent tumors develop which are almost invariably preceded by a rise in detectable PSA (referred to as “biochemical failure”). This stage of disease, for which there is no durable cure, is known as castrate-resistant prostate cancer (CRPC), and arises as a result of restored AR activity that is refractory to ADT and AR-directed therapeutics(Chen et al., 2004, 2008; Knudsen and Penning, 2010; Knudsen and Scher, 2009).

An extensive body of literature has addressed the multiple mechanisms by which AR is reactivated to promote therapeutic bypass, and these pathways have been recently reviewed (Knudsen and Penning, 2010). At least five major, non-mutually exclusive categories have been identified through which cells adapt to ADT and AR-directed therapeutics. Most frequently, deregulation of AR is observed, as can be achieved through amplification of the AR gene locus, alternative mechanisms that induce high level AR gene expression, and/or mechanisms that induce AR protein stabilization. Significantly, it has been shown in multiple model systems that up-regulation of AR alone is sufficient to drive the transition to CRPC, and high nuclear AR levels are predictive for increased risk of death from prostate cancer (Chen et al., 2004; Donovan et al., 2010). Secondly, it has been recently shown that prostate cancers up-regulate enzymes that convert weak adrenal androgens to testosterone, and thus engage in intracrine androgen synthesis (Labrie et al., 1995, 2000; Locke et al., 2008; Montgomery et al., 2008; Penning et al., 2006; Stanbrough et al., 2006; Tran et al., 2009). These events therefore supply the receptor with sufficient ligand to outcompete AR antagonists, restore AR activity, and promote CRPC growth (Locke et al., 2008; Montgomery et al., 2008; Stanbrough et al., 2006; Tran et al., 2009). New pharmacological agents (*e.g.* abiraterone acetate) directed against this pathway show positive results in clinical trials (Attard et al., 2009, 2008; Pal and Sartor, 2011). Third, somatic mutation of AR, or development of splice variants, are known to facilitate CRPC. ADT is known to select for AR mutations that broaden the spectrum of ligands able to be utilized as agonists and/or convert antagonists into agonists (Brooke and Bevan, 2009; Knudsen and Penning, 2010; Steinkamp et al., 2009; Yuan and Balk, 2009). Not surprisingly, these mutations generally cluster to the ligand binding domain (Knudsen and Penning, 2010). Alternatively, production of constitutively active AR splice variants that lack the ligand binding domain occurs in CRPC; these variants are not amenable to inhibition by ADT or established AR antagonists (Dehm et al., 2008; Guo et al., 2009; Hu et al., 2009; Knudsen and Penning, 2010). Fourth, alterations in pathways that regulate AR post-translational modifications that

alter AR activity in a no or low ligand environment have been observed, and are thought to promote CRPC (Faus and Haendler, 2006; Knudsen and Penning, 2010; Yuan and Balk, 2009). Finally, alterations in the levels and/or action of cofactors that modulate AR function have been reported, and play diverse roles in CRPC (Heemers et al., 2009; Knudsen and Penning, 2010). Irrespective of the mechanism(s) utilized to bypass therapeutic intervention, AR activity resumes the capacity to drive cellular proliferation in CRPC. As such, it is imperative to delineate the mechanisms by which AR governs cell cycle transitions in both early stage and castrate-resistant disease. As will be discussed herein, investigation of the mechanisms by which AR controls the cell cycle led to discovery of elegant crosstalk between the AR signaling axis and the cell cycle machinery that, when altered, significantly influence tumor cell phenotypes and disease progression.

### 1.1. AR regulates cell cycle control

The means by which ligand-activated AR initiates the cell cycle has been generally defined. ADT-sensitive cells deprived of androgen exit the cell cycle and arrest in G0 (Agus et al., 1999; Huggins and Hodges, 1972; Knudsen et al., 1998). Transitions into and within the cell cycle are controlled by cyclin/cyclin-dependent kinase (cdk) complexes, which act sequentially to maintain ordered progression from G1 to mitosis (Lee and Sicinski, 2006; Malumbres and Barbacid, 2007; Sherr and Roberts, 2004). Typically, cdk activity is influenced by limited availability of the required cyclin subunit and/or the presence of cdk-inhibitors, and both processes assist in controlling androgen-dependent cell cycle progression (Fig. 1). In early G1, cdk4 or cdk6 activity is induced in most cell types, as achieved by growth factor- mediated D-type cyclin accumulation (Lee and Sicinski, 2006; Sherr and Roberts, 2004). Indeed, expression of major D-type cyclins (cyclins D1 and D3) is suppressed in ADT-responsive prostate cancer cells after steroid deprivation, and contributes to cell cycle arrest (Knudsen et al., 1998; Xu et al., 2006). There is little evidence that androgen deprivation alters $p16^{ink4a}$ levels, a known cdk4/6 inhibitor. As such, it is thought that androgen regulation of D-cyclin accumulation serves as the major underpinning means by which AR regulates cdk4/6 activity and early G1 transitions. Accordingly, androgen stimulation induces mTOR-dependent translation of D-cyclins, resulting in protein accumulation sufficient to activate cdk4/6 (Xu et al., 2006). These events are independent of D-cyclin gene expression; distinct from what is observed in breast cancer cells, D-cyclin mRNA levels are unchanged by hormone deprivation in prostate cancer cells (Comstock et al., 2009, 2011). Thus, AR regulates early G1 progression primarily through controlling D-type cyclin protein levels.

Activated D-cyclin/cdk4 or 6 complexes initiate phosphorylation/inactivation of the retinoblastoma tumor suppressor (RB), which negatively regulates cell cycle transitions and the onset of DNA replication (Knudsen and Knudsen, 2008). RB function is envisaged as a “rheostat” to govern all stages of the cell cycle, wherein cdk4/6-mediated phosphorylation events compromise RB activity, and subsequent cdks increase RB phosphorylation status as a function of cell cycle progression (Knudsen and Knudsen, 2006). Active RB is recruited to chromatin at sites that regulate expression of genes important for cellular proliferation, including genes essential for DNA replication (*e.g.* MCM7) and S-phase entry (*e.g.* cyclin A2). Many RB-regulated genes are activated by the E2F family of transcription factors, and RB counterbalances E2F-mediated gene expression by assembling transcriptional repressor complexes that dampen transcriptional transactivation. Thus, a major function of G1 cdk complexes is to attenuate these functions of RB through direct phosphorylation and inactivation (Burkhart and Sage, 2008; Knudsen and Knudsen, 2008).

Toward this end, cdk2 activity promotes completion of G1 and transitions into S-phase, as mediated by sequential partnering with cyclins E1 and A2 (Knudsen and Knudsen, 2008). Similar to what is observed with cdk4/6, androgen ablation or stimulation has little influence

on cdk2 protein levels (Knudsen et al., 1998). However, at least two gatekeepers place cdk2 activity under AR control. First, while cyclin E1 protein levels are unchanged by the presence or absence of androgen, cyclin E1/cdk2 activity is strongly suppressed by ADT *in vitro* (Knudsen et al., 1998). This effect is likely attributed to androgen-mediated regulation of $p27^{kip1}$, a potent suppressor of cdk2 activity and bona fide tumor suppressor protein. $p27^{kip1}$ levels are induced by androgen deprivation (Knudsen et al., 1998); conversely, androgen stimulation is known to promote rapid $p27^{kip1}$ degradation (Lu et al., 2002). Although the mechanisms by which androgen promotes $p27^{kip1}$ degradation and subsequent cdk2 activity is incompletely defined, it is thought that these events serve to promote G1 progression and commitment to the mitotic cell cycle. A related protein with no known tumor suppressor function, $p21^{cip1}$, exerts both pro-proliferative effects (as mediated through its capacity to assist in assembly of D-cyclin/cdk4 complexes) and anti-proliferative effects (through association with and suppression of cdk2 complexes) on the cell cycle (Alt et al., 2002; Balk and Knudsen, 2008; Cheng et al., 1999; LaBaer et al., 1997; Sherr and Roberts, 1999). Interestingly, p21 levels are directly up-regulated by androgen, indicating that $p21^{cip1}$ may serve to facilitate G1 progression in this tumor type (Lu et al., 1999). Second, androgen deprivation reduces cyclin A2 levels, as a result of ADT-mediated suppression of cdk4/6 function and resultant engagement of RB transcriptional repressive capacity (Knudsen et al., 1998). Recently, it has been shown that the DNA replication factor Cdc6 is under direct control of AR activity, indicating that both G1 and S-phase components of the cell cycle machinery may be under AR regulation (Jin and Fondell, 2009; Mallik et al., 2008). Based on these collective findings, it is evident that a major function of AR is to control the G1-S transition. Additionally, AR could potentially serve to assist in DNA replication licensing, as in some systems, AR is degraded in mitosis (Litvinov et al., 2006). Whether the putative link between AR and the mechanics of DNA synthesis control impinge upon cell cycle transitions mediated by endogenous AR or in an in vivo setting will be of interest to discern.

While androgen utilizes divergent mechanisms to induce cdk4/6 and cdk2 activation, suppress RB activity, and thereby permit commitment to the mitotic cell cycle in ADT-sensitive cells, distinct mechanisms may be invoked in CRPC which further enhance AR-dependent cell cycle progression. Through genome-wide analyses of AR binding in CRPC cells, novel AR occupancy sites were observed near the regulatory loci of genes associated with mitotic progression (Wang et al., 2009). Consistent with the postulate that the AR program is altered in CRPC so as to strengthen the capacity of AR to drive cellular proliferation, AR was shown to up-regulate expression of UBE2C, whose gene product has been associated with regulation of cyclin levels. Upregulation of UBE2C was not sufficient to induce castrate-resistant cell growth in ADT-responsive cells, but silencing of UBE2C slowed cell proliferation rates in CRPC cells (Wang et al., 2009). These findings suggest that CRPC cells develop additional means to foster AR-dependent cell cycle control, and further delineation of these mechanisms may be of benefit for the design of novel therapeutic agents.

## 2. Cell cycle feedback regulation of AR activity

Investigation of the mechanisms by which AR governs cell cycle control led to the unexpected discovery of feedback pathways that influence AR activity (Fig. 2). Delineation of the interplay between AR and cell cycle machinery has both illuminated the cellular consequence of pathway crosstalk and underscored the importance of cdk and cyclin functions in transcriptional control.

### 2.1. cdks

A subset of cdks proximal to cell cycle regulation have been shown to enhance AR activity, thus indicating that initiation of the androgen dependent cell cycle engine may induce signals that further support AR-dependent cellular proliferation. In early G1, cdk6 has been shown to associate with AR in prostate cancer cells and to enhance ligand-dependent AR activity as monitored by reporter assays (Lim et al., 2005). These effects of cdk6 do not require cyclin D1 or intrinsic kinase activity, and are not shared by cdk4. Subsequently, cytoplasmic localization of the p44 AR cofactor was shown to up-regulate cdk6 levels, and it would be of interest to determine if the co-activator functions of p44 require cdk6 action (Peng et al., 2008). In latter phases of the cell cycle, expression of the G2/M kinase cdk1 has been shown to promote phosphorylation of AR at multiple sites including Ser-81, which has been implicated in bolstering AR stability and function (Chen et al., 2006). It will be intriguing to determine whether the effects of cdk1 on AR phosphorylation are direct, and whether cdk1 function alters cell cycle stage-specific AR signaling and/or resultant cell cycle progression. In addition, it may be of benefit to determine what phosphatases may counterbalance cdk1-mediated AR activity, whether cdk1-dependent phosphorylation events are up-regulated in human disease, and to assess whether or not this pathway could be developed as a means to suppress AR activity in human tumors. Finally, cdk5 was predicted (based on cdk1 homology) to harbor potential catalytic activity utilizing AR as a substrate, yet was shown in ectopic expression studies to function in a manner distinct from that of cdk1 (Chen et al., 2006). Independent mass spectrometry studies subsequently confirmed association of AR with both cdk1 and cdk5 (Gordon et al., 2010). Thus, it is apparent that cdks differentially regulate AR, and may compete for phosphorylation sites and result in divergent biological outcomes.

