Androgen receptor signaling in prostate cancer development and progression

Peter E Lonergan, Donald J Tindall

Abstract

The androgen receptor (AR) signaling axis plays a critical role in the development, function and homeostasis of the prostate. The classical action of AR is to regulate gene transcriptional processes via AR nuclear translocation, binding to androgen response elements on target genes and recruitment of or crosstalk with transcription factors. Prostate cancer initiation and progression is also uniquely dependent on AR. Androgen deprivation therapy remains the standard of care for treatment of advanced prostate cancer. Despite an initial favorable response, almost all patients invariably progress to a more aggressive, castrate-resistant phenotype. Considerable evidence now supports the concept that development of castrate-resistant prostate cancer (CRPC) is causally related to continued transactivation of AR. Understanding the critical events and complexities of AR signaling in the progression to CRPC is essential in developing successful future therapies. This review provides a synopsis of AR structure and signaling in prostate cancer progression, with a special focus on recent findings on the role of AR in CRPC. Clinical implications of these findings and potential directions for future research are also outlined.

Keywords: Androgen receptor; castrate-resistant prostate cancer; signaling.

BACKGROUND

Prostate cancer is the most frequently diagnosed non-cutaneous malignancy in men, and the second leading cause of male cancer-related mortality in the United States. Clinically localized prostate cancer is managed primarily through surgery or radiation therapy. For patients who recur systemically following definitive treatment or who present with locally advanced or metastatic disease, the mainstay of treatment is androgen-deprivation therapy (ADT), typically with a luteinizing-hormone-releasing hormone (LRHR) agonist. However, this effect is temporary and after a median of 18-24 months, there is disease progression, heralded by rising serum prostate-specific antigen (PSA), increasing tumor size, new metastatic spread and disease-related symptoms. This represents the lethal phenotype of the disease, and is referred to as castration-resistant prostate cancer (CRPC).

The androgen receptor (AR) plays a pivotal role in the normal growth and development of the prostate gland, and also in prostate carcinogenesis and progression to androgen-independent disease. The AR is expressed to some degree in nearly all primary prostate cancers. Studies in both
ANDROGEN RECEPTOR STRUCTURE

The human AR gene is a nuclear transcription factor and a member of the steroid hormone receptor superfamily of genes. It is located on the X chromosome (q11-12) and consists of 8 exons. It codes for a protein of 919 amino acids with a mass of 110 kDa. The AR consists of four structurally and functionally distinct domains [Figure 1], a poorly conserved N-terminal domain (NTD), a highly conserved deoxyribonucleic acid (DNA) -binding domain (DBD) and a moderately conserved ligand-binding domain (LBD). A short amino acid sequence called the ‘hinge region’ separates the LBD from the DBD and also contains part of a bipartite ligand-dependent nuclear localization signal (NLS) for AR nuclear import. AR domain structure and function has been extensively reviewed elsewhere.\[21-23\]

N-terminal domain

The NTD (amino acids 1-537 coded by exon 1) is considered to be constitutively active, and can activate transcription independently of androgenic stimulus in LBD-deletion mutants.\[24,25\] The NTD also harbors transcriptional activation function (AF)-1, which encompasses two transcriptional activation units (TAU): TAU-1 and TAU-5.\[26\] The core domain mediating TAU1 transcriptional activity has been mapped to a discreet LKDIL motif within the NTD.\[27,28\] However, TAU5 is responsible for the majority of constitutive transcriptional activity within the NTD, and is mediated through the core sequence WHTLF,\[29\] accounting for approximately 50% aberrant AR activity in CRPC cells.\[29\]

Figure 1: Schematic representation of the androgen receptor gene and protein, with indications of its specific motifs and domains

Deoxyribonucleic acid-binding domain

The DBD (68 amino acids coded by exons 2 and 3) consists primarily of two zinc finger domains. The first zinc finger contains a conserved P-box motif that co-ordinates gene specific nucleotide contacts within the DNA groove. The second zinc finger contains a conserved D-box motif, which functions as a DBD/DBD binding site for receptor homodimer formation.\[30\]

Hinge region

A short sequence of approximately 50 amino acids (625-669) called the hinge region separates the LBD from the DBD. This region also contains part of a bipartite ligand-dependent nuclear localization signal (NLS) for AR nuclear import. A cytoskeletal protein Filamin-A (FlnA) interacts with the hinge, DBD and LBD of AR, facilitating AR translocation to the nucleus. FlnA negative cell lines do not show nuclear translocation of AR, and AR remains cytoplasmic even after prolonged androgen exposure.\[31\] FlnA cytoplasmic localization in clinical specimens also correlates with increased metastatic potential and a hormone-refractory phenotype.\[32\] Two additional NLS exist in the NTD and LBD with distinct pathways for nuclear import: the NLS of DBD is Ran and importin /β-dependent, whereas the NLSs of NTD and LBD are Ran dependent but importin /β-independent.\[33\] This suggests that the nuclear import of AR is regulated by interplay between each domain of the AR.

Ligand-binding domain

The AR LBD (amino acids 669-919) facilitates binding of the AR ligands, testosterone and dihydrotestosterone (DHT), which represents the primary control mechanism of the androgen-signaling axis. Similar to the AF1 region in the
Androgenic steroids are 19-carbon steroids of which, testosterone is the prototype. It is produced primarily by the testes in males with a small contribution from the adrenal glands. Androgens play a role in a wide range of developmental and physiological responses. The cytochrome P450 enzyme, 5α-reductase, which converts testosterone to DHT, is highly expressed within the prostate and genital tissues. Both testosterone and DHT can bind to and activate AR under physiological conditions, with DHT having a significantly greater affinity for AR, therefore activating target genes at lower concentrations than testosterone.

In the absence of ligand, the AR is located primarily in the cytoplasm (Figure 2), where it associates with heat shock proteins (HSP)-90, -70, -56, cytoskeletal proteins and other chaperones (reviewed in [44]). HSPs are believed to be tethered to cytoskeletal proteins, such as FlnA. FlnA interacts directly with the hinge-region of AR, thereby modulating nuclear translocation and transcriptional action of AR as well as androgen dependence of LNCaP cells. [31,45,46] Androgens enhance association and co-localization of AR with FlnA. This complex also recruits integrin beta 1 and induces activation of Rac1 and focal adhesion kinase (FAK), which both coordinate cell migration. [47] This complex appears to act as a link between androgen signaling and actin cytoskeleton, thereby driving cell migration and may affect prostate cancer progression and metastasis.

