Reviews

Molecular Alterations during Progression of Prostate Cancer to Androgen Independence

Punit Saraon,[1,2] Keith Jarvi,[1,3] and Eleftherios P. Diamandis[1,2,3,4*]

BACKGROUND: Prostate cancer is the most commonly diagnosed cancer among men in North America and is a leading cause of death. Standard treatments include androgen deprivation therapy, which leads to improved clinical outcomes. However, over time, most tumors become androgen independent and no longer respond to hormonal therapies. Several mechanisms have been implicated in the progression of prostate cancer to androgen independence.

CONTENT: Most tumors that have become androgen independent still rely on androgen receptor (AR) signaling. Mechanisms that enhance AR signaling in androgen-depleted conditions include: AR gene amplification, AR mutations, changes in the balance of AR cofactors, increases in steroidogenic precursors, and activation via "outlaw" pathways. Along with AR signaling, various other AR-independent "bypass" pathways have been shown to operate aberrantly during androgen independence. Changes in the epigenetic signatures and microRNA concentrations have also been implicated in the development of androgen-independent prostate cancer.

SUMMARY: Understanding of the molecular mechanisms that lead to the development of androgen-independent prostate cancer will allow for improved therapeutic strategies that target key pathways and molecules that are essential for these cells to survive.

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Prostate cancer is the most commonly diagnosed cancer in men and is the second leading cause of death due to cancer in men (1). Almost all prostate cancer cells, as well as normal prostate tissues, require androgens for growth and survival. Localized cancers are usually treated with radical prostatectomy or radiation. For more advanced cancers that have either recurred or metastasized, the gold standard treatment is androgen ablation therapy (2). Although very efficient at reducing cancer growth and volume, this treatment eventually selects for cells that are no longer responsive to such therapy, resulting in a recurring lethal cancer termed androgen-independent prostate cancer (AIPC)[5] (3). In advanced-stage prostate cancer, hormone therapy is no longer effective because cancerous cells have gained the ability to grow in the absence of androgens, and this is the stage at which most patients develop AIPC. Although androgen independence occurs through a gradual process, most cancers will become androgen independent because these cancer cells transform themselves in a manner that promotes their growth in the absence of key survival factors such as androgens. Interestingly, the term AIPC is becoming less widely accepted because these aggressive forms of the cancer still rely on androgen signaling (4). Instead, hormone refractory prostate cancer (HRPC) is becoming a more widely accepted term.

The most prominent player in the progression of AIPC is the androgen receptor (AR), a protein that binds to androgens and acts as a transcription factor to regulate a wide array of genes involved in various processes, including proliferation and growth (5–7). The role of the AR in prostate cancer progression has been investigated in many studies; however, the precise molecular mechanisms that occur in the progression to androgen independence remain largely unknown, and as a result, there are no effective therapies against AIPC.

In this review, we focus on current and prior work on deciphering the mechanisms of androgen independence in prostate cancer (8–10). We discuss AR signaling and other pathways that are involved in the evolution and progression of prostate cancer, as well as other

[1] Samuel Lunenfeld Research Institute and Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada; [2] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; [3] Department of Surgery (Division of Urology), Mount Sinai Hospital, Toronto, ON, Canada; [4] Department of Clinical Biochemistry, University Health Network, Toronto, ON, Canada.