**2.1.1. Transcriptional cdks—**Distinct from the cdks proximal to cell cycle control, several cdks associated with transcriptional regulation (cdk9, cdk7, and cdk11) have been shown to modulate AR activity. Serine 81 of AR is phosphorylated by ectopic expression of cdk9 in AR-negative cells re-engineered to express the receptor (Gordon et al., 2010). In this model system, mutation of Ser81 to alanine resulted in decreased cell proliferation, consistent with the observation that Ser81 phosphorylation is important for AR activity. In cells with endogenous AR, ectopic expression of the S81A mutant similarly inhibited cell growth, even in the presence of AR agonists; conversely, the pan-cdk inhibitor flavopiridol suppressed cell proliferation in AR-positive cells, and cdk9 silencing decreased AR phosphorylation (Gordon et al., 2010). Less is known mechanistically about how cdk7 impinges on AR phosphorylation, but this transcriptional cdk has also been shown to heighten AR function. cdk7 is part of the CAK (cdk activating kinase) complex, which modulates both cdk function and general transcriptional activity. Over-expression of cdk7 in AR-negative cell lines enhanced AR activity in transient assays, thus implicating the alterations in the general transcription machinery as a putative means to alter AR activity (Lee et al., 2000). Finally, cdk11, which is involved in diverse biological functions including RNA processing and apoptosis, exists in two isoforms that can bind cyclin D3 (p58 and p110). Intriguingly, each isoform regulates AR but yields opposing effects on receptor function; cyclin D3/cdk11$^{p58}$ phosphorylates the AR N-terminus at Ser-308, suppresses AR activity, and dampens prostate cell proliferation, whereas cdk11$^{p110}$ activates AR function and lacks the ability to phosphorylate AR (Zong et al., 2007). To further advance these findings, delineation of the mechanisms that control the “switch” between the cdk11 isoforms would be relevance, as would assessment of cdk11 function during disease progression.

### 2.2. Effectors of cdk activity

Consistent with the concept that cdks can modulate AR activity, there is evidence that positive regulators of cdks can further enhance AR function. Cdc25b, a phosphatase that primarily acts in G2/M to dephosphorylate and activate cdk1, was shown in mammalian 2-hybrid assays to interact with AR, and in transient transcriptional assays to induce AR activity (Chua et al., 2004; Ma et al., 2001). As Cdc25b is a proto-oncogene and is upregulated in a number of tumor types, it would be of interest, to determine whether Cdc25 protein alterations affect AR phosphorylation status, alter function of AR at endogenous sites of action, and/or contribute to AR-mediated cell cycle control. Whether other Cdc25s with proto-oncogenic activity serve to facilitate AR function or the transition to CRPC remains unaddressed. Delineation of overall Cdc25 action should be discerned more rigorously, as opposing functions were observed with Cdc25a (Chiu et al., 2009); this Cdc25 family phosphatase was found to bind to AR *in vitro* and inhibit endogenous AR activity.

### 2.3. G1 cyclins

A rich body of evidence supports the concept that AR is modulated by G1 cyclins, resulting in both positive and negative effects on AR function (Burd et al., 2005, 2006; Knudsen, 2006; Knudsen et al., 1999; Kobayashi et al., 2009; Olshavsky et al., 2008; Petre et al., 2002; Petre-Draviam et al., 2003, 2005; Reutens et al., 2001; Schiewer et al., 2009; Yamamoto et al., 2000; Zong et al., 2007). The most widely studied cyclins that modulate AR belong to the D-cyclin family, and are negative regulators of ligand-dependent AR activity (Burd et al., 2005, 2006; Knudsen et al., 1999; Kobayashi et al., 2009; Olshavsky et al., 2008; Petre et al., 2002; Petre-Draviam et al., 2003, 2005; Reutens et al., 2001; Schiewer et al., 2009; Zong et al., 2007). *In vivo* analyses of cyclin D1 function confirmed that cyclin D1 not only associates with cdks, but is associated with a large number of transcriptional modulators (Bienvenu et al., 2010). Strikingly, the ability of cyclin D1 to alter transcription factor activity appears to underpin major cyclin D1 functions. For example, loss of cyclin D1-mediated Notch1 activity results in the long-observed retinal hypoplasia of the cyclin D1 knockout mouse. Moreover, ChIP-promoter chip analyses determined that cyclin D1 is found on chromatin at a large number of regulatory loci (Bienvenu et al., 2010). Thus, it is apparent that cyclin D1 harbors significant functions independent from cdk control that serve to modulate transcription factor activity. With regard to AR, cyclin D1 interacts directly with the receptor N-terminal domain, requiring the FxxLF motif (Burd et al., 2005). This motif mediates ligand-dependent N-to-C terminal interactions that facilitate chromatin binding and resultant AR activity (He et al., 1999; Wong et al., 1993); cyclin D1 association suppresses both events (Burd et al., 2005). In addition, cyclin D1 has been shown to associate with a number of histone deacetylases (HDACs) (Fu et al., 2005; Li et al., 2002; Lin et al., 2002; Petre et al., 2002), thus providing a means of transcriptional repression (Bienvenu et al., 2010). Accordingly, the ability of cyclin D1 to dampen ligand-dependent AR function is partially reversed by HDAC inhibitors (Petre et al., 2002). These combined functions of cyclin D1 put forth a model of elegant cell cycle control, wherein androgen up-regulates cyclin D1 through mTOR-dependent mechanisms, thus allowing for cdk4 activation and initiation of the cell cycle engine (Knudsen et al., 1998; Xu et al., 2006). However, as cyclin D1 levels rise, cdk4-independent binding to AR provides a negative feedback switch to govern subsequent AR-dependent signaling and cell cycle transitions. The ability of cyclin D1 to bind HDACs and negatively regulate AR is dependent on a defined motif, deemed the repressor domain (RD) (Petre-Draviam et al., 2005). Introduction of the RD domain allows for separation of the cyclin D1 cdk4-modulatory and AR-modulatory functions, and leads to not only suppression of AR-dependent cell growth but also triggers loss of cell viability, thus illustrating the importance of cyclin D1 on cellular outcomes (Schiewer et al., 2009). As will be discussed below, the ability of cyclin D1 to dampen AR activity appears to be altered in prostate cancer, as mediated by down-

regulation of cyclin D1 expression, mislocalization, and/or a shift to production of the cyclin D1b variant, which is compromised for AR-regulatory functions (Burd et al., 2006; Comstock et al., 2009, 2007; Knudsen, 2006; Olshavsky et al., 2010). As would be expected based on conservation of the RD domain, cyclin D3 elicits similar functions with regard to AR control (Olshavsky et al., 2008); in addition, cdk11-mediated functions of cyclin D3 also contribute to negative regulation of AR (Zong et al., 2007). Although limited in study, preliminary findings indicate that cyclin D2, which is silenced in a subset prostate cancers, also negatively regulates AR activity (Henrique et al., 2006; Kobayashi et al., 2009; Padar et al., 2003). Collectively, these observations strongly suggest that a function of D-cyclins is to control AR output, and that these functions are dysregulated. Given these observations, it will be of relevance to further delineate the overall impact of D-cyclin mediated AR regulation, in the context of both the untransformed and tumorigenic cells.

Distinct from the functions of D-cyclins, up-regulation of cyclin E can enrich AR activity both in transient assays as well as at endogenous sites of action. Cyclin E also binds the AR N-terminus, and therefore could conceivably compete with D-cyclins for controlling AR (Yamamoto et al., 2000). Accordingly, in the TRAMP (T-antigen driven) model of prostate cancer, development of prostatic intraepithelial hyperplasia (PIN) is associated with down-regulation of D-cyclins and up-regulation of cyclins E1 and A2 (Maddison et al., 2004). A tempting hypothesis is that a shift in cell cycle control toward dependence on the later G1 cdk-cyclin complexes facilitates both rapid cell cycling and aberrant AR activity.

### 2.4. RB: gatekeeper of G1/S control

Recent evidence demonstrates that RB is a major effector of AR levels, AR function, and the ability of prostate cancer cells to transition to the CRPC stage. Initial studies with RB suggested that transiently over-expressed RB and AR (in cells with no endogenous AR), can allow for formation of a complex between the transcriptional regulators and enhanced AR activity in reporter gene assays (Lu and Danielsen, 1998; Yeh et al., 1998). These initial findings prompted further analyses of RB function in an endogenous setting, which yielded surprising outcomes. Remarkably, it was found that RB is lost in the transition to CRPC with high frequency, whereas there was low evidence of RB loss in primary disease (Sharma et al., 2010). Mimicry of this event in prostate cancer cells demonstrated that silencing of RB resulted in marked up-regulation of AR mRNA and AR protein sufficient to drive castration resistance both *in vitro* and *in vivo* (Sharma et al., 2010). Mechanistically, it was found that the AR locus is under RB/E2F1 control; loss of RB results in E2F1 deregulation and resultant upregulation of AR and AR activity. The importance of this event was evident in exploration of CRPC specimens, wherein loss of RB was significantly associated with high AR levels (Sharma et al., 2010). Thus, in AR-positive PCa cells, RB serves not only to suppress cell cycle progression in the absence of androgen, but actively attenuates AR expression (Sharma et al., 2010). These findings identify RB as utilizing pleiotropic mechanisms to suppress tumor progression in prostate cancer. Further interplay between the RB/E2F1 axis and AR may alter the activity of EZH2, a polycomb group associated transcription factor that is deregulated in human disease. Specifically, androgens repress EZH2 expression in concert with RB family members (including p130), thereby altering phenotypes associated with cell migration (Bohrer et al., 2010). These combined observations illustrate the importance of delineating the underlying mechanisms and consequence of crosstalk between the RB/E2F axis and AR.

Collectively, these studies provide strong evidence that interplay between the cell cycle machinery and the AR signaling axis leads to profound cellular outcomes using *in vitro* and *in vivo* models of human disease. The contributions of these interactions to disease development and progression in human prostate cancer have only begun to be explored. Early advances toward this end will be discussed below, and lay the foundation for future

studies directed at harnessing this information for the purpose of identifying new nodes of therapeutic intervention.