Binding of ligand to the AR ligand-binding pocket induces a conformational change in AR whereby helices 3, 4 and 12 within the LBD, form the AF-2 binding surface. AF-2 is the principal protein-protein interaction surface used by nuclear receptors to recruit LxxLL-motif containing coactivators. [34] However, AR differs from other nuclear receptors in this respect and interacts with coactivators in a unique manner. This pocket in the LBD binds preferentially to FxxLF motifs found in the NTD, and interacts poorly with LxxLL motifs commonly found in coactivators. [46,49]

As a result, the hydrophobic pocket within the AR LBD facilitates intramolecular and intermolecular interaction between the AR NTD and its carboxy-terminal domain (CTD), resulting in the dimerization of AR. This NTD/CTD interaction occurs predominately when AR is not bound to DNA. [50] Several AR-associated coactivators that contain FxxLF motifs have been isolated, [51] suggesting that competition exists between these regulatory proteins and the NTD for binding to the AF-2. The significance of this is unclear, but suggests that additional binding sites outside this well-defined coactivator pocket enable AR to interact with its coactivators, and that different classes of coactivators may interact with different AR surfaces. [18] These interactions facilitate the nuclear targeting of AR and AR homodimer formation. Once inside the nucleus, AR binds to specific recognition sequences known as androgen response elements (AREs) in the promoter and enhancer regions of target genes. The AR transcriptional complex is completed by recruitment of coregulators, which ultimately results in modulation of gene expression. [52]

**Androgen receptor coregulatory proteins**

Almost 200 AR coregulators have now been identified (reviewed by Heemers and Tindall. [18]) These proteins have specific and distinct functions, either enhancing (coactivators) or repressing (corepressors) AR activity, depending on the target gene. [53] However, unlike general and specific transcription factors, they do not significantly alter the basal transcription rate and do not typically possess DNA binding capabilities. Instead, coregulators act at AR target gene promoter and/or enhancer regions to facilitate transcriptional activation.

![Figure 2: Summary of the major androgen receptor signaling pathways in prostate cancer. Upon binding to dihydrotestosterone, androgen receptor translocates to the nucleus, binds to its target genes and regulates their expression. Androgen receptor can also be transactivated in the absence, or in very low levels of dihydrotestosterone. Activating signals arise from several, non-mutually-exclusive mechanisms including extracellular peptides such as Insulin-like growth factor, Epidermal growth factor and Interleukin-6](http://www.carcinogenesis.com/content/10/1/20)
DNA occupancy, induce chromatin remodeling, and/or recruit general transcription factors associated with RNA polymerase activity. These proteins are broadly divided into 4 main types: (i) molecular chaperones that coordinate AR maturation and movement, (ii) histone modifiers, (iii) coordinators of transcription and (iv) DNA structural modifiers.

The formation of an active AR-directed transcription pre-initiation complex occurs via the sequential recruitment of coregulators with distinct activities on the ligand-bound nuclear AR. The first identified and most widely understood of these coregulatory proteins is the p160 coactivator family, which consists of three 160 kDa proteins: SRC1, transcription intermediary factor 2 (TIF2; and its mouse homologue GRIP1) and SRC3. Immunohistochemical studies have shown that SRC1 expression is increased in 50% of androgen-dependent prostate cancer samples, compared to benign or normal prostate tissues. Moreover, SRC1 and TIF2 expression are increased in 63% of CRPC samples. A correlation between increased levels of SRC3 and prostate tumor grade and stage has been identified in clinically localized disease. The p160 coactivators interact with the AR NTD and also the LBD, thereby enhancing ligand-dependent, AR-mediated transcription of target genes. Their recruitment directly influences AR transactivation capacity via intrinsic histone acetyltransferase activity, and indirectly by acting as platforms for the recruitment of secondary coactivators possessing chromatin remodeling and protein acetyltransferase capabilities such as p300. In tissue samples from patients with biopsy-proven prostate cancer who underwent prostatectomy, p300 levels not only correlated with proliferation in vivo, but also predicted larger tumor volumes, the likelihood of extraprostatic extension, seminal vesicle involvement at surgery as well as progression after surgery. Further evidence in vitro has shown that an increase in p300 expression, fostered by androgen deprivation, offers a growth advantage to androgen-insensitive prostate cancer cells.

The role of the non-AR specific coregulator, ARA70, is less clear. ARA70 protein has been found to be overexpressed in high grade prostate carcinomas, prostate cancer cell lines and xenografts, whereas ARA70 mRNA expression was found to be decreased in prostate tissue. Increased in response to hormone deprivation or unchanged between normal and prostate cancer in the same tissue. As a result of ARA70’s ability to interact with other nuclear receptors, the significance of ARA70-AR interaction remains to be fully elucidated. ARA24/Ran interacts with the TAU-1 region of the NTD, leading to polyglutamine repeat expansion in AR. The role of ARA24 in prostate cancer progression remains inconclusive. In one study, ARA24 mRNA expression was found to be increased in early primary prostate cancer specimens, whereas another study found ARA24 expression to be similar between benign prostate hypertrophy, primary prostate tumors and CRPC tumors.

Overall, these studies provide evidence that prostate cancer is associated with overexpression of certain AR coregulators, which may contribute to disease progression. However, the simultaneous involvement of multiple coregulators and their overlapping interaction, suggest that additional studies are required to determine the full contribution of AR coregulators in prostate carcinogenesis.

Androgen receptor target genes

Defining the androgen-regulated gene expression program in normal and malignant prostate cancer cells has been an area of intense investigation over the last number of years. This has been facilitated by advances in high throughput gene expression analysis. The majority of these studies have been performed in LNCaP cells, with other models systems including rat ventral prostate, rat ventral prostate epithelial cells (rVPECs) and a variety of other human prostate cancer cell lines such as 22Rv1, MDACaP2a, MDACaP2b and LAPC4 (reviewed by Dehm and Tindall). Studies have estimated the LNCaP transcriptome to be anywhere from 10,570 to 23,448 polyadenylated RNAs, with 1.5-4.3% of the transcriptome either directly or indirectly regulated by androgens.

More recently, ChIP-on-chip analysis, a technique that combines chromatin immunoprecipitation (“ChIP”) with microarray technology (“chip”), has been used to screen for novel androgen responsive genes. Wang and colleagues mapped the AR-binding sites on chromosomes 21 and 22 in LNCaP cells by combining ChIP with tiled oligonucleotide microarrays and expanded on this with comparisons between LNCaP and castrate-resistant LNCaP- abl cells, in an attempt to identify direct AR-dependent target genes in both androgen-dependent disease as well as in CRPC. Ultimately, they determined that the role of the AR in CRPC is to execute a distinct program resulting in androgen-independent growth, involving mitotic phase (M-phase) regulatory genes and in particular, UBE2C which is overexpressed in CRPC tissues. Significantly, silencing of UBE2C significantly decreases growth in CRPC cells by arresting Gap 2 (G2)/M and synthesis phases (S phases), providing an exciting potential therapeutic target.

One of the most significant findings with respect to prostate cancer development and progression was the identification of chromosomal rearrangements leading to novel fusions
between the androgen-regulated promoter of the TMPRSS2 gene to the 3’ end of the oncogenic ETS transcription factor family members, ERG or ETV1.\textsuperscript{74} TMPRSS2 was identified using complementary DNA (cDNA) microarrays derived from human prostate tissues to examine the transcript expression profiles of androgen-responsive LNCaP cells under conditions of androgen deprivation or androgen supplementation. TMPRSS2 mRNA is induced within 2 hours of androgen stimulation and reaches a maximum level within 24 hours.\textsuperscript{73} Subsequent studies have reported that TMPRSS2 is highly specific to prostatic tissue and localized to prostate luminal epithelial cells.\textsuperscript{76,77} Fusions between TMPRSS2 and ERG or ETV1 were identified using a novel bioinformatics approach called cancer outlier profile analysis (COPA)\textsuperscript{74}. COPA was applied to databases of microarray data to identify overexpressed oncogenes in subsets of malignant versus normal tissues. Strong outlier profiles were identified for ERG and ETV1, and overexpression of these genes was confirmed for a subset of malignant prostate tissue. Using a combination of genomic analysis techniques, ERG or ETV1 overexpression was attributed to chromosomal translocations, which result in various fusions between the 5’ end of the TMPRSS2 gene and the 3’ end of either ERG or ETV1. In microarray datasets, ERG1 or ETV1 was overexpressed in 57% of prostate cancer cases but not in benign tissues, and the fusion with TMPRSS2 was found in 20 of 22 cases that overexpressed ERG or ETV.\textsuperscript{74} Data from larger cohorts support these findings, and indicate that TMPRSS2 fusion with ETS members is the most frequent rearrangement in prostate cancer.\textsuperscript{78,79} Recently, it has been reported that androgen treatment can induce these fusion events.\textsuperscript{80,81} Interestingly, TMPRSS2:ERG fusion has been detected in non-malignant prostate cancer epithelial cells following long-term exposure to DHT,\textsuperscript{82} suggesting that these fusions may be an early event in prostate carcinogenesis and play a pivotal role in prostate cancer progression. These landmark findings have positioned chromosomal rearrangements as critical initiating events in prostate cancer, elaborating our understanding the mechanisms of carcinogenesis and potentially opening new avenues for therapeutic intervention.\textsuperscript{83,84}