* Address correspondence to this author at: Mount Sinai Hospital, Joseph and Wolf Lebovic Complex, 60 Murray St., Box 32, Flr 6–Rm L6–201, Toronto, ON, M5T 3L9, Canada. Fax 416-619-5521; e-mail ediamandis@mtsinai.on.ca.
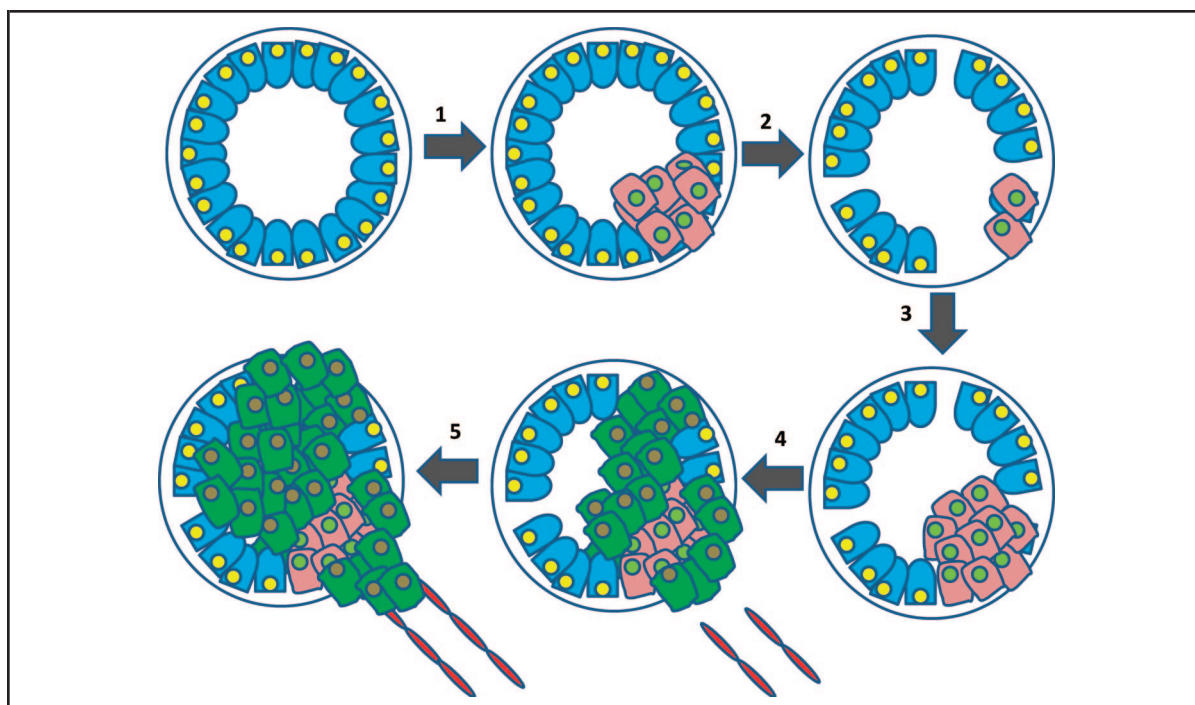
Fig. 1. Progression to androgen independence in prostate cancers.

1. Various carcinogenic processes occur whereby some prostate cells proliferate out of control. 2. Prostate cancer cells are initially androgen dependent; therefore, androgen-deprivation therapy is successful in destroying these cancer cells. 3. Some cells are able to survive this treatment and continue proliferating. 4. Cells are now androgen independent and gain subsequent changes resulting in increased angiogenesis. 5. AIPC begins to metastasize to distant sites.

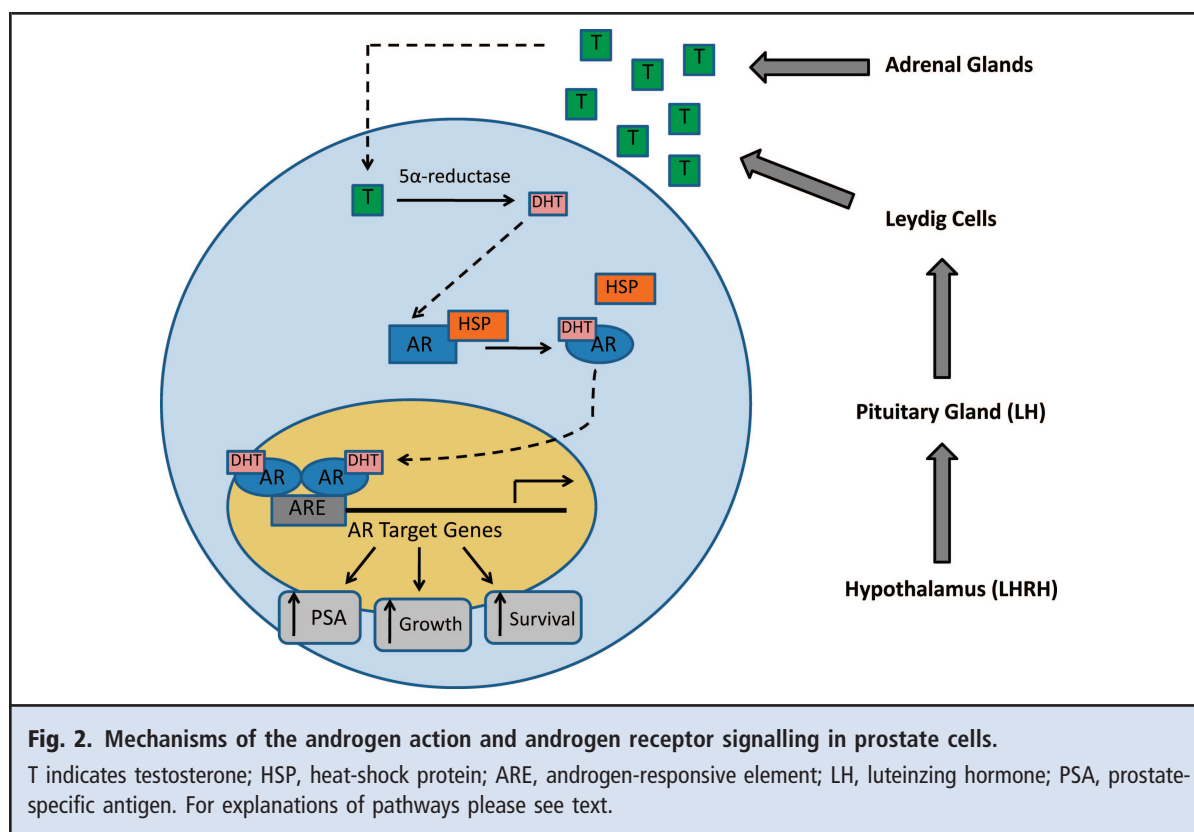factors, such as epigenetic alterations and microRNA (miRNA) regulation.