## 3. Clinical relevance of cell cycle-AR crosstalk

Assessment of AR–cell cycle crosstalk in the context of clinical samples has been limited. The current state of the field is summarized in Tables 1, 2 and 3 and Supplementary Table 1, and reveals open questions germane to human disease. Those studies that examined any part of AR biology are highlighted in light gray with dark borders, demonstrating how few studies have actually examined AR in the context of cell cycle effector perturbation. For example, cell cycle-associated and transcriptional-regulatory cdks have been scantly examined in clinical specimens; given the depth of *in vitro* and *in vivo* studies that illustrate putative pathway and disease relevance of these players, these effectors should be prioritized for further investigation. A preliminary resource to potentially assist in this endeavor is the Human Protein Atlas, which provides useful but limited insight into the relative expression profiles (including frequency and distribution) in human disease (Uhlen et al., 2010). Similarly, the clinical relevance of the Cdc25 proteins should be determined, given the putative divergent roles of individual isoforms on AR regulation and cell cycle progression and dearth of information regarding the impact of Cdc25s on disease phenotypes (Ozen and Ittmann, 2005; Ngan et al., 2003). With regard to the cyclins, it remains unclear whether the E-, A-, and B-family cyclins merely serve as indicators of increased cellular proliferation, or hold predictive value beyond that of proliferative indices. Evidence to date with the D-type cyclins demonstrate roles for these proteins that are distinct from mere cell cycle control, as may be mediated through functions in transcriptional regulation. Discerning the proximal targets of cyclin D action and the resultant impact on human disease behavior is of the utmost importance.

By contrast, much emphasis has been placed on understanding the impact of cdk inhibitors in the context of both ADT-sensitive and castrate resistant disease. With few exceptions, clear and diverse clinical correlates are associated with alterations of $p27^{kip1}$ (Ben-Izhak et al., 2003; Chang et al., 2004; Cheng et al., 2000; Cheville et al., 1998; Claudio et al., 2002; Cordon-Cardo et al., 1998; Cote et al., 1998; De Marzo et al., 1998; Doganavsargil et al., 2006; Dreher et al., 2004; Drobnjak et al., 2003; Erdamar et al., 1999; Fernandez et al., 1999; Freedland et al., 2003; Guo et al., 1997; Halvorsen et al., 2003; Huang et al., 2008; Kibel et al., 2001, 2000, 2003; Kuczyk et al., 1999, 2001; Li et al., 2006; Nguyen et al., 2009; Nikoleishvili et al., 2008; Ribal et al., 2003; Romics et al., 2008; Thomas et al., 2000; Tsihlias et al., 1998; Vis et al., 2000, 2002; Wolters et al., 2010; Wu et al., 2007; Yang et al., 2002, 1998; Zeng et al., 2004) versus $p21^{cip1}$ (Aaltomaa et al., 1999; Baretton et al., 1999; Cheng et al., 2000; Facher et al., 1997; Lacombe et al., 2001; Matsushima et al., 1998; Omar et al., 2001; Sarkar et al., 1999). As detailed in Supplementary Table 1, low or no $p27^{kip1}$ is generally associated with poor outcomes, including shorter time to biochemical recurrence and reduced survival. These observations are consistent with the well-known and validated role of $p27^{kip1}$ as a tumor suppressor. Conversely, cytoplasmic $p27^{kip1}$, which has oncogenic functions associated with metastasis, is associated with poor outcome in human disease (Claudio et al., 2002; Dreher et al., 2004; Li et al., 2006). Based on these findings, it will be critical to determine the overall impact not only of $p27^{kip1}$ levels on clinical outcomes, but the utility of assessing $p27^{kip1}$ sub-cellular distribution in prognostic or predictive studies.

Despite conservation of cdk2 inhibitory functions, the related protein $p21^{cip1}$ shows remarkably distinct expression profiles and association with clinical correlates as compare to $p27^{kip1}$. These observations may not be surprising, as $p21^{cip1}$ holds no validated tumor suppressive activity in any tissue type. In the context of prostate cancer, $p21^{cip1}$ is generally

associated with proliferation, higher grade, and poor outcomes (Aaltomaa et al., 1999; Baretton et al., 1999; Lacombe et al., 2001; Matsushima et al., 1998; Omar et al., 2001; Sarkar et al., 1999), consistent with the *in vitro* observation that $p21^{cip1}$ levels are increased upon AR activation. Thus, prostate cancer provides an intriguing platform with which to study divergent roles of cdk2 inhibitors on cellular and tumor outcomes, and the mechanisms underlying differential effects on cellular proliferation should be discerned. Finally, the role of $p16^{ink4a}$ remains an enigma. While $p16^{ink4a}$ shows clear tumor suppressor function in a number of tissue types, $p16^{ink4a}$ levels in prostate cancer show a wide range of expression (Cairns et al., 1995; Chakravarti et al., 2007, 2003; Chen et al., 1996; Chi et al., 1997; Cho et al., 2007; Faith et al., 2005; Gu et al., 1998; Halvorsen et al., 2000; Heidenreich et al., 2000; Henshall et al., 2001; Iemely-nova et al., 2009; Jarrard et al., 1997, 2002; Jeronimo et al., 2004; Komiya et al., 1995; Konishi et al., 2002; Lee et al., 1999; Maruyama et al., 2002; Nguyen et al., 2000; Perinchery et al., 1999; Roach et al., 2009; Tamimi et al., 1996; Zhang et al., 2006). Moreover, no clear patterns of associated clinical correlates have emerged from studies to date. This lack of clarity may be attributed to the confounding effects of the RB pathway on $p16^{ink4a}$ expression, as loss of RB function typically results in marked up-regulation of $p16^{ink4a}$. Up-regulation of $p16^{ink4a}$ in the absence of functional RB does not impinge on cell cycle progression (since $p16^{ink4a}$ requires RB to elicit cell cycle arrest); therefore, the cellular or tumor relevance of this event remains uncertain and needs to be explored (Knudsen and Knudsen, 2008).

Finally, the role of the RB family members in prostate cancer progression has been recently addressed. While there is little evidence that p107 and p130 are altered in prostate cancer, recent findings implicate alternation of the RB/E2F1 axis as playing major roles in human disease. As described above, tracking RB expression and RB loss of function (as achieved through a well established RB loss gene signature) in human specimens revealed that inactivation of the RB axis is highly overrepresented in castration resistant disease, and is strongly associated with AR deregulation (Sharma et al., 2010). Since perturbation of RB function can lead to AR-dependent castration resistance (Sharma et al., 2007, 2010), it will be imperative to discern the mechanisms underlying this event, and to determine alternative mechanism(s) for treating RB-deficient tumors. Further underscoring this postulate, recent findings in genetically engineered mice suggested that RB can control progression to invasive carcinoma (Sun et al., 2011). Collectively, these findings identify disruption of RB tumor suppressor and AR regulatory functions as significantly contributing to disease progression.

## 4. Conclusion and future directions

Inability to durably control AR activity plays a major role in the transition to lethal prostate cancer. While it has been long understood that AR activation drives prostate cancer cell proliferation, the realization that significant cross-communication between AR and the cell cycle machinery modifies this process has been only recently brought to light. Gains in our understanding of AR–cell cycle interplay not only bring new appreciation of the mechanisms that support AR function in human disease, but raise important questions that should be prioritized for future study. *First, are the mechanisms by which AR promotes cell cycle control altered in castrate- resistant disease?* Most studies to date have examined the impact of AR on cell cycle control in hormone-therapy sensitive systems, wherein AR requires ligand for activation. As a subset of CRPC demonstrates AR activity in the absence of ligand or in the presence of alternative ligands, it will be imperative to assess the impact of these events on AR crosstalk with the cell cycle machinery. *Second, given the number of cell cycle alterations observed in human tumors, it will be essential to determine whether these events alter AR function or AR regulation in the clinical setting.* Precedent has been established in tumors exhibiting loss of RB or expression, for which tumor suppressor loss

results in AR deregulation. *Third, data in model systems suggest that cell cycle regulation of AR may result in cell cycle phase-specific AR activity.* It is tempting to speculate that AR function (with regard to both chromatin binding and downstream signaling) is altered as a function of cell cycle position, and preliminary studies support this concept (Knudsen et al., in preparation). *Fourth, numerous mouse models of cell cycle alterations have been generated which perturb factors important for AR–cell cycle crosstalk, yet few studies have addressed the impact of these events on prostate-specific AR function*. It will be of interest to determine the *in vivo* impact of these events on AR activity and AR-dependent tumor development using these defined genetic systems. *Finally, and perhaps most importantly, current understanding of AR–cell cycle interplay affords the possibility for therapeutic intervention, so as to determine whether cell cycle alterations that promote AR activity can serve as targets to prevent tumor development or progression*. Such studies are increasingly tractable, as new agents for therapeutic modulation are in advanced stages of development or in clinical trial. Overall, it is apparent that cross-communication between AR and the cell cycle machinery plays a significant role in controlling hormone-dependent cellular proliferation; next steps will be to obtain greater mechanistic understanding of these events, discern specificity, and leverage this combined information for therapeutic gain.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

The authors are grateful to Drs. S. Balk, C. Comstock, D. Gioeli, E. Knudsen, and R. Schrecengost for critical commentary and ongoing discussions. Additional thanks are given to E. Schade for technical and artistic assistance.

## References

Aaltomaa S, Lipponen P, Eskelinen M, Ala-Opas M, Kosma VM. Prognostic value and expression of p21(waf1/cip1) protein in prostate cancer. Prostate. 1999; 39:8–15. [PubMed: 10221260]

Agus DB, Cordon-Cardo C, Fox W, Drobnjak M, Koff A, Golde DW, et al. Prostate cancer cell cycle regulators: response to androgen withdrawal and development of androgen independence. J Natl Cancer Inst. 1999; 91:1869–1876. [PubMed: 10547394]

Alt JR, Gladden AB, Diehl JA. P21(Cip1) Promotes cyclin D1 nuclear accumulation via direct inhibition of nuclear export. J Biol Chem. 2002; 277:8517–8523. [PubMed: 11751903]

Attard G, Reid AH, A'Hern R, Parker C, Oommen NB, Folkerd E, et al. Selective inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. J Clin Oncol. 2009; 27:3742–3748. [PubMed: 19470933]

Attard G, Reid AH, Yap TA, Raynaud F, Dowsett M, Settatree S, et al. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. J Clin Oncol. 2008; 26:4563–4571. [PubMed: 18645193]

Balk SP. Androgen receptor as a target in androgen-independent prostate cancer. Urology. 2002; 60:132–138. discussion 138–9. [PubMed: 12231070]

Balk SP, Knudsen KE. AR, the cell cycle, and prostate cancer. Nucl Recept Signal. 2008; 6:e001. [PubMed: 18301781]

Baretton GB, Klenk U, Diebold J, Schmeller N, Lohrs U. Proliferation- and apoptosis-associated factors in advanced prostatic carcinomas before and after androgen deprivation therapy: prognostic significance of p21/WAF1/CIP1 expression. Br J Cancer. 1999; 80:546–555. [PubMed: 10408865]

Ben-Izhak O, Lahav-Baratz S, Meretyk S, Ben-Eliezer S, Sabo E, Dirnfeld M, et al. Inverse relationship between Skp2 ubiquitin ligase and the cyclin dependent kinase inhibitor p27Kip1 in prostate cancer. J Urol. 2003; 170:241–245. [PubMed: 12796697]

Bienvenu F, Jirawatnotai S, Elias JE, Meyer CA, Mizeracka K, Marson A, et al. Transcriptional role of cyclin D1 in development revealed by a genetic-proteomic screen. Nature. 2010; 463:374–378. [PubMed: 20090754]

Bohrer LR, Chen S, Hallstrom TC, Huang H. Androgens suppress EZH2 expression via retinoblastoma (RB) and p130-dependent pathways: a potential mechanism of androgen-refractory progression of prostate cancer. Endocrinology. 2010; 151:5136–5145. [PubMed: 20881251]

Brooke GN, Bevan CL. The role of androgen receptor mutations in prostate cancer progression. Curr Genomics. 2009; 10:18–25. [PubMed: 19721807]