**ALTERNATIVE ANDROGEN RECEPTOR SIGNALING PATHWAYS**

Considerable evidence now exists that AR remains transcriptionally active in CRPC. Numerous immunohistochemical studies have shown that AR protein is expressed at high levels (compared to levels in untreated tumors), in most cases of CRPC.\textsuperscript{5,85,86} Consistent with this finding, real-time quantitative reverse transcription-polymerase chain reaction assay studies have shown that expression of AR mRNA in CRPC is higher compared to primary untreated tumors.\textsuperscript{87,88} At least one mechanism for the increased AR mRNA expression is AR gene amplification, which occurs in 20-33% of CRPC cases.\textsuperscript{87,89,90} In addition to AR, multiple AR regulated genes (such as PSA) are expressed, indicating that AR transcriptional activity is active in CRPC.\textsuperscript{91} AR mutations that occur in CRPC is a possible mechanism for this AR transcriptional activity, however the overall frequency of CRPC that harbor somatic mutations in the AR gene is approximately 10%,\textsuperscript{92} and unlikely to account for most cases of CRPC.

**Interleukins**

A considerable body of evidence now exists that implicates extracellular peptide signals in the form of growth factors and cytokines in the maintenance of the transcriptional activity of AR in CRPC. The role of interleukins (IL), and in particular IL-6 and IL-8, in the regulation of cellular events in different cancers has been extensively investigated (reviewed by Culig\textsuperscript{91}). IL-6 is a multifunctional cytokine produced by many cells including prostate, immune cells and osteoblasts. IL-6 binds to the IL-6 receptor, which is composed of the ligand binding subunit gp80 and the ubiquitously expressed signal-transducing subunit gp130. This leads to phosphorylation of Janus kinases (JAK), after which signal transducer and activator of transcription (STAT) factor-3 is phosphorylated and translocated to the nucleus. However, activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K) pathways can also occur depending on the cell type.\textsuperscript{92} The rationale for studies on AR regulation by IL-6 is that elevated circulating IL-6 levels have been associated with advanced stage,\textsuperscript{93,94} distant metastases,\textsuperscript{95} metastasis-related morbidity\textsuperscript{96,97} and decreased survival.\textsuperscript{95} IL-6 can transactivate AR in prostate cancer cells; however, the effect of IL-6 on ligand-independent AR activation, tumor formation and castrate-resistant growth is variable depending on the status of AR, as well as crosstalk with other signaling pathways.\textsuperscript{97,98} IL-6 enhances proliferation in LAPC-4 cells (which expresses wild-type AR) and PCa-2b (which expresses two AR substitution mutants at T877A and L701H), however IL-6 inhibits ligand independent AR activity in LNCaP cells (which express the AR T877A mutant in the LBD).\textsuperscript{98} PSA transcription is inhibited by IL-6 treatment in the presence of androgen in LNCaP cells, by preventing recruitment of p300 to the PSA promoter.\textsuperscript{99} However, prolonged IL-6 treatment of LNCaP cells leads to activation of AR and PSA expression, via STAT signaling, even in the absence of androgen.\textsuperscript{100} STAT3 interacts directly with amino acids 234-558 in the NTD of AR,\textsuperscript{101} and AR transcriptional activation is dependent on phosphorylation of STAT3 at Serine-772.\textsuperscript{102} As expression of IL-6 is increased in tissues and sera of CRPC, it has been explored as a target for therapy. Siltuximab
With increased expression of both EGF and AR translocation into the nucleus of prostate cancer cells that express endogenous AR such as LNCaP cells, treatment with EGF in the absence of androgen induces phosphorylation of AR at Tyr-267 and -534 by Src and Ack1 kinases. Androgen-activated AR activates the MAPK pathway and conversely, EGF-activated MAPK signaling cascade interferes with AR function, modulating the androgen response. MAPK extracellular kinase (MEK) inhibition reverses the EGF-mediated AR down regulation in differentiated cells, thus suggesting the existence of an inverse correlation between EGF and androgen signaling in non-tumor epithelial cells. EGF can also induce IL-6 upregulation in prostate cancer cells.

Increased levels of STAT3 leads to STAT3-AR complex formation in response to EGF and IL-6. Moreover, STAT3 increases the EGF-induced transcriptional activation of AR, while androgen pre-treatment increases STAT3 levels in an IL-6 autocrine/paracrine-dependent manner, suggesting an intracellular feedback loop. With increased expression of EGFR in metastatic disease, and correlation with disease progression, EGFR has potential as a therapeutic target. Gefitinib, an EGFR-selective tyrosine kinase inhibitor, exhibits antitumor activity in xenograft models of both androgen-dependent and androgen-independent human prostate cancer. However, as a single agent, gefitinib, failed to demonstrate any objective or PSA responses in phase II studies in patients with either metastatic or non-metastatic CRPC. A Phase II study in CRPC of lapatinib, an inhibitor of EGFR and human epidermal growth factor receptor 2 (HER2), showed a PSA response only in a very small number of patients.

Insulin-like growth factor (IGF)-1 signaling is of significant biological importance. Large-scale epidemiological studies have suggested a correlation between elevated serum levels of IGF-1 and low levels of IGFBP-3 (Insulin-like growth factor-binding protein 3, a serum protein that regulates the binding of free IGF-1 to the IGF receptor), and increased risk of developing prostate cancer. However, several subsequent studies have failed to show this effect. None-the-less, the IFG pathway has been implicated in the modulation of AR signaling. Potential mechanisms include AR phosphorylation and translocation into the nucleus or simulation of expression/activity of AR cofactors, thereby affecting AR transcriptional activation both in the presence and absence of androgens. Activation of androgen-responsive promoters with IGF-1 treatment is equivalent to androgen treatment in ectopic AR-expressing DU-145 cells. However, the cross-talk between IGF-1 and AR is apparent in other cell types, including non-transformed cells. Therefore, the action of IGF-1 on AR activity appears
to be non-specific. As a result, the IGF-1 signaling pathway is not an attractive pathway as a therapeutic target.