Progression Model of Prostate Cancer

A normal prostate requires basal concentrations of androgen for growth and survival. Likewise, during prostate cancer development, the cancerous cells are initially dependant on androgens. A simplified model of prostate cancer progression is illustrated in Fig. 1. Initially, multiple carcinogenic processes occur, whereby some cells are altered and begin to proliferate out of control. If the cancer is detected early, androgen ablation can be used for therapy either via chemical castration by use of antiandrogens, or surgical removal of the testicles, the major producers of androgens. This therapy is very effective in the destruction of androgen-dependent cells. However, over time, this continuous androgen ablation results in the selection of cell subpopulations that can survive in the absence of androgens, leading to an androgen-independent cancer. Further evolution of these androgen-independent cells can result in increased angiogenesis, whereby the cells metastasize and migrate to distant sites, primarily the bone and lymph nodes. The specific molecular alterations that govern these changes still remain unknown, but one thing remains clear: the AR is a major player involved in this process.

Androgen Receptor Signaling: A Key Regulator of Prostate Cancer Progression

Testosterone, the main androgen produced by the body, is predominantly released by the Leydig cells of the testes *(11)*. Small amounts are also produced in the adrenal glands. Free circulating testosterone, which is not bound to steroid hormone binding protein, can enter prostate cells, where it can be converted to its more potent metabolite, dihydrotestosterone (DHT), which in turn binds to the AR protein (Fig. 2) *(11)*. Testosterone itself can bind to the AR as well, but DHT has a much higher affinity *(11)*. The AR is a nuclear transcription factor that can activate and regulate the expression of many genes involved in growth and proliferation. The AR protein consists of 3 domains: DNA-binding domain, ligand-binding domain, and N-terminal domain. Inhibitory studies of the N-terminal domain resulted in decreased AR transcriptional activity, demonstrating

Fig. 2. Mechanisms of the androgen action and androgen receptor signalling in prostate cells.
T indicates testosterone; HSP, heat-shock protein; ARE, androgen-responsive element; LH, luteinzing hormone; PSA, prostate-specific antigen. For explanations of pathways please see text.