Brooks JD, Bova GS, Isaacs WB. Allelic loss of the retinoblastoma gene in primary human prostatic adenocarcinomas. Prostate. 1995; 26:35–39. [PubMed: 7845865]

Burd CJ, Petre CE, Moghadam H, Wilson EM, Knudsen KE. Cyclin D1 binding to the androgen receptor (AR) $NH_2$-terminal domain inhibits activation function 2 association and reveals dual roles for AR corepression. Mol Endocrinol. 2005; 19:607–620. [PubMed: 15539430]

Burd CJ, Petre CE, Morey LM, Wang Y, Revelo MP, Haiman CA, et al. Cyclin D1b variant influences prostate cancer growth through aberrant androgen receptor regulation. Proc Natl Acad Sci USA. 2006; 103:2190–2195. [PubMed: 16461912]

Burkhart DL, Sage J. Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nat Rev Cancer. 2008; 8:671–682. [PubMed: 18650841]

Cairns P, Polascik TJ, Eby Y, Tokino K, Califano J, Merlo A, et al. Frequency of homozygous deletion at p16/CDKN2 in primary human tumours. Nat Genet. 1995; 11:210–212. [PubMed: 7550353]

Chakravarti A, DeSilvio M, Zhang M, Grignon D, Rosenthal S, Asbell SO, et al. Prognostic value of p16 in locally advanced prostate cancer: a study based on Radiation Therapy Oncology Group Protocol 9202. J Clin Oncol. 2007; 25:3082–3089. [PubMed: 17634487]

Chakravarti A, Heydon K, Wu CL, Hammond E, Pollack A, Roach M, et al. Loss of p16 expression is of prognostic significance in locally advanced prostate cancer: an analysis from the Radiation Therapy Oncology Group protocol 86–10. J Clin Oncol. 2003; 21:3328–3334. [PubMed: 12947069]

Chang BL, Zheng SL, Isaacs SD, Wiley KE, Turner A, Li G, et al. A polymorphism in the CDKN1B gene is associated with increased risk of hereditary prostate cancer. Cancer Res. 2004; 64:1997–1999. [PubMed: 15026335]

Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004; 10:33–39. [PubMed: 14702632]

Chen S, Xu Y, Yuan X, Bubley GJ, Balk SP. Androgen receptor phosphorylation and stabilization in prostate cancer by cyclin-dependent kinase 1. Proc Natl Acad Sci USA. 2006; 103:15969–15974. [PubMed: 17043241]

Chen W, Weghorst CM, Sabourin CL, Wang Y, Wang D, Bostwick DG, et al. Absence of p16/MTS1 gene mutations in human prostate cancer. Carcinogenesis. 1996; 17:2603–2607. [PubMed: 9006095]

Chen Y, Sawyers CL, Scher HI. Targeting the androgen receptor pathway in prostate cancer. Curr Opin Pharmacol. 2008; 8:440–448. [PubMed: 18674639]

Cheng L, Lloyd RV, Weaver AL, Pisansky TM, Cheville JC, Ramnani DM, et al. The cell cycle inhibitors p21WAF1 and p27KIP1 are associated with survival in patients treated by salvage prostatectomy after radiation therapy. Clin Cancer Res. 2000; 6:1896–1899. [PubMed: 10815913]

Cheng M, Olivier P, Diehl JA, Fero M, Roussel MF, Roberts JM, et al. The p21(Cip1) and p27(Kip1) CDK ‘inhibitors’ are essential activators of cyclin D-dependent kinases in murine fibroblasts. EMBO J. 1999; 18:1571–1583. [PubMed: 10075928]

Cheville JC, Lloyd RV, Sebo TJ, Cheng L, Erickson L, Bostwick DG, et al. Expression of p27kip1 in prostatic adenocarcinoma. Mod Pathol. 1998; 11:324–328. [PubMed: 9578081]

Chi SG, de Vere White RW, Muenzer JT, Gumerlock PH. Frequent alteration of CDKN2 (p16(INK4A)/MTS1) expression in human primary prostate carcinomas. Clin Cancer Res. 1997; 3:1889–1897. [PubMed: 9815578]

Chiu YT, Han HY, Leung SC, Yuen HF, Chau CW, Guo Z, et al. CDC25A functions as a novel Ar corepressor in prostate cancer cells. J Mol Biol. 2009; 385:446–456. [PubMed: 19013180]

Cho NY, Kim BH, Choi M, Yoo EJ, Moon KC, Cho YM, et al. Hypermethylation of CpG island loci and hypomethylation of LINE-1 and Alu repeats in prostate adenocarcinoma and their relationship to clinicopathological features. J Pathol. 2007; 211:269–277. [PubMed: 17139617]

Chodak GW. Maximum androgen blockade: a clinical update. Rev Urol. 2005; 7(Suppl 5):S13–S17. [PubMed: 16985879]

Chua SS, Ma Z, Ngan E, Tsai SY. Cdc25B as a steroid receptor coactivator. Vitam Horm. 2004; 68:231–256. [PubMed: 15193457]

Claudio PP, Zamparelli A, Garcia FU, Claudio L, Ammirati G, Farina A, et al. Expression of cell-cycle-regulated proteins pRb2/p130, p107, p27(kip1), p53, mdm-2, and Ki-67 (MIB-1) in prostatic gland adenocarcinoma. Clin Cancer Res. 2002; 8:1808–1815. [PubMed: 12060621]

Comstock CE, Augello MA, Benito RP, Karch J, Tran TH, Utama FE, et al. Cyclin D1 splice variants: polymorphism, risk, and isoform-specific regulation in prostate cancer. Clin Cancer Res. 2009; 15:5338–5349. [PubMed: 19706803]

Comstock CE, Augello MA, Schiewer MJ, Karch J, Burd CJ, Ertel A, et al. Cyclin D1 is a selective modifier of androgen-dependent signaling and androgen receptor function. J Biol Chem. 2011

Comstock CE, Revelo MP, Buncher CR, Knudsen KE. Impact of differential cyclin D1 expression and localisation in prostate cancer. Br J Cancer. 2007; 96:970–979. [PubMed: 17375037]

Cooney KA, Wetzel JC, Merajver SD, Macoska JA, Singleton TP, Wojno KJ. Distinct regions of allelic loss on 13q in prostate cancer. Cancer Res. 1996; 56:1142–1145. [PubMed: 8640774]

Cordon-Cardo C, Koff A, Drobnjak M, Capodieci P, Osman I, Millard SS, et al. Distinct altered patterns of p27KIP1 gene expression in benign prostatic hyperplasia and prostatic carcinoma. J Natl Cancer Inst. 1998; 90:1284–1291. [PubMed: 9731735]

Cote RJ, Shi Y, Groshen S, Feng AC, Cordon-Cardo C, Skinner D, et al. Association of p27Kip1 levels with recurrence and survival in patients with stage C prostate carcinoma. J Natl Cancer Inst. 1998; 90:916–920. [PubMed: 9637141]

Culig Z, Bartsch G. Androgen axis in prostate cancer. J Cell Biochem. 2006; 99:373–381. [PubMed: 16598769]

De Marzo AM, Meeker AK, Epstein JI, Coffey DS. Prostate stem cell compartments: expression of the cell cycle inhibitor p27Kip1 in normal, hyperplastic, and neoplastic cells. Am J Pathol. 1998; 153:911–919. [PubMed: 9736039]

Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. Cancer Res. 2008; 68:5469–5477. [PubMed: 18593950]

Ding Z, Wu CJ, Chu GC, Xiao Y, Ho D, Zhang J, et al. SMAD4-dependent barrier constrains prostate cancer growth and metastatic progression. Nature. 2011; 470:269–273. [PubMed: 21289624]

Doganavsargil B, Simsir A, Boyacioglu H, Cal C, Hekimgil M. A comparison of p21 and p27 immunoexpression in benign glands, prostatic intraepithelial neoplasia and prostate adenocarcinoma. BJU Int. 2006; 97:644–648. [PubMed: 16469041]

Donovan MJ, Osman I, Khan FM, Vengrenyuk Y, Capodieci P, Koscuiszka M, et al. Androgen receptor expression is associated with prostate cancer-specific survival in castrate patients with metastatic disease. BJU Int. 2010; 105:462–467. [PubMed: 19624594]

Dreher T, Zentgraf H, Abel U, Kappeler A, Michel MS, Bleyl U, et al. Reduction of PTEN and p27kip1 expression correlates with tumor grade in prostate cancer. Analysis in radical prostatectomy specimens and needle biopsies. Virchows Arch. 2004; 444:509–517. [PubMed: 15118854]

Drobnjak M, Melamed J, Taneja S, Melzer K, Wieczorek R, Levinson B, et al. Altered expression of p27 and Skp2 proteins in prostate cancer of African-American patients. Clin Cancer Res. 2003; 9:2613–2619. [PubMed: 12855638]

Drobnjak M, Osman I, Scher HI, Fazzari M, Cordon-Cardo C. Overexpression of cyclin D1 is associated with metastatic prostate cancer to bone. Clin Cancer Res. 2000; 6:1891–1895. [PubMed: 10815912]

Dunsmuir WD, Gillett CE, Meyer LC, Young MP, Corbishley C, Eeles RA, et al. Molecular markers for predicting prostate cancer stage and survival. BJU Int. 2000; 86:869–878. [PubMed: 11069416]

Erdamar S, Yang G, Harper JW, Lu X, Kattan MW, Thompson TC, et al. Levels of expression of p27KIP1 protein in human prostate and prostate cancer: an immunohistochemical analysis. Mod Pathol. 1999; 12:751–755. [PubMed: 10463475]

Evans RM. The steroid and thyroid hormone receptor superfamily. Science. 1988; 240:889–895. [PubMed: 3283939]

Facher EA, Becich MJ, Deka A, Law JC. Association between human cancer and two polymorphisms occurring together in the p21Waf1/Cip1 cyclin-dependent kinase inhibitor gene. Cancer. 1997; 79:2424–2429. [PubMed: 9191533]

Faith D, Han S, Lee DK, Friedl A, Hicks JL, De Marzo AM, et al. P16 Is upregulated in proliferative inflammatory atrophy of the prostate. Prostate. 2005; 65:73–82. [PubMed: 15880529]

Faus H, Haendler B. Post-translational modifications of steroid receptors. Biomed Pharmacother. 2006; 60:520–528. [PubMed: 16949786]

Fernandez PL, Arce Y, Farre X, Martinez A, Nadal A, Rey MJ, et al. Expression of p27/Kip1 is down-regulated in human prostate carcinoma progression. J Pathol. 1999; 187:563–566. [PubMed: 10398122]

Freedland SJ, de Gregorio F, Sacoolidge JC, Elshimali YI, Csathy GS, Elashoff DA, et al. Predicting biochemical recurrence after radical prostatectomy for patients with organ-confined disease using p27 expression. Urology. 2003; 61:1187–1192. [PubMed: 12809895]

Fu M, Rao M, Bouras T, Wang C, Wu K, Zhang X, et al. Cyclin D1 inhibits peroxisome proliferator-activated receptor gamma-mediated adipogenesis through histone deacetylase recruitment. J Biol Chem. 2005; 280:16934–16941. [PubMed: 15713663]

Gelmann EP. Molecular biology of the androgen receptor. J Clin Oncol. 2002; 20:3001–3015. [PubMed: 12089231]

Gnanapragasam VJ, Robson CN, Leung HY, Neal DE. Androgen receptor signalling in the prostate. BJU Int. 2000; 86:1001–1013. [PubMed: 11119093]