**Intracellular Kinase Signaling**

The extracellular signals discussed in the previous section represent just a small part of the complex mechanism of AR transactivation in the absence of androgens. These factors work in isolation or, more likely, in concert with each other to activate intracellular kinase signaling cascades that target fundamental cellular processes such as proliferation and transcription initiation. Kinase signal cascades are rapidly transduced and mediated through protein-protein interactions. The deregulated cell growth observed in cancer occurs as a result of perturbed signal transduction, with alterations in the activities of certain kinases driving the development and progression of many cancers, including those of the prostate. Therapeutically, targeting these complex protein-protein interactions or phosphorylation events is difficult and requires vigorous validation and confirmation of specificity. However, an understanding of the principal intracellular signaling cascades responsible for prostate cancer progression provides important insights into the role of AR during progression to CRPC.

The MAPK signaling pathway has been implicated in many cancers, including prostate cancer. As discussed in the previous section, a variety of extracellular stimuli can activate the MAPK signaling pathway. The many downstream targets of the MAPK pathway provide a huge number of potential regulators of AR activity, only a few of which have been characterized, including sarcoma-related kinase (Src) and p42/44 extracellular-signal-regulated kinases (ERK).

Src is a powerful oncogene that is activated in a variety of tumors. In LNCaP and LAPC-4 cells, Src phosphorylates AR at Tyrosine-534, which induces transcriptional activity by promoting nuclear translocation and DNA binding in the absence of androgen. Activation of Src occurs as a result of a number of stimuli, including EGF, described earlier. The activity of Src on AR transactivation is modulated, at least in part through its binding partner and associated scaffold protein, receptor for activated protein kinase C-1 (RACK1). Treatment of C4-2 cells with the Src inhibitor, protein phosphatase-2 in androgen free conditions results in decreased AR activation of reporter activities and reduced recruitment of AR to the PSA enhancer region.

p42/44 (ERK1 and ERK2, respectively) is another key effector of the MAPK pathway. Immunohistochemical studies of prostate tumors have shown the level of activated MAPK increases with increasing Gleason score and tumor stage. High levels of activated MAP kinase were also detected in CRPC, suggesting an increase in the activation of the MAP kinase signal pathway during prostate cancer progression.

Treatment of C4-2 cells with the MAP kinase inhibitor, U0126 in steroid depleted conditions, substantially decreases AR stability and protein expression, suggesting that enhanced autocrine growth factor signaling contributes to the maintenance of AR expression and facilitates AR activity in the absence of androgens. G protein-related factors have also been shown to regulate AR activity through stimulation of the MAP kinase pathway. The expression of Vav3, a Rho GTPase guanine nucleotide exchange factor, is upregulated in the progression of LNCaP cells to the androgen independent phenotype of LNCaP-R1 cells. Vav3 has also been shown to enhance EGF activation of AR in the absence of androgens.

This signaling pathway induces nuclear localization of AR via the activation of Rho GTPase, Rac1, whose downstream signaling includes members of the MAP kinase family, including ERK.

The phosphatidylinositol 3-kinase (PI3K) pathway has been implicated in prostate carcinogenesis to CRPC, however its precise function remains to be fully elucidated (reviewed by Sarker and colleagues). Activation of PI3K leads to the generation of second messenger phosphatidylinositol 3,5-triphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate (PIP2). This results in recruitment of pleckstrin homology domains of a number of signaling kinases including Akt (Akt/PKB is a serine/threonine protein kinase), driving their conformational change and phosphorylation by the constitutively active phosphoinositide-dependent kinase 1 and 2. Activated Akt translocates to the nucleus and activates downstream targets involved in fundamental cellular processes such as proliferation, cell cycle progression, growth and angiogenesis. Akt is negatively regulated by the tumor suppressor, phosphatase and tensin homolog deleted on chromosome 10 (PTEN).

Immunohistochemical studies indicate that loss of PTEN expression is found in 20-27% of primary tumors and in 79% of CRPC specimens. PTEN loss correlates with advanced stage and Gleason score. Functional loss of PTEN is associated with increased activity of Akt-1 and correlates with prognosis, predicting disease recurrence after primary treatment. Targeted Cre-mediated PTEN homozygous deletion in mice leads to a significant shortened latency of prostate intraepithelial neoplasia (PIN) formation and results in prostate cancer progression to a metastatic stage, despite androgen deprivation therapy, mimicking the disease progression seen in humans. These data support the concept that PI3K signaling induces continued AR gain of function despite reduced androgen levels, possibly though activation by posttranslational modification or reduced corepressor activity.
As discussed previously, the role of the TMPRSS2:ERG fusion in prostate carcinogenesis has gained considerable interest. Recent data has shown that loss of PTEN cooperates with TMPRSS2:ERG in prostate cancer. Transgenic mice expressing TMPRSS2:ERG in the prostate failed to develop PIN or invasive cancer. However, when they were crossed with either PTEN+/- mice or prostate-specific Akt transgenic mice, PIN but not invasive cancer developed.\[140] Other studies have found transgenic overexpression of ERG in mouse prostate tissue promotes marked acceleration and progression of high-grade PIN to adenocarcinoma when crossed with PTEN+/- mice.\[141] This study also showed prostate cancer specimens containing the TMPRSS2:ERG rearrangement are significantly enriched for PTEN loss.\[141] Together these data suggest cooperativity between PI3K-pathway activation and ERG aberration in induction of PIN, but suggest that additional events are likely required for invasive malignancy.

Nuclear factor-κB (NF-κB) transcription factor members are important mediators of oncogenesis in many cancers, including prostate cancer. The activity of NF-κB is higher in androgen-independent cell lines, and androgen-independent xenografts compared with androgen-dependent grafts,\[142] as well as in metastatic prostate cancer compared to localized disease.\[143] Elevation of NF-κB activity in primary prostate cancer correlates with poor prognosis and predicts biochemical relapse.\[144] Activation of NF-κB in transgenic mouse models leads to continued tumor growth despite surgical castration,\[145] indicating that activation of the NF-κB pathway may play a critical role in progression of the tumor to androgen independence. The mechanism of this remains largely unknown; however, recent data suggests that NF-κB/p52 can activate AR, leading to increased transcription of AR-responsive genes in a ligand independent manner.\[146] NF-κB/p52 enhances nuclear translocation of AR by binding to the AR NTD and enhancing recruitment of AR coactivators such as p300 to the promoter regions of AR target genes.\[146]"

Protein kinase A, which is regulated by intracellular cyclic adenosine monophosphate (cAMP) levels, can also modulate AR activity in the absence of androgens. This was first demonstrated over a decade ago.\[147] AR can be activated in the absence of androgens through an alternate signaling pathway involving forskolin, which activates adenyl cyclase, thus increasing intracellular levels of cAMP and consequently protein kinase A (PKA).\[148] This activation is blocked by bicalutamide, confirming that this ligand-independent pathway is AR dependent.\[147] Aurora-A is a serine-threonine protein kinase frequently overexpressed in several cancers, including prostate. It is overexpressed in high-grade PIN lesions,\[149] primary prostate cancer specimens and also several prostate cancer cell lines.\[150] In addition, Aurora-A expression correlates with tumorigenicity and invasive potential as well as the clinical staging, surgical margin status, and seminal vesicle invasion in radical prostatectomy specimens.\[150] Aurora-A interacts with AR, phosphorylates AR at Threonine 282 and Serine 293 and induces AR transactivation in a phosphorylation dependent-manner. Overexpression of Aurora-A in LNCaP cells induces PSA and cell survival. However, inhibition of Aurora-A sensitizes LNCaP-RF cells (an androgen independent subline) to apoptosis and cell growth arrest.\[151] This data suggests that AR is a substrate of Aurora-A and that elevated Aurora-A may contribute to androgen-independent growth by phosphorylation and activation of AR. The Aurora kinase inhibitor, VX680, attenuates phosphorylation of histone H3 and reduces survival in PC3 and LNCaP cells.\[150] Intracellular kinase signaling represents an important mediator of extra-cellular signals that promote cancer cell proliferation and survival. The ability of kinases such as those discussed here, to directly modify AR and promote AR activity in CRPC provides attractive targets for therapeutic intervention. The redundancy between many of these pathways makes achieving specific and durable inhibition difficult. This is further complicated by the uniquely heterogeneous nature of CRPC. Further investigation into the interplay between these pathways and AR [Figure 2], may lead to the identification of distinct subsets of CRPC patients who may benefit from a multi-drug approach.