Gordon V, Bhadel S, Wunderlich W, Zhang J, Ficarro SB, Mollah SA, et al. CDK9 regulates AR promoter selectivity and cell growth through serine 81 phosphorylation. Mol Endocrinol. 2010; 24:2267–2280. [PubMed: 20980437]

Gu K, Mes-Masson AM, Gauthier J, Saad F. Analysis of the p16 tumor suppressor gene in early-stage prostate cancer. Mol Carcinog. 1998; 21:164–170. [PubMed: 9537647]

Guo Y, Sklar GN, Borkowski A, Kyprianou N. Loss of the cyclin-dependent kinase inhibitor p27(Kip1) protein in human prostate cancer correlates with tumor grade. Clin Cancer Res. 1997; 3:2269–2274. [PubMed: 9815624]

Guo Z, Yang X, Sun F, Jiang R, Linn DE, Chen H, et al. A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. Cancer Res. 2009; 69:2305–2313. [PubMed: 19244107]

Halvorsen OJ, Haukaas SA, Akslen LA. Combined loss of PTEN and p27 expression is associated with tumor cell proliferation by Ki-67 and increased risk of recurrent disease in localized prostate cancer. Clin Cancer Res. 2003; 9:1474–1479. [PubMed: 12684422]

Halvorsen OJ, Hostmark J, Haukaas S, Hoisaeter PA, Akslen LA. Prognostic significance of p16 and CDK4 proteins in localized prostate carcinoma. Cancer. 2000; 88:416–424. [PubMed: 10640976]

He B, Kemppainen JA, Voegel JJ, Gronemeyer H, Wilson EM. Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH(2)-terminal domain. J Biol Chem. 1999; 274:37219–37225. [PubMed: 10601285]

Heemers HV, Regan KM, Schmidt LJ, Anderson SK, Ballman KV, Tindall DJ. Androgen modulation of coregulator expression in prostate cancer cells. Mol Endocrinol. 2009; 23:572–583. [PubMed: 19164447]

Heidenreich B, Heidenreich A, Sesterhenn A, Srivastava S, Moul JW, Sesterhenn IA. Aneuploidy of chromosome 9 and the tumor suppressor genes p16(INK4) and p15(INK4B) detected by in situ hybridization in locally advanced prostate cancer. Eur Urol. 2000; 38:475–482. [PubMed: 11025389]

Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. Endocr Rev. 2002; 23:175–200. [PubMed: 11943742]

Heinlein CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev. 2004; 25:276–308. [PubMed: 15082523]

Henrique R, Costa VL, Cerveira N, Carvalho AL, Hoque MO, Ribeiro FR, et al. Hypermethylation of Cyclin D2 is associated with loss of mRNA expression and tumor development in prostate cancer. J Mol Med. 2006; 84:911–918. [PubMed: 17016690]

Henshall SM, Quinn DI, Lee CS, Head DR, Golovsky D, Brenner PC, et al. Overexpression of the cell cycle inhibitor p16INK4A in high-grade prostatic intraepithelial neoplasia predicts early relapse in prostate cancer patients. Clin Cancer Res. 2001; 7:544–550. [PubMed: 11297246]

Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res. 2009; 69:16–22. [PubMed: 19117982]

Huang SP, Yu CC, Liu CC, Wu TT, Huang CH, Wu MT. CDKN1B V109G polymorphism frequency and prostate cancer risk in Taiwan. Urol Int. 2008; 81:36–40. [PubMed: 18645269]

Huggins C, Hodges CV. Studies on prostatic cancer. I The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. CA Cancer J Clin. 1972; 22:232–240. [PubMed: 4625049]

Iemelynova AA, Grygorenko VM, Cheremuha SV, Romanenko AM. Correlation between histological type and immunohistochemical profile of prostate cancer and gleason scale gradation. Exp Oncol. 2009; 31:246–249. [PubMed: 20010526]

Ittmann MM, Wieczorek R. Alterations of the retinoblastoma gene in clinically localized, stage B prostate adenocarcinomas. Hum Pathol. 1996; 27:28–34. [PubMed: 8543307]

Jarrard DF, Bova GS, Ewing CM, Pin SS, Nguyen SH, Baylin SB, et al. Deletional, mutational, and methylation analyses of CDKN2 (p16/MTS1) in primary and metastatic prostate cancer. Genes Chromosomes Cancer. 1997; 19:90–96. [PubMed: 9171999]

Jarrard DF, Modder J, Fadden P, Fu V, Sebree L, Heisey D, et al. Alterations in the p16/pRb cell cycle checkpoint occur commonly in primary and metastatic human prostate cancer. Cancer Lett. 2002; 185:191–199. [PubMed: 12169393]

Jeronimo C, Henrique R, Hoque MO, Mambo E, Ribeiro FR, Varzim G, et al. A quantitative promoter methylation profile of prostate cancer. Clin Cancer Res. 2004; 10:8472–8478. [PubMed: 15623627]

Jin F, Fondell JD. A novel androgen receptor-binding element modulates Cdc6 transcription in prostate cancer cells during cell-cycle progression. Nucleic Acids Res. 2009; 37:4826–4838. [PubMed: 19520769]

Kallakury BV, Sheehan CE, Ambros RA, Fisher HA, Kaufman RP Jr, Ross JS. The prognostic significance of p34cdc2 and cyclin D1 protein expression in prostate adenocarcinoma. Cancer. 1997; 80:753–763. [PubMed: 9264360]

Kallakury BV, Sheehan CE, Rhee SJ, Fisher HA, Kaufman RP Jr, Rifkin MD, et al. The prognostic significance of proliferation-associated nucleolar protein p120 expression in prostate adenocarcinoma: a comparison with cyclins A and B1, Ki-67, proliferating cell nuclear antigen, and p34cdc2. Cancer. 1999; 85:1569–1576. [PubMed: 10193948]

Kaltz-Wittmer C, Klenk U, Glaessgen A, Aust DE, Diebold J, Lohrs U, et al. FISH analysis of gene aberrations (MYC, CCND1, ERBB2, RB, and AR) in advanced prostatic carcinomas before and after androgen deprivation therapy. Lab Invest. 2000; 80:1455–1464. [PubMed: 11005213]

Kibel AS, Christopher M, Faith DA, Bova GS, Goodfellow PJ, Isaacs WB. Methylation and mutational analysis of p27(kip1) in prostate carcinoma. Prostate. 2001; 48:248–253. [PubMed: 11536304]

Kibel AS, Faith DA, Bova GS, Isaacs WB. Loss of heterozygosity at 12P12-13 in primary and metastatic prostate adenocarcinoma. J Urol. 2000; 164:192–196. [PubMed: 10840458]

Kibel AS, Suarez BK, Belani J, Oh J, Webster R, Brophy-Ebbers M, et al. CDKN1A and CDKN1B polymorphisms and risk of advanced prostate carcinoma. Cancer Res. 2003; 63:2033–2036. [PubMed: 12727815]

Klotz L. Hormone therapy for patients with prostate carcinoma. Cancer. 2000; 88:3009–3014. [PubMed: 10898345]

Klotz L. Combined androgen blockade: an update. Urol Clin North Am. 2006; 33:161–166. v–vi. [PubMed: 16631454]

Knudsen ES, Knudsen KE. Retinoblastoma tumor suppressor: where cancer meets the cell cycle. Exp Biol Med (Maywood). 2006; 231:1271–1281. [PubMed: 16816134]

Knudsen ES, Knudsen KE. Tailoring to RB: tumour suppressor status and therapeutic response. Nat Rev Cancer. 2008; 8:714–724. [PubMed: 19143056]

Knudsen KE. The cyclin D1b splice variant: an old oncogene learns new tricks. Cell Div. 2006; 1:15. [PubMed: 16863592]

Knudsen KE, Arden KC, Cavenee WK. Multiple G1 regulatory elements control the androgen-dependent proliferation of prostatic carcinoma cells. J Biol Chem. 1998; 273:20213–20222. [PubMed: 9685369]

Knudsen KE, Cavenee WK, Arden KC. D-type cyclins complex with the androgen receptor and inhibit its transcriptional transactivation ability. Cancer Res. 1999; 59:2297–2301. [PubMed: 10344732]

Knudsen KE, Penning TM. Partners in crime: deregulation of AR activity and androgen synthesis in prostate cancer. Trends Endocrinol Metab. 2010; 21:315–324. [PubMed: 20138542]

Knudsen KE, Scher HI. Starving the addiction: new opportunities for durable suppression of AR signaling in prostate cancer. Clin Cancer Res. 2009; 15:4792–4798. [PubMed: 19638458]

Kobayashi T, Nakamura E, Shimizu Y, Terada N, Maeno A, Kobori G, et al. Restoration of cyclin D2 has an inhibitory potential on the proliferation of LNCaP cells. Biochem Biophys Res Commun. 2009; 387:196–201. [PubMed: 19577536]

Komiya A, Suzuki H, Aida S, Yatani R, Shimazaki J. Mutational analysis of CDKN2 (CDK4I/MTS1) gene in tissues and cell lines of human prostate cancer. Jpn J Cancer Res. 1995; 86:622–625. [PubMed: 7559077]

Konishi N, Nakamura M, Kishi M, Nishimine M, Ishida E, Shimada K. DNA hypermethylation status of multiple genes in prostate adenocarcinomas. Jpn J Cancer Res. 2002; 93:767–773. [PubMed: 12149142]

Kuczyk M, Machtens S, Hradil K, Schubach J, Christian W, Knuchel R, et al. Predictive value of decreased p27Kip1 protein expression for the recurrence-free and long-term survival of prostate cancer patients. Br J Cancer. 1999; 81:1052–1058. [PubMed: 10576664]

Kuczyk MA, Bokemeyer C, Hartmann J, Schubach J, Walter C, Machtens S, et al. Predictive value of altered p27Kip1 and p21WAF/Cip1 protein expression for the clinical prognosis of patients with localized prostate cancer. Oncol Rep. 2001; 8:1401–1407. [PubMed: 11605074]

LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, et al. New functional activities for the p21 family of CDK inhibitors. Genes Dev. 1997; 11:847–862. [PubMed: 9106657]

Labrie F, Belanger A, Simard J, Luu-The V, Labrie C. “Intracrinology” Autonomy and freedom of peripheral tissues. Ann Endocrinol” (Paris). 1995; 56:23–29. [PubMed: 7747916]

Labrie F, Luu-The V, Lin SX, Simard J, Labrie C, El-Alfy M, et al. Intracrinology: role of the family of 17 beta-hydroxysteroid dehydrogenases in human physiology and disease. J Mol Endocrinol. 2000; 25:1–16. [PubMed: 10915214]

Lacombe L, Maillette A, Meyer F, Veilleux C, Moore L, Fradet Y. Expression of p21 predicts PSA failure in locally advanced prostate cancer treated by prostatectomy. Int J Cancer. 2001; 95:135–139. [PubMed: 11307144]

Lee CT, Capodieci P, Osman I, Fazzari M, Ferrara J, Scher HI, et al. Overexpression of the cyclin-dependent kinase inhibitor p16 is associated with tumor recurrence in human prostate cancer. Clin Cancer Res. 1999; 5:977–983. [PubMed: 10353729]

Lee DK, Duan HO, Chang C. From androgen receptor to the general transcription factor TFIIH. Identification of cdk activating kinase (CAK) as an androgen receptor NH(2)-terminal associated coactivator. J Biol Chem. 2000; 275:9308–9313. [PubMed: 10734072]

Lee YM, Sicinski P. Targeting cyclins and cyclin-dependent kinases in cancer: lessons from mice, hopes for therapeutic applications in human. Cell Cycle. 2006; 5:2110–2114. [PubMed: 16969111]

Li C, Larsson C, Futreal A, Lancaster J, Phelan C, Aspenblad U, et al. Identification of two distinct deleted regions on chromosome 13 in prostate cancer. Oncogene. 1998; 16:481–487. [PubMed: 9484837]

Li J, Lin Q, Wang W, Wade P, Wong J. Specific targeting and constitutive association of histone deacetylase complexes during transcriptional repression. Genes Dev. 2002; 16:687–692. [PubMed: 11914274]

Li R, Wheeler TM, Dai H, Sayeeduddin M, Scardino PT, Frolov A, et al. Biological correlates of p27 compartmental expression in prostate cancer. J Urol. 2006; 175:528–532. [PubMed: 16406988]

Lim JT, Mansukhani M, Weinstein IB. Cyclin-dependent kinase 6 associates with the androgen receptor and enhances its transcriptional activity in prostate cancer cells. Proc Natl Acad Sci USA. 2005; 102:5156–5161. [PubMed: 15790678]

Lin HM, Zhao L, Cheng SY. Cyclin D1 Is a Ligand-independent Co-repressor for Thyroid Hormone Receptors. J Biol Chem. 2002; 277:28733–28741. [PubMed: 12048199]

Linja MJ, Porkka KP, Kang Z, Savinainen KJ, Janne OA, Tammela TL, et al. Expression of androgen receptor coregulators in prostate cancer. Clin Cancer Res. 2004; 10:1032–1040. [PubMed: 14871982]

Litvinov IV, Vander Griend DJ, Antony L, Dalrymple S, De Marzo AM, Drake CG, et al. Androgen receptor as a licensing factor for DNA replication in androgen-sensitive prostate cancer cells. Proc Natl Acad Sci USA. 2006; 103:15085–15090. [PubMed: 17015840]

Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res. 2008; 68:6407–6415. [PubMed: 18676866]

Lu J, Danielsen M. Differential regulation of androgen and glucocorticoid receptors by retinoblastoma protein. J Biol Chem. 1998; 273:31528–31533. [PubMed: 9813067]

Lu L, Schulz H, Wolf DA. The F-box protein SKP2 mediates androgen control of p27 stability in LNCaP human prostate cancer cells. BMC Cell Biol. 2002; 3:22. [PubMed: 12188931]

Lu S, Liu M, Epner DE, Tsai SY, Tsai MJ. Androgen regulation of the cyclin-dependent kinase inhibitor p21 gene through an androgen response element in the proximal promoter. Mol Endocrinol. 1999; 13:376–384. [PubMed: 10076995]

Ma ZQ, Liu Z, Ngan ES, Tsai SY. Cdc25B functions as a novel coactivator for the steroid receptors. Mol Cell Biol. 2001; 21:8056–8067. [PubMed: 11689696]

Mack PC, Chi SG, Meyers FJ, Stewart SL, deVere White RW, Gumerlock PH. Increased RB1 abnormalities in human primary prostate cancer following combined androgen blockade. Prostate. 1998; 34:145–151. [PubMed: 9465946]

Maddison LA, Huss WJ, Barrios RM, Greenberg NM. Differential expression of cell cycle regulatory molecules and evidence for a “cyclin switch” during progression of prostate cancer. Prostate. 2004; 58:335–344. [PubMed: 14968434]

Mallik I, Davila M, Tapia T, Schanen B, Chakrabarti R. Androgen regulates Cdc6 transcription through interactions between androgen receptor and E2F transcription factor in prostate cancer cells. Biochim Biophys Acta. 2008; 1783:1737–1744. [PubMed: 18541154]

Malumbres M, Barbacid M. Cell cycle kinases in cancer. Curr Opin Genet Dev. 2007; 17:60–65. [PubMed: 17208431]

Marivoet S, Van Dijck P, Verhoeven G, Heyns W. Interaction of the 90-kDa heat shock protein with native and in vitro translated androgen receptor and receptor fragments. Mol Cell Endocrinol. 1992; 88:165–174. [PubMed: 1459337]

Maruyama R, Toyooka S, Toyooka KO, Virmani AK, Zochbauer-Muller S, Farinas AJ, et al. Aberrant promoter methylation profile of prostate cancers and its relationship to clinicopathological features. Clin Cancer Res. 2002; 8:514–519. [PubMed: 11839671]

Mashal RD, Lester S, Corless C, Richie JP, Chandra R, Propert KJ, et al. Expression of cell cycle-regulated proteins in prostate cancer. Cancer Res. 1996; 56:4159–4163. [PubMed: 8797586]

Matsushima H, Sasaki T, Goto T, Hosaka Y, Homma Y, Kitamura T, et al. Immunohistochemical study of p21WAF1 and p53 proteins in prostatic cancer and their prognostic significance. Hum Pathol. 1998; 29:778–783. [PubMed: 9712417]

Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res. 2008; 68:4447–4454. [PubMed: 18519708]

Nash AF, Melezinek I. The role of prostate specific antigen measurement in the detection and management of prostate cancer. Endocr Relat Cancer. 2000; 7:37–51. [PubMed: 10808195]

Ngan ES, Hashimoto Y, Ma ZQ, Tsai MJ, Tsai SY. Overexpression of Cdc25B, an androgen receptor coactivator, in prostate cancer. Oncogene. 2003; 22:734–739. [PubMed: 12569365]

Nguyen PL, Lin DI, Lei J, Fiorentino M, Mueller E, Weinstein MH, et al. The impact of Skp2 overexpression on recurrence-free survival following radical prostatectomy. Urol Oncol. 2009

Nguyen TT, Nguyen CT, Gonzales FA, Nichols PW, Yu MC, Jones PA. Analysis of cyclin-dependent kinase inhibitor expression and methylation patterns in human prostate cancers. Prostate. 2000; 43:233–242. [PubMed: 10797499]

Nikoleishvili D, Pertia A, Trsintsadze O, Gogokhia N, Managadze L, Chkhotua A. Expression of p27((Kip1)), cyclin D3 and Ki67 in BPH, prostate cancer and hormone-treated prostate cancer cells. Int Urol Nephrol. 2008; 40:953–959. [PubMed: 18317945]

Olshavsky NA, Comstock CE, Schiewer MJ, Augello MA, Hyslop T, Sette C, et al. Identification of ASF/SF2 as a critical, allele-specific effector of the cyclin D1b oncogene. Cancer Res. 2010; 70:3975–3984. [PubMed: 20460515]

Olshavsky NA, Groh EM, Comstock CE, Morey LM, Wang Y, Revelo MP, et al. Cyclin D3 action in androgen receptor regulation and prostate cancer. Oncogene. 2008; 27:3111–3121. [PubMed: 18084330]

Omar EA, Behlouli H, Chevalier S, Aprikian AG. Relationship of p21(WAFI) protein expression with prognosis in advanced prostate cancer treated by androgen ablation. Prostate. 2001; 49:191–199. [PubMed: 11746264]

Ozen M, Ittmann M. Increased expression and activity of CDC25C phosphatase and an alternatively spliced variant in prostate cancer. Clin Cancer Res. 2005; 11:4701–4706. [PubMed: 16000564]

Padar A, Sathyanarayana UG, Suzuki M, Maruyama R, Hsieh JT, Frenkel EP, et al. Inactivation of cyclin D2 gene in prostate cancers by aberrant promoter methylation. Clin Cancer Res. 2003; 9:4730–4734. [PubMed: 14581343]

Pal SK, Sartor O. Phase III data for abiraterone in an evolving landscape for castration-resistant prostate cancer. Maturitas. 2011; 68:103–105. [PubMed: 21093995]

Peng Y, Chen F, Melamed J, Chiriboga L, Wei J, Kong X, et al. Distinct nuclear and cytoplasmic functions of androgen receptor cofactor p44 and association with androgen-independent prostate cancer. Proc Natl Acad Sci USA. 2008; 105:5236–5241. [PubMed: 18356297]

Penning TM, Steckelbroeck S, Bauman DR, Miller MW, Jin Y, Peehl DM, et al. Aldo-keto reductase (AKR) 1C3: role in prostate disease and the development of specific inhibitors. Mol Cell Endocrinol. 2006; 248:182–191. [PubMed: 16417966]

Perinchery G, Bukurov N, Nakajima K, Chang J, Li LC, Dahiya R. High frequency of deletion on chromosome 9p21 may harbor several tumor-suppressor genes in human prostate cancer. Int J Cancer. 1999; 83:610–614. [PubMed: 10521795]

Petre CE, Wetherill YB, Danielsen M, Knudsen KE. Cyclin D1: mechanism and consequence of androgen receptor co-repressor activity. J Biol Chem. 2002; 277:2207–2215. [PubMed: 11714699]

Petre-Draviam CE, Cook SL, Burd CJ, Marshall TW, Wetherill YB, Knudsen KE. Specificity of cyclin D1 for androgen receptor regulation. Cancer Res. 2003; 63:4903–4913. [PubMed: 12941814]

Petre-Draviam CE, Williams EB, Burd CJ, Gladden A, Moghadam H, Meller J, et al. A central domain of cyclin D1 mediates nuclear receptor corepressor activity. Oncogene. 2005; 24:431–444. [PubMed: 15558026]

Reutens AT, Fu M, Wang C, Albanese C, McPhaul MJ, Sun Z, et al. Cyclin D1 binds the androgen receptor and regulates hormone-dependent signaling in a p300/CBP-associated factor (P/CAF)-dependent manner. Mol Endocrinol. 2001; 15:797–811. [PubMed: 11328859]

Ribal MJ, Fernandez PL, Lopez-Guillermo A, Farre X, Santos Y, Gibanel R, et al. Low p27 expression predicts biochemical relapse after radical prostatectomy in patients with clinically localised prostate cancer. Anticancer Res. 2003; 23:5101–5106. [PubMed: 14981973]

Riegman PH, Vlietstra RJ, van der Korput JA, Brinkmann AO, Trapman J. The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol. 1991; 5:1921–1930. [PubMed: 1724287]

Roach M 3rd, Waldman F, Pollack A. Predictive models in external beam radiotherapy for clinically localized prostate cancer. Cancer. 2009; 115:3112–3120. [PubMed: 19544539]

Romics I, Banfi G, Szekely E, Krenacs T, Szende B. Expression of p21(waf1/cip1), p27 (kip1), p63 and androgen receptor in low and high Gleason score prostate cancer. Pathol Oncol Res. 2008; 14:307–311. [PubMed: 18415709]

Rosenbaum E, Hoque MO, Cohen Y, Zahurak M, Eisenberger MA, Epstein JI, et al. Promoter hypermethylation as an independent prognostic factor for relapse in patients with prostate cancer following radical prostatectomy. Clin Cancer Res. 2005; 11:8321–8325. [PubMed: 16322291]

Ryan CJ, Smith A, Lal P, Satagopan J, Reuter V, Scardino P, et al. Persistent prostate-specific antigen expression after neoadjuvant androgen depletion: an early predictor of relapse or incomplete androgen suppression. Urology. 2006; 68:834–839. [PubMed: 17070363]