**CONCLUSIONS AND FUTURE DIRECTIONS**

Ever since the seminal observation by Huggins and Hodges in 1941 that castration was beneficial in CRPC, abrogation of AR action has remained the therapeutic objective in clinical management of the disease. There is now incontrovertible evidence that the onset of CRPC coincides with renewed AR signaling. Given that metastatic prostate cancer is a molecularly heterogeneous disease even within a single patient,\[152] multiple mechanisms may simultaneously and ultimately give rise to a molecularly diverse group of CRPCs. Despite these challenges, a new generation of AR antagonists such as MDV3100 and EPI-001 has shown promise. MDV3100 which binds to the AR LBD, impairs nuclear translocation, DNA binding, and coactivator recruitment,\[153] and has shown encouraging antitumor activity in phase I-2 studies in patients.\[154] In contrast to MDV3100, EPI-001 binds to the AR NTD. Data from preclinical studies has shown that EPI-001 treatment leads to cytoreduction of CRPC in xenografts dependent on AR for growth and survival, without causing toxicity.\[155] These emerging next-generation AR antagonists, used either alone or in combination with existing treatments
have huge potential to significantly change treatment options in metastatic disease in the future.

DISCLOSURES

The authors declare no conflict of interests.

ACKNOWLEDGEMENTS

This work is supported by the NIH (CA125747, CA091956 and CA121277) and the T.J. Martell Foundation (DJT). PEL is supported by a Fulbright Scholarship and the Postgraduate Traveling Scholarship in Medicine from Trinity College, Dublin.

REFERENCES

1. Jemal A, Siegel R, Xu J, Ward E. Cancer Statistics, 2010. CA Cancer J Clin 2010;60:277-300.
2. Klein EA, Ciezki J, Kupelian PA, Mahadevan A. Outcomes for intermediate risk prostate cancer: are there advantages for surgery, external radiation, or brachytherapy? Urol Oncol 2009;27:67-71.
3. Loblaw DA, Virgo KS, Nam R, Sommerfield MR, Ben-Josef E, Mendelson DS, et al. Initial hormonal management of androgen-sensitive metastatic, recurrent, or progressive prostate cancer: 2006 update of an American Society of Clinical Oncology practice guideline. J Clin Oncol 2007;25:1596-605.
4. Pienta KJ, Bradley D. Mechanisms underlying the development of androgen-independent prostate cancer. Clin Cancer Res 2006;12:1663-71.
5. Ruizeved de Winter JA, Janssen PJ, Slaedens HP, Verleen-Moolijan MC, Trapani J, Brinkmann AO, et al. Androgen receptor status in localized and locally progressive hormone refractory human prostate cancer. Am J Pathol 1999;144:735-46.
6. Chodak GW, Kranc DM, Puy LA, Takeda H, Johnson K, Chang C. Nuclear localization of androgen receptor in heterogeneous samples of normal, hyperplastic and neoplastic human prostate. J Urol 1992;147:798-803.
7. Sadi MV, Walsh PC, Barrack ER. Immunohistochemical study of androgen receptors in metastatic prostate cancer: Comparison of receptor content and response to hormonal therapy. Cancer 1991;67:3057-64.
8. Henshall SM, Quinn DJ, Lee CS, Head DR, Golovsky D, Brenner PC, et al. Altered expression of androgen receptor in the malignant epithelium and adjacent stroma is associated with early relapse in prostate cancer. Cancer Res 2001;61:423-7.
9. Ricciardelli C, Choong CS, Buchanan G, Vivekanandan S, Neufang P, Stahl J, et al. Androgen receptor levels in prostate cancer epithelial and peritumoral stromal cells identify non-organ confined disease. Prostate 2005;63:19-28.
10. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, et al. Molecular determinants of resistance to androgenant therapy. Nat Med 2004;10:33-9.
11. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer 2001;1:34-45.
12. Scher HI, Buchanang N, Gerald W, ButlerLM, Tilley WD. Targeting the androgen receptor: improving outcomes for castration-resistant prostate cancer. Endocr Relat Cancer 2004;11:459-76.
13. Scher HI, Sawyer CL. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. J Clin Oncol 2005;23:8253-61.
14. Ryan CJ, Smith A, Lai P, Satagopan J, Reuter V, Scardino P, et al. Persistent prostate-specific antigen expression after neoadjuvant androgen deprivation: an early predictor of relapse or incomplete androgen suppression. Urology 2006;68:834-9.
15. Buchanang G, Irvine RA, Coetzee GA, Tilley WD. Contribution of the androgen receptor to prostate cancer predisposition and progression. Cancer Metastasis Rev 2001;20:207-23.
16. Steinke MP, O’Malony OA, Brogley M, Rehman H, Lapensee EW, Dhanakaran S, et al. Treatment-dependent androgen receptor mutations in prostate cancer exploit multiple mechanisms to evade therapy. Cancer Res 2009;69:4434-42.
17. 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;68:6407-15.
18. Heemers HV, Tindall DJ. Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. Endocr Rev 2007;28:778-808.
19. Zhu ML, Kyriianou N. Androgen receptor and growth factor signaling cross-talk in prostate cancer cells. Endocr Relat Cancer 2008;15:841-9.
20. Bergerat JP, Ceraloni J. Pleiotropic functional properties of androgen receptor mutants in prostate cancer. Hum Mutat 2009;30:145-57.
21. Claessens F, Denayer S, Van Tilborgh N, Kirkhofs S, Helsen C, Haelen A. Diverse roles of androgen receptor (AR) domains in AR-mediated signaling. Nucl Recept Signal 2008;6:6008.
22. Gelmann EP. Molecular biology of the androgen receptor. J Clin Oncol 2002;20:3001-15.
23. Heinlein CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev 2004;25:276-308.
24. Jenster G, van der Korput HA, van Vroonhoven C, van der KiswT, Trapani J, Brinkmann AO. Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. Mol Endocrinol 1991;5:1396-404.
25. Sinnotal JA, Sar M, Lane MV, French FS, Wilson EM. Transcriptional activation and nuclear targeting signals of the human androgen receptor. J Biol Chem 1991;266:510-8.
26. Jenster G, van der Korput HA, Trapani J, Brinkmann AO. Identification of two transcription activation units in the N-terminal domain of the human androgen receptor. J Biol Chem 1995;270:7341-6.
27. Callewaert L, Van Tilborgh N, Claessens F. Interplay between two hormone-independent activation domains in the androgen receptor. Cancer Res 2006;66:543-53.
28. Chamberlain NL, Whitacre DC, Miesfeld RL. Delineation of two distinct type I activation functions in the androgen receptor amino-terminal domain. J Biol Chem 1996;271:26772-8.
29. Dehm SM, Regan KM, Schmidt LJ, Tindall DJ. Selective role of an NH2-terminal WxxLF motif for aberrant androgen receptor activation in androgen-depletion-independent prostate cancer cells. Cancer Res 2007;67:10067-77.
30. Umesono K, Evans RM. Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell 1989;57:1139-46.
31. Ozanne DM, Brady ME, Cook S, Gaughan L, Neal DE, Robson CN. Androgen receptor nuclear translocation is facilitated by the factin cross-linking protein filamin. Mol Endocrinol 2000;14:1618-26.
32. Bedolla RG, Wang Y, Asuncion A, Chamie K, Siddiqui S, Mudryj MM, et al. Nuclear versus cytoplasmic localization of filamin A in prostate cancer: immunohistochemical correlation with metastases. Clin Cancer Res 2007;13:795-804.
33. Kaku N, Matsuda K, Tsuchiura A, Kawata M. Characterization of nuclear import of the domain-specific androgen receptor in association with the impotin alpha/beta and Ran-guanosine 5’-triphosphate systems. Endocrinology 2008;149:3960-9.
34. Heery DM, Kalkhooven E, Hoare S, Parker MG. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 1997;387:733-6.
35. Duff J, Davies PW, Watt K, McEwan I. Structural dynamics of the human androgen receptor: implications for prostate cancer and neurodegenerative disease. Biochem Soc Trans 2006;34:1098-102.
36. Buchanan G, Greenberg NM, Scher HI, Harris JM, Marshall VR, Tilley WD. Colocalization of androgen receptor gene mutations in prostate cancer. Clin Cancer Res 2001;17:1273-81.
37. Vissakori T, Hyytinien E, Koivistoo T, Marnin K, Raiman T, Palmberg C, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 1995;9:401-6.
38. Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer: N Engl J Med 1995;332:1393-8.
39. Taplin ME, Rajeshkumar B, Halabi S, Werner CP, Woda BA, Picus J, et al. Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663. J Clin Oncol 2003;21:2673-8.
40. Wilson JD. The role of 5 alpha-reduction in steroid hormone physiology. Reprod Fertil Dev 2001;13:673-8.
41. Schmidt LJ, Tindall DJ. Steroid 5 alpha-reductase inhibitors targeting BPH and prostate cancer. J Steroid Biochem Mol Biol 2011;115:32-8.
42. Zhou ZX, Lane MV, Kempainen JA, French FS, Wilson EM. Specificity of ligand-dependent androgen receptor stabilization: receptor domain interactions influence ligand dissociation and receptor stability. Mol Endocrinol 1995;9:208-18.
43. Wright AS, Thomas LN, Douglas RC, Lazier CB, Rittmaster RS. Relative potency of testosterone and dihydrotestosterone in preventing atrophy and apoptosis in the prostate of the castrated rat. J Clin Invest 1996;98:2558-63.
44. Smith DC, Toft DO. Minireview: the intersection of steroid receptors with molecular chaperones: observations and questions. Mol Endocrinol 2008;22:2229-40.
45. Loy CJ, Sim KS, Yong EL. Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions. Proc Natl Acad Sci U S A 2003;100:4562-7.
46. Wang Y, Krebsig JL, Bedolla RG, Mikhailova M, deVere-White RW, Ghosh PM. A 90 kDa fragment of filamin A promotes Casodex-induced growth inhibition in Casodex-resistant androgen receptor positive C4-2 prostate cancer cells. Oncogene 2007;26:606-10.
47. Castoria G, D'Amato L, Ciociola A, Giovannelli P, Giraldi T, Sepe L, et al. Androgen-induced cell migration: role of androgen receptor/filamin A association. PLoS One 2011;6:e17218.
48. He B, Kempainen LA, Wilson EM. FXXLF and WXXLF sequences mediate the NH2-terminal interaction with the ligand binding domain of the androgen receptor. J Biol Chem 2000;275:22986-94.
49. Chang CY, McDonnell DP. Evaluation of ligand-dependent changes in AR structure using peptide probes. Mol Endocrinol 2002;16:647-60.
50. van Royen ME, Cunha SM, Brink MC, Mastern KA, Nigg AL, Dubbink HJ, et al. Compartmentalization of androgen receptor protein–protein interactions in living cells. J Cell Biol 2007;177:63-72.
51. He B, Minges JT, Lee LW, Wilson EM. The FXXLF motif mediates androgen receptor-specific interactions with coregulators. J Biol Chem 2002;277:10226-35.
52. Dehm SM, Tindall DJ. Molecular regulation of androgen action in prostate cancer. J Cell Biochem 2006;99:333-44.
53. Agoulnik IU, Weigel NL. Coactivator selective regulation of androgen receptor activity. Steroids 2009;74:669-74.
54. Gregory CW, He B, Johnson RT, Ford OH, Mohler JL, French FS, et al. A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. Cancer Res 2001;61:4315-9.
55. Zhou HJ, Yan J, Luo W, Ayala G, Lin SH, Erdem H, et al. SRC-3 is required for prostate cancer cell proliferation and survival. Cancer Res 2005;65:7967-83.
56. Gnanapragasam VJ, Leung HY, Pulimood AS, Neal DE, Robson CN. Expression of RAC 3, a steroid hormone receptor co-activator in prostate cancer. Brit J Cancer 1995;71:1928-36.
57. Ma H, Hong H, Huang SM, Irvin RA, Webb P, Kushner PJ, et al. Multiple signal input and output domains of the 160-kilodalton nuclear receptor coactivator proteins. Mol Cell Biol 1999;19:6164-73.
58. Alen P, Claessen F, Verhoeven G, Rombauts W, Peeters B. The androgen receptor amino-terminal domain plays a key role in p160 coactivator-stimulated gene transcription. Mol Cell Biol 1999;19:6085-97.
59. Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Miesen CA, et al. Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 1997;389:194-8.
60. Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schuman I, Gilman H, et al. Role of CBP/P300 in nuclear receptor signalling. Nature 1996;383:99-103.
61. Debes JD, Sebo TJ, Lohse CM, Murphy LM, Haugen DA, Tindall DJ. p300 in prostate cancer proliferation and progression. Cancer Res 2003;63:7638-40.
62. Heemers HV, Sebo TJ, Debes JD, Regan KM, Raclaw KA, Murphy LM, et al. Androgen deprivation increases p300 expression in prostate cancer cells. Cancer Res 2007;67:3422-30.
63. Hu YC, Yeh SY, Sampson ER, Huang J, Li P, et al. Functional domain and motif analyses of androgen receptor coregulator ARA70 and its differential expression in prostate cancer. J Biol Chem 2004;279:33438-46.
64. Li PY, Xu X, Ge K, Melsmed J, Roeder RG, Wang Z. Heterogeneous expression and functions of androgen receptor co-factors in primary prostate cancer. Am J Pathol 2002;161:1467-74.
65. Chang HC, Chen SC, Chen J, Hsieh JT. In vitro gene expression changes of androgen receptor coactivators after hormone deprivation in an androgen-dependent prostate cancer cell line. J Formos Med Assoc 2005;104:652-8.
66. Edelstein D, Schnitt SJ, Visvanathan K, Harlow E. Androgen receptor coactivator in prostate cancer. Recent Results Cancer Res 2005;201:62-73.
67. van der Kwast TH, Schalken JA, Wijkstra CM, Mattern KA, van Vroonhoven OJ. Expression of Androgen receptor coregulators in prostate cancer. Cancer Invest 2005;23:1-11.
68. Lin J, Ma H, Hong H, Huang SM, Irvine RA, Webb P, Kushner PJ, et al. Androgen receptor coactivator selective regulation of androgen receptor activity. Cancer Res 2001;61:1686-92.
69. Vaaraa MH, Porvari KS, Kelloluoppa S, Kyllonen AP, Vilhko PT. Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. J Pathol 2001;193:134-40.
70. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644-8.
71. Lin B, Ferguson CW, White JT, Wang S, Vessella R, True LD, et al. Prostate-localized androgen-regulated expression of the membrane-bound serine protease TMPRSS2. Cancer Res 1999;59:4180-4.
72. Aflar DE, Vancocco I, Hubert RS, Kuo J, Chen E, Saffran DC, et al. Catalytic cleavage of the androgen-regulated TMPRSS2 protease results in its secretion by prostate and prostate cancer epithelial. Cancer Res 2001;61:1686-92.
73. Vaaraa MH, Porvari KS, Kelloluoppa S, Kyllonen AP, Vilhko PT. Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. J Pathol 2001;193:134-40.
74. Mehra R, Tomlins SA, Shen R, Nadeem O, Wang L, Wei JT, et al. Comprehensive assessment of TMPRSS2 and ETS family gene aberrations in clinically localized prostate cancer: Mod Pathol 2007;20:338-44.
75. Rajput AB, Miller MA, De Luca A, Boyd N, Leung S, Hurtado-Coll A, et al. Frequency of the TMPRSS2-ERG gene fusion is increased in moderate to poorly differentiated prostate cancers. J Clin Pathol 2007;60:1218-23.
76. Lin C, Yang L, Tanasa B, Hurst K, Ju BG, Ohgi K, et al. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. Cell 2009;139:1069-83.
77. Mani RS, Tomlins SA, Callahan K, Ghosh A, Nyati MK, Varambally S, et al. Induced chromosomal proximity and genes fusions in prostate cancer. Science 2009;326:1230.
78. Berger MF, Lawrence MS, Demechene F, Drier Y, Chibutski S, Sivachenko AY, et al. The genomic complexity of primary human prostate cancer. Nature 2011;470:214-20.
79. Bastus NC, Boyd LK, Mao X, Stankiewicz E, Kudahetti SC, Oliver RT, et al. Androgen-induced TMPRSS2:ERG fusion in nonmalignant prostate epithelial cells. Cancer Res 2009;70:654-48.
80. Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BC, Cao X, Morris DS, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 2007;448:595-9.
81. van der Kwast TH, Schalken JA, de Ruiter J, van Vroonhoven CC, Mulder E, Boersma W, et al. Androgen receptors in endocrine-therapy-resistant human prostate cancer. J Clin Cancer 1991;48:189-93.
82. Tilley WD, Lim-Tio SS, Horsfall DJ, Aspinall JO, Marshall VR, Skinner JM. Detection of discrete androgen receptor epitopes in prostate cancer by immunostaining: measurement by color video image analysis. Cancer Research 1994;54:4096-102.
83. Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BC, Cao X, Morris DS, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Science 2007;326:1230.
84. Berger MF, Lawrence MS, Demechene F, Drier Y, Chibutski S, Sivachenko AY, et al. The genomic complexity of primary human prostate cancer. Nature 2011;470:214-20.
126. Linja MJ, Savinainen KJ, Saramäki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 2001;61:3550-5.
125. Latli A, Bieche I, Vidua D, Lidereau R, Berthon P, Cussenot O, et al. Evaluation of androgen, estrogen (ER alpha and ER beta), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse transcription-polymerase chain reaction assays. Cancer Res 2001;61:1919-26.
124. Bubendorf L, Kononen J, Koivisto P, Schraml P, Moeh H, Gasser TC, Willi N, Mihtats M, Sauter G, Kallioniemi OP. Survey of gene amplifications during prostate cancer progression by high-throughput fluorescence in situ hybridization on tissue microarrays. Cancer Res 1999;59:803-806.
123. Edwards J, Krishna NS, Grigor KM, Bartlett JM. Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer. Br J Cancer 2003;89:552-6.
122. Cugil Z. Cytokine disbalance in common human cancers. Biochim Biophys Acta 2011;1813:308-14.
121. Cugil Z, Steiner H, Barttsch G, Hobisch A. Interleukin-6 regulation of prostate cancer cell growth. J Cell Biochem 2005;95:497-505.
120. Adler H, McCurdy MA, Kattan MW, Timme TL, Scardino PT, Thompson TC. Elevated levels of circulating interleukin-6 and transforming growth factor-beta 1 in patients with metastatic prostatic carcinoma. J Urol 1999;161:182-7.
119. Shariat SF, Andrews B, Kattan MW, Wilson C, Pettigrew J, Gallagher R, et al. Interleukin-8 signaling promotes androgen-independent proliferation of prostate cancer cells via induction of androgen receptor expression and activation. Carcinogenesis 2008;29:1148-56.
118. Araki S, Omori Y, Lyn D, Singh RK, Meinbach DM, Sandman Y, et al. Interleukin-8 is a molecular determinant of androgen independence and progression in prostate cancer. Cancer Res 2007;67:6854-62.
117. Singh RK, Lokeshwar BL. Depletion of intrinsic expression of Interleukin-8 in prostate cancer cells causes cell cycle arrest, spontaneous apoptosis and increases the efficacy of chemotherapeutic drugs. Mol Cancer 2009;8:57.
116. Trask AM, Morganental A. Epidural growth factor receptor expression escapes androgen regulation in prostate cancer: a potential molecular switch for tumour growth. Br J Cancer 2009;101:1949-56.
115. Di Lorenzo G, Tortora G, D’Armiento FP, De Rosa G, Staibano S, Autorino R, et al. Expression of epidermal growth factor receptor correlates with disease relapse and progression to androgen-independence in human prostate cancer. Clin Cancer Res 2002;8:3438-44.
114. Abreu-Martın MT, Chari A, Palladino AA, Craft NA, Sawyers CL. Mitogen-activated protein kinase kinase kinase 1 activates androgen receptor-dependent transcription and apoptosis in prostate cancer. Mol Cell Biol 1999;19:143-54.
113. Cugil Z, Hobisch A, Cronauer MV, Radmayer C, Trapman J, Hittmair A, et al. Androgen receptor activation in prostatic tumour cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor Cancer Res 1994;54:5474-8.
112. Liu Y, Karaca M, Zhang Z, Gieoli D, Earp HS, Whang YW. Dastatinib inhibits sited-specific tyrosine phosphorylation of androgen receptor by Ack1 and Src kinases. Oncogene 2010;29:3208-16.
111. Peterziel H, Mink S, Schonert A, Becker M, Klocker H, Cato AC. Rapid signalling by androgen receptor in prostate cancer cells. Oncogene 1999;18:6322-9.
110. Léotoing L, Manin M, Monté D, Baroum S, Communell Y, Lours C, et al. Crossstalk between androgen receptor and epidermal growth factor receptor-signalling pathways: a molecular switch for epithelial cell differentiation. J Mol Endocrinol 2007;39:151-62.
109. Sirotznik PM, She Y, Lee F, Chen J, Scher HI. Studies with CCR2b xenografts in nude mice suggest that ZD1839 may have a role in the treatment of both androgen-dependent and androgen-independent human prostate cancer. Clin Cancer Res 2002;8:3870-6.
108. Canil CM, Moore MJ, Winquist E, Baetz T, Pollak M, Chi KN, et al. Randomized phase II study of two doses of gefitinib in hormone-refractory prostate cancer: a trial of the National Cancer Institute of Canada-Clinical Trials Group. J Clin Oncol 2005;23:455-60.
107. Whang YE, Armstrong AJ, Rathmell WK, Godley PA, Kim WY, Pruthi RS, et al. Phase II study of lapatinib, a dual EGFR and HER-2 tyrosine kinase inhibitor, in patients with castration-resistant prostate cancer. Urol Oncol 2011;29:40-4.
106. Chau JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 1998;279:563-6.
105. Chen C, Lewis SK, Voigt L, Fitzpatrick A, Plymyer SR, Weiss NS. Prostate carcinoma incidence in relation to prediagnostic circulating levels of insulin-like growth factor I, insulin-like growth factor binding protein 3, and insulin. Cancer 2005;103:76-84.
104. Harman SM, Metter EJ, Blackman MR, Landis PK, Carter HB. Serum levels of insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-3, and prostate-specific antigen as predictors of clinical prostate cancer. J Clin Endocrinol Metab 2000;85:4258-65.
103. Wu JD, Haugk K, Woodle N, Nelson P, Coleman I, Plymyer SR. Interaction of IGF signaling and the androgen receptor in prostate cancer progression. J Cell Biochem 2006;99:392-401.
102. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature 2001;411:355-63.
101. Guo Z, Dai B, Jiang T, Xu K, Xie Y, Kim O, et al. Regulation of androgen receptor activity by tyrosine phosphorylation. Cancer Cell 2006;10:309-19.
100. Kraus S, Gieoli D, Vomastek T, Gordon V, Webster MJ. Receptor for activated C kinase 1 (RACK1) and Src regulate the tyrosine phosphorylation and function of the androgen receptor. Cancer Res 2006;66:11047-54.
99. Asim M, Siddiqui IA, Hafeez BB, Banniahmad A, Mukhtar H. Src kinase potentiates androgen receptor transactivation function and invasion
of androgen-independent prostate cancer C4-2 cells. Oncogene 2008;27:3596-604.