Sarkar FH, Li Y, Sakr WA, Grignon DJ, Madan SS, Wood DP Jr, et al. Relationship of p21(WAF1) expression with disease-free survival and biochemical recurrence in prostate adenocarcinomas (PCa). Prostate. 1999; 40:256–260. [PubMed: 10420154]

Schiewer MJ, Morey LM, Burd CJ, Liu Y, Merry DE, Ho SM, et al. Cyclin D1 repressor domain mediates proliferation and survival in prostate cancer. Oncogene. 2009; 28:1016–1027. [PubMed: 19079343]

Shand RL, Gelmann EP. Molecular biology of prostate-cancer pathogenesis. Curr Opin Urol. 2006; 16:123–131. [PubMed: 16679847]

Shang Y, Myers M, Brown M. Formation of the androgen receptor transcription complex. Mol Cell. 2002; 9:601–610. [PubMed: 11931767]

Sharma A, Comstock CE, Knudsen ES, Cao KH, Hess-Wilson JK, Morey LM, et al. Retinoblastoma tumor suppressor status is a critical determinant of therapeutic response in prostate cancer cells. Cancer Res. 2007; 67:6192–6203. [PubMed: 17616676]

Sharma A, Yeow WS, Ertel A, Coleman I, Clegg N, Thangavel C, et al. The retinoblastoma tumor suppressor controls androgen signaling and human prostate cancer progression. J Clin Invest. 2010; 120:4478–4492. [PubMed: 21099110]

Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev. 1999; 13:1501–1512. [PubMed: 10385618]

Sherr CJ, Roberts JM. Living with or without cyclins and cyclin-dependent kinases. Genes Dev. 2004; 18:2699–2711. [PubMed: 15545627]

Shiraishi T, Watanabe M, Muneyuki T, Nakayama T, Morita J, Ito H, et al. A clinicopathological study of p53, p21 (WAF1/CIP1) and cyclin D1 expression in human prostate cancers. Urol Int. 1998; 61:90–94. [PubMed: 9873247]

Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res. 2006; 66:2815–2825. [PubMed: 16510604]

Steinkamp MP, O'Mahony OA, Brogley M, Rehman H, Lapensee EW, Dhanasekaran S, et al. Treatment-dependent androgen receptor mutations in prostate cancer exploit multiple mechanisms to evade therapy. Cancer Res. 2009; 69:4434–4442. [PubMed: 19366804]

Sun H, Wang Y, Chinnam M, Zhang X, Hayward SW, Foster BA, et al. E2f binding-deficient Rb1 protein suppresses prostate tumor progression in vivo. Proc Natl Acad Sci USA. 2011; 108:704–709. [PubMed: 21187395]

Tamimi Y, Bringuier PP, Smit F, van Bokhoven A, Debruyne FM, Schalken JA. P16 mutations/deletions are not frequent events in prostate cancer. Br J Cancer. 1996; 74:120–122. [PubMed: 8679444]

Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010; 18:11–22. [PubMed: 20579941]

Thomas GV, Schrage MI, Rosenfelt L, Kim JH, Salur G, de Kernion JB, et al. Preoperative prostate needle biopsy p27 correlates with subsequent radical prostatectomy p27, Gleason grade and pathological stage. J Urol. 2000; 164:1987–1991. [PubMed: 11061897]

Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science. 2009; 324:787–790. [PubMed: 19359544]

Trapman J, Brinkmann AO. The androgen receptor in prostate cancer. Pathol Res Pract. 1996; 192:752–760. [PubMed: 8880876]

Tsihlias J, Kapusta LR, DeBoer G, Morava-Protzner I, Zbieranowski I, Bhattacharya N, et al. Loss of cyclin-dependent kinase inhibitor p27Kip1 is a novel prognostic factor in localized human prostate adenocarcinoma. Cancer Res. 1998; 58:542–548. [PubMed: 9458103]

Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, et al. Towards a knowledge-based Human Protein Atlas. Nat Biotechnol. 2010; 28:1248–1250. [PubMed: 21139605]

Vis AN, Noordzij MA, Fitoz K, Wildhagen MF, Schroder FH, van der Kwast TH. Prognostic value of cell cycle proteins p27(kip1) and MIB-1, and the cell adhesion protein CD44s in surgically treated patients with prostate cancer. J Urol. 2000; 164:2156–2161. [PubMed: 11061947]

Vis AN, van Rhijn BW, Noordzij MA, Schroder FH, van der Kwast TH. Value of tissue markers p27(kip1), MIB-1, and CD44s for the pre-operative prediction of tumour features in screen-detected prostate cancer. J Pathol. 2002; 197:148–154. [PubMed: 12015737]

Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, et al. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell. 2009; 138:245–256. [PubMed: 19632176]

Wolters T, Vissers KJ, Bangma CH, Schroder FH, van Leenders GJ. The value of EZH2, p27(kip1), BMI-1 and MIB-1 on biopsy specimens with low-risk prostate cancer in selecting men with significant prostate cancer at prostatectomy. BJU Int. 2010; 106:280–286. [PubMed: 19888978]

Wong CI, Zhou ZX, Sar M, Wilson EM. Steroid requirement for androgen receptor dimerization and DNA binding. Modulation by intramolecular interactions between the $NH_2$-terminal and steroid-binding domains. J Biol Chem. 1993; 268:19004–19012. [PubMed: 8360187]

Wu TT, Wang JS, Jiaan BP, Yu CC, Tsai JY, Lin JT, et al. Role of p21(WAF1) and p27(KIP1) in predicting biochemical recurrence for organ-confined prostate adenocarcinoma. J Chin Med Assoc. 2007; 70:11–15. [PubMed: 17276927]

Xu Y, Chen SY, Ross KN, Balk SP. Androgens induce prostate cancer cell proliferation through mammalian target of rapamycin activation and post-transcriptional increases in cyclin D proteins. Cancer Res. 2006; 66:7783–7792. [PubMed: 16885382]

Yamamoto A, Hashimoto Y, Kohri K, Ogata E, Kato S, Ikeda K, et al. Cyclin E as a coactivator of the androgen receptor. J Cell Biol. 2000; 150:873–880. [PubMed: 10953010]

Yang G, Ayala G, De Marzo A, Tian W, Frolov A, Wheeler TM, et al. Elevated Skp2 protein expression in human prostate cancer: association with loss of the cyclin-dependent kinase inhibitor p27 and PTEN and with reduced recurrence-free survival. Clin Cancer Res. 2002; 8:3419–3426. [PubMed: 12429629]

Yang RM, Naitoh J, Murphy M, Wang HJ, Phillipson J, de Kernion JB, et al. Low p27 expression predicts poor disease-free survival in patients with prostate cancer. J Urol. 1998; 159:941–945. [PubMed: 9474188]

Yeh S, Miyamoto H, Nishimura K, Kang H, Ludlow J, Hsiao P, et al. Retinoblastoma, a tumor suppressor, is a coactivator for the androgen receptor in human prostate cancer DU145 cells. Biochem Biophys Res Commun. 1998; 248:361–367. [PubMed: 9675141]

Yuan X, Balk SP. Mechanisms mediating androgen receptor reactivation after castration. Urol Oncol. 2009; 27:36–41. [PubMed: 19111796]

Zeng L, Rowland RG, Lele SM, Kyprianou N. Apoptosis incidence and protein expression of p53, TGF-beta receptor II, p27Kip1, and Smad4 in benign, premalignant, and malignant human prostate. Hum Pathol. 2004; 35:290–297. [PubMed: 15017584]

Zhang Z, Rosen DG, Yao JL, Huang J, Liu J. Expression of p14ARF, p15INK4b, p16INK4a, and DCR2 increases during prostate cancer progression. Mod Pathol. 2006; 19:1339–1343. [PubMed: 16799475]

Zong H, Chi Y, Wang Y, Yang Y, Zhang L, Chen H, et al. Cyclin D3/CDK11p58 complex is involved in the repression of androgen receptor. Mol Cell Biol. 2007; 27:7125–7142. [PubMed: 17698582]

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.mce.2011.06.033.

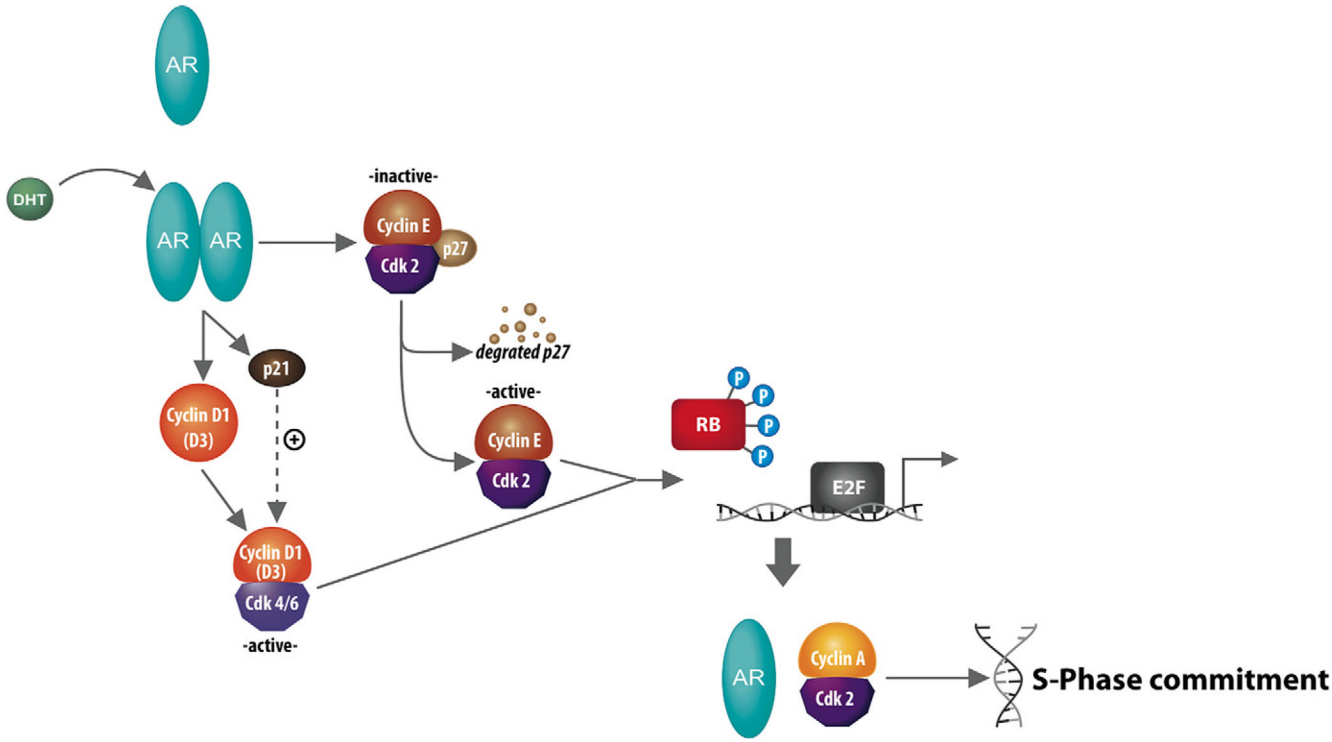


**Fig. 1.**

Regulation of cell cycle by the androgen receptor. Liganded AR induces the translation and accumulation of D-type cyclins by engaging the mammalian Target of Rapamycin (mTOR) complex, which mediates cdk4/6 activation and subsequent phosphorylation and inactivation of the retinoblastoma tumor suppressor (RB). Concomitantly, AR further impinges on G1-S progression by inducing expression of $p21^{cip1}$ and degradation of $p27^{kip1}$, promoting enhanced cdk4/6 and cdk2 dependent inactivation of RB and progression through G1. Pathways requisite for entry into S phase are initiated upon RB inactivation, which induce the activity of the E2F family of transcription factors responsible for the production cyclin A, activation of cdk2 (via cyclin A binding), and entry into S-phase. Additionally, E2F1 directly induces the expression of AR itself, potentially enhancing progression through the cell cycle.