129. Giocoli D, Mandell JW, Petroni GR, Frierson HF Jr, Weber MJ. Activation of mitogen-activated protein kinase associated with prostate cancer progression. Cancer Res 1999;59:279-84.

130. Agoulnik IU, Bingman WE 3rd, Nakka M, Li W, Wang Q, Liu XS, et al. Target gene-specific regulation of androgen receptor activity by p42/p44 mitogen-activated protein kinase. Mol Endocrinol 2008;22:2420-32.

131. Lyons LS, Burnstein KL. Var3, a Rho GTPase guanine nucleotide exchange factor, increases during progression to androgen independence in prostate cancer cells and potentiates androgen receptor transcriptional activity. Mol Endocrinol 2006;20:1061-72.

132. Lyons LS, Rao S, Balkan W, Faysal J, Maiorino CA, Burnstein KL. Ligand-independent activation of androgen receptors by Rho GTPase signaling in prostate cancer. Mol Endocrinol 2008;22:597-608.

133. Sarker D, Reid AH, Yap TA, de Bono JS. Targeting the PI3K/AKT pathway for the treatment of prostate cancer. Clin Cancer Res 2009;15:4799-805.

134. 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;9:1474-9.

135. Bertram J, Peacock JW, Fazli L, Mui AL, Chung SW, Cox ME, et al. Loss of PTEN is associated with progression to androgen independence. Prostate 2006;66:895-902.

136. McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. Cancer Res 1999;59:4291-6.

137. Ayala G, Thompson T, Yang G, Froluv A, Li R, Scardino P, et al. High levels of phosphorylated form of Akt-1 in prostate cancer and non-neoplastic prostate tissues are strong predictors of biochemical recurrence. Clin Cancer Res 2004;10:6572-8.

138. Kreisberg JJ, Malik SN, Prihoda TJ, Bedolla RG, Trower DA, Kreisberg S, et al. Phosphorylation of Akt (Ser-473) is an excellent predictor of poor clinical outcome in prostate cancer. Cancer Res 2004;64:5232-6.

139. Wang S, Gao J, Li Q, Rozengurt N, Pritchard C, Jiao J, et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 2003;4:209-21.

140. King JC, Xu J, Wongvipat J, Hieronymus H, Carver BS, Leung DH, et al. Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis. Nat Genet 2009;41:524-6.

141. Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, et al. aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nat Genet 2009;41:16-9.

142. Chen CD, Sawyers CL. NF-kappaB activates prostate-specific antigen expression and is upregulated in androgen-independent prostate cancer. Mol Cell Biol 2002;22:2862-70.

143. Imsl HA, Lessard L, Mes-Masson AM, Saad F. Expression of NF-kappaB in prostate cancer lymph node metastases. Prostate 2004;58:308-13.

144. Lessard L, Benin LR, Gleave ME, Mes-Masson AM, Saad F. Nuclear localization of nuclear factor-kappaB transcription factors in prostate cancer: an immunohistochemical study. Br J Cancer 2005;93:1019-23.

145. Jin RJ, Lho Y, Connelly L, Wang Y, Xu X, Saint Jean L, et al. The nuclear factor-kappaB pathway controls the progression of prostate cancer to androgen-independent growth. Cancer Res 2008;68:6762-9.

146. Nadiminty N, Lou W, Sun M, Chen J, Yue J, Kung HJ, et al. Aberrant activation of the androgen receptor by NF-kappaB in prostate cancer cells. Cancer Res 2010;70:3309-19.

147. Nazareth LV, Weigel NL. Activation of the human androgen receptor through a protein kinase A signaling pathway. J Biol Chem 1996;271:19900-7.

148. Sadar MD. Androgen-independent induction of prostate-specific antigen gene expression via cross-talk between the androgen receptor and protein kinase A signal transduction pathways. J Biol Chem 1999;274:7777-83.

149. Buschhorn HM, Klein RR, Chambers SM, Hardy MC, Green S, Beards D, et al. Aurora-A overexpression in high-grade PIN lesions and prostate cancer. Prostate 2005;64:341-6.

150. Lee EC, Frolov A, Li R, Ayala G, Greenberg NM. Targeting Aurora kinases for the treatment of prostate cancer. Cancer Res 2006;66:4996-5002.

151. Shu SK, Liu Q, Coppola D, Cheng JQ. Phosphorylation and activation of androgen receptor by Aurora-A. J Biol Chem 2010;283:33045-53.

152. Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghoish D, Zhou M, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. Cancer Res 2004;64:9209-16.

153. 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;324:787-90.

154. Scher HI, Beer TM, Higano CS, Anand A, Taplin ME, Efstathiou E, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. Lancet 2010;375:1437-46.

155. Andersen RJ, Mawji NR, Wang J, Wang G, Haile S, Myung JK, et al. Regulation of castrate-resistant prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. Cancer Cell 2010;17:535-46.