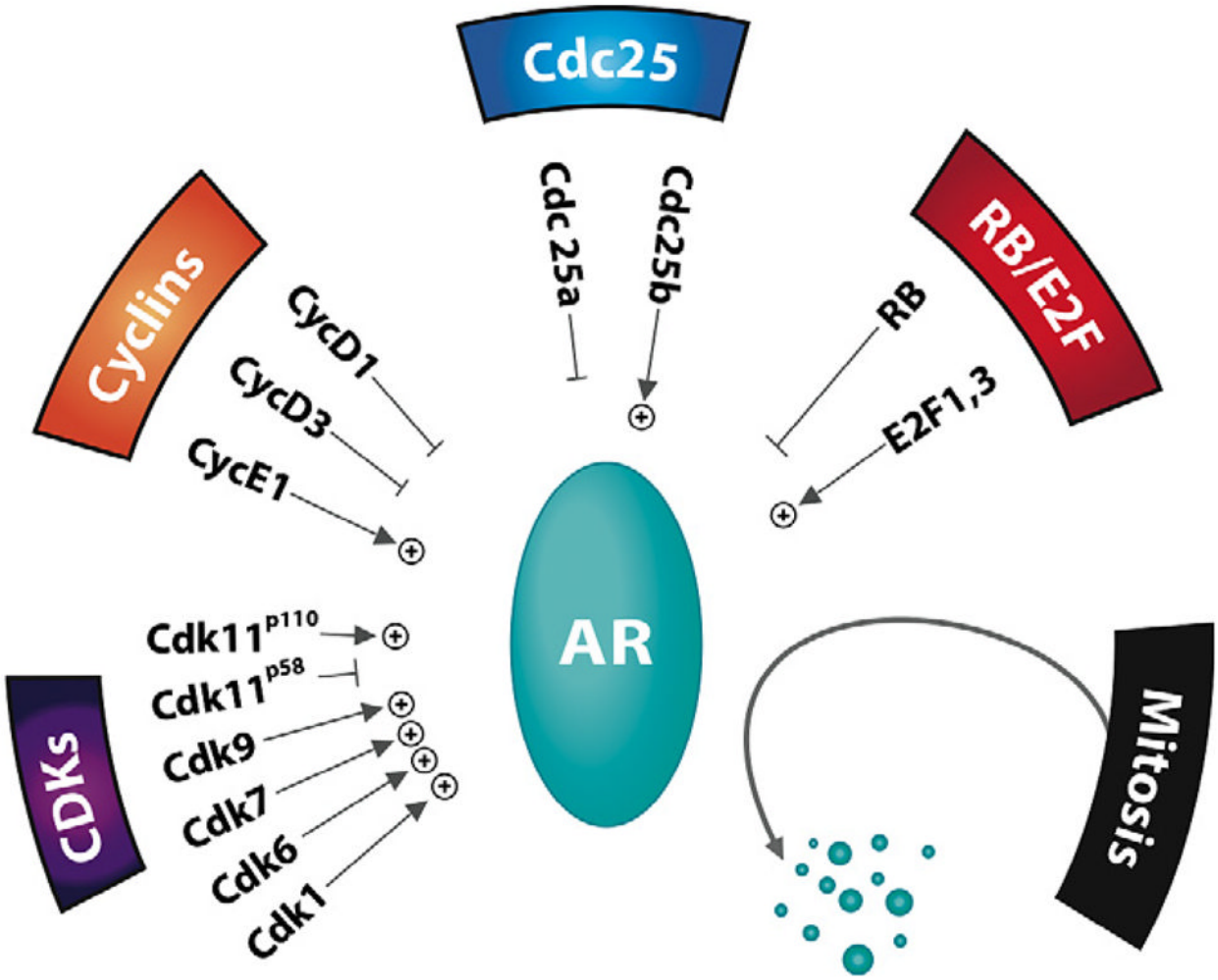


**Fig. 2.**

Contribution of cell cycle machinery to androgen receptor activity. The androgen receptor (AR) is regulated by many factors whose functions are also responsible for modulating cell cycle control. Cyclin-dependent kinases (CDKs), cyclins, members of the Cdc25 family of phosphatases, and the RB/E2F axis all contribute to this regulation. AR is degraded as cell progress through mitosis. Arrows terminating in "+" indicate activities resulting in upregulation of AR activity, while negative regulation is indicated by "−".

**Table 1**

CDKs.

| Cell cycle factor | Study size (*n*) | Change observed | Impact on AR activity or PSA levels | Clinical correlates | Citation |
|---|---|---|---|---|---|
| CDK4 | *n* = 104 | No change from BPH to PCa | No correlation | No correlation | Halvorsen et al. (2000) |
| CDK1 | *n* = 140 | 60% positive for high cdk1 | | Associated with increased grade, stage, DNA ploidy, and disease recurrence | Kallakury et al. (1997) |

**Table 2**

Cyclins.

| Cell cycle factor | Study Size (*n*) | Change observed | Impact on AR activity or PSA levels | Clinical correlates | Citation |
|---|---|---|---|---|---|
| Cyclin D1 | *n* = 140 | 78% showed no or low cyclin D1 | | No correlation with grade, stage, or DNA ploidy | Kallakury et al. (1997) |
| | *n* = 66 | 70% no or low cyclin D1 staining | | No correlation with Gleason | Shiraishi et al. (1998) |
| | *n* = 86 PCa<br>*n* = 22 Met | 11% with >20% cyclin D1 positive in primary and 68% in bone metastases | | • High cyclin D1 in bone metastases, associated with high Ki67<br>• No association with Gleason score or time to PSA Failure | Drobnjak et al. (2000) |
| | *n* = 156 | • No or low cyclin D1 was associated with reduced survival<br>• Overexpression rare and observed only in advanced disease states | | | Dunsmuir et al. (2000) |
| | Not specified | mRNA trends lower in primary and CRPC as a function of progression | | No correlation with Ki67 | Linja et al. (2004) |
| | *n* = 68 | • Reduced expression<br>• Cytoplasmic localization | Low cyclin D1 associated with high pre-operative PSA | | Comstock et al. (2007) |
| | *n* = 1600 PCa, PIN, normal | • Reduced expression (1°PCa)<br>• Splice to produce cyclin D1b | | No correlation with Ki67 | Comstock et al. (2009) |
| | *n* = 79 | | | High expression (along with low PTEN and SMAD4, and high SPP1) separated patients into low and high risk for biochemical recurrence | Ding et al. (2011) |
| | *n* = 600 | | | This four gene signature improves the prognostic value of Gleason score | |
| Cyclin D2 | *n* = 101 PCa | 32% show CCND2 methylation | Methylation correlates with high PSA | Increased methylation correlated with higher Gleason score | Padar et al. (2003) |
| | *n* = 74 PCa | Methylation observed | | If co-observed with APC methylation, correlates with decreased time to progression | Rosenbaum et al. (2005) |
| | *n* = 118 PCa<br>*n* = 38 PIN<br>*n* = 11 Normal | Cyclin D2 encoding gene (CCND2) is methylated in all tissues, but is hypermethylated in cancer | | Higher methylation associated with increased grade and non-organ confined disease | Henrique et al. (2006) |

| Cell cycle factor | Study Size (*n*) | Change observed | Impact on AR activity or PSA levels | Clinical correlates | Citation |
|---|---|---|---|---|---|
| | *n* = 4 cell lines | | | | |
| Cyclin D3 | *n* = 32 BPH<br>*n* = 20 PCa<br>*n* = 10 hormone treated | Increased expression in PCa vs. BPH | | Cyclin D3 correlates with Ki67 | Nikoleishvili et al. (2008) |
| | *n* = 67 PCa | Expression decreased as a function of disease progression | | High cyclin D3 inversely correlates with Ki67 | Olshavsky et al. (2008) |
| Cyclin E1 | *n* = 28 PCa | Low expression (14% pos, 86% negative) | | None | Mashal et al. (1996) |
| Cyclin A2 | *n* = 28 | 100% positive | | Correlated with Ki67 expression (0.71) | Mashal et al. (1996) |
| | *n* = 132 | 35% positive | | Cell-cycle-associated markers and clinical outcome in human epithelial cancers: a tissue microarray study. | Kallakury et al. (1999) |
| Cyclin B1 | *n* = 28 | 71% positive | | Correlated with Ki67 | Mashal et al. (1996) |
| | *n* = 132 | 46% positive | | Correlated with ploidy and grade $p < 0.05$ and PCNA $p < 0.0001$ | Kallakury et al. (1999) |

## Table 3

The RB/E2F axis.

| Cell cycle effector | Study size (n) | Change observed | Impact on AR activity or PSA levels | Clinical correlates | Citation |
|---|---|---|---|---|---|
| RB | *n* = 41 PCa | 27% show loss of a single Rb1 allele | | | Brooks et al. (1995) |
| | *n* = 40 PCa | • 48% showed allelic loss of Rb1<br>• 22% showed no RB detection<br>• LOH did not correlate with low RB expression | | | Cooney et al. (1996) |
| | *n* = 26 localized stage B PCa | • 35% showed LOH of the Rb1 locus<br>• 33% of specimens with LOH showed low or no RB protein | | | Ittmann and Wieczorek (1996) |
| | *n* = 36 PCa | • Analyzed for 13q deletions<br>• Found in 50% tumors<br>• Deletion did not correlate with absent RB protein | | • 13q deletion did not correlate with grade, stage, or DNA ploidy<br>• Tetraploidy found in 56% tumors with altered RB expression | Li et al. (1998) |
| | *n* = 81 | Low expression observed in 36% of tumors from patients treated with combined androgen blockade, as compared to 13% in tumors from untreated patients | | Low RB correlated with higher Gleason grade | Mack et al. (1998) |
| | *n* = 63 tumors after ADT<br>*n* = 22 matched pre-ADT controls | FISH analyses showed that loss of the Rb1 locus was four times more frequent after ADT failure | | | Kaltz-Wittmer et al. (2000) |
| | n = 72 PCa | 19% showed low RB expression | | • RB status did not correlate with p16<br>• Loss of either p16 or RB found more commonly in metastatic disease (55%) | Jarrard et al. (2002) |
| | *n* = 156 CRPC specimens<br>*n* = 22 human xenografts (LuCaP) gene expression assays | RB loss is highly overrepresented in CRPC | | • RB loss associated with hormone therapy failure<br>• RB loss associated with poor outcome | Sharma et al. (2010) |
| | *n* = 218 prostate cancers | Downregulation of RB gene expression observed in 5% of primary but 37% of metastatic samples | | | Taylor et al. (2010) |

| Cell cycle effector | Study size (n) | Change observed | Impact on AR activity or PSA levels | Clinical correlates | Citation |
|---|---|---|---|---|---|
| p130 | *n* = 24 matched normal PCa | Low expression in cancer | | | Claudio et al. (2002) |
| p107 | *n* = 24 matched normal and PCa | | | Low p107 showed an inverse correlation with Ki67 | Claudio et al. (2002) |