that this domain plays a crucial role in the transcriptional transactivation activity of the AR *(12)*. The ligand-binding domain, where androgens bind, results in a conformational change in the AR, where it dissociates from heat shock proteins in the cytoplasm, and localizes to the nucleus *(13)*. In the nucleus, the AR binds to specific DNA sequences, called androgen responsive elements, via the DNA-binding domain, promoting further association of factors into a complex, which leads to gene transcription *(14)*. Various genes are regulated by the AR, including kallikrein-related peptidase 3 (*KLK3*),[6] which encodes kallikrein-3 (formerly known as prostate-specific antigen), one of the best cancer biomarkers available.
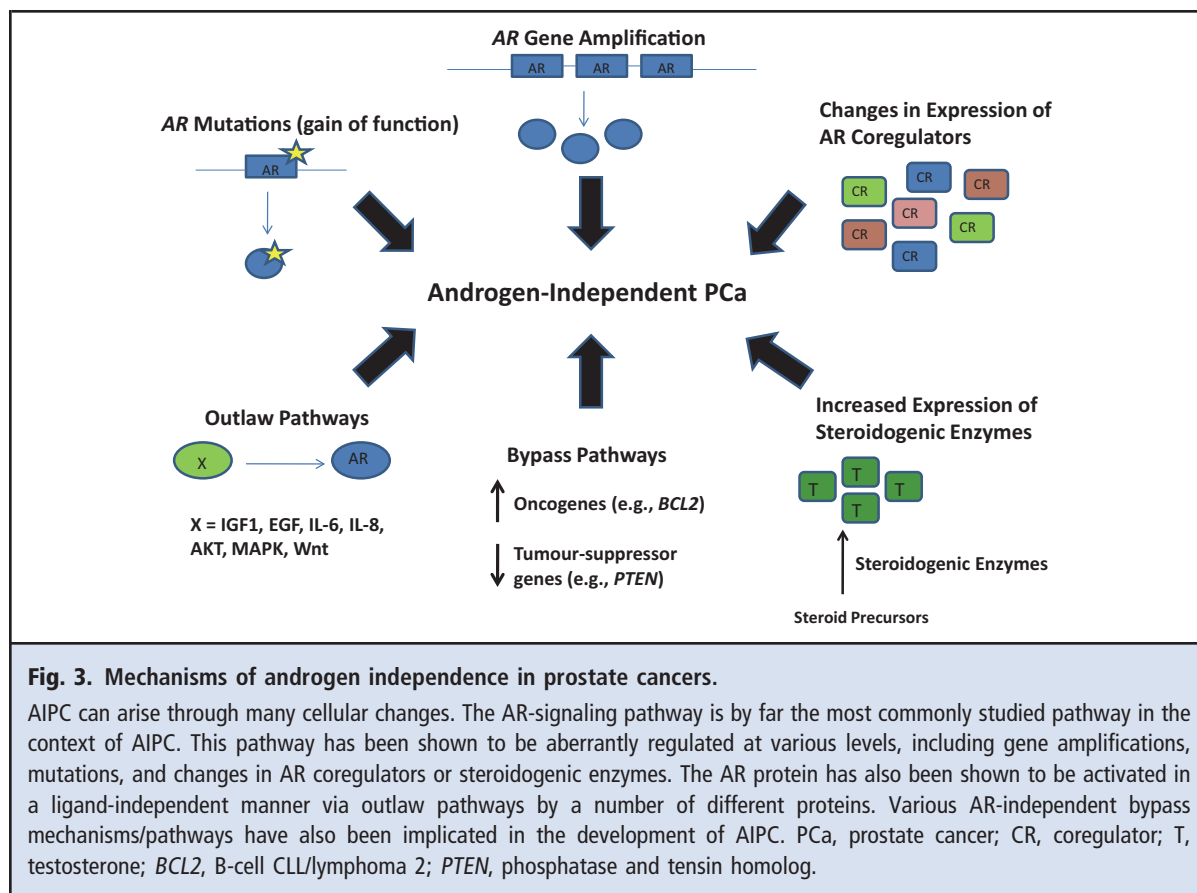
As alluded to earlier, most prostate cancers begin in an androgen-dependent state, in which androgens are required for the AR to be activated and regulate its downstream effectors. However, during androgen deprivation, the AR is not activated as prominently, which results in decreased growth and proliferation of cancerous cells *(15)*. The AR, as well as factors of the AR signaling pathway, have been found to be aberrantly expressed or mutated in many prostate cancers. This finding has led to speculation that these proteins are key players in prostate cancer progression *(8, 16–19)*. Many models have been proposed for alteration of this signaling cascade, including amplification and mutation of the androgen receptor (*AR*) gene, changes in AR coregulator concentrations, alterations in steroidogenic pathways, and activation in a ligand-independent manner via alternative "outlaw" pathways (Fig. 3).

AR GENE AMPLIFICATIONS

AR overexpression has been implicated in many AIPC cases, both in vitro and in vivo. Together, gene and protein expression data show that the AR is overexpressed at the mRNA and protein levels, respectively *(20–22)*. Studies have revealed that approximately 25%–30% of androgen-independent tumors have AR amplifications *(22)*. Interestingly, AR amplification has not been found in any untreated prostate cancer samples, suggesting that AR amplification is one by-

[6] Human genes: *KLK3*, kallikrein-related peptidase 3; *AR*, androgen receptor; *CYP17A1*, cytochrome P450, family 17, subfamily A, polypeptide 1; *CDKN2A*, cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4); *CDH1*, cadherin 1, type 1, E-cadherin (epithelial); *CD44*, CD44 molecule (Indian blood group); *MGMT*, O-6-methylguanine-DNA methyltransferase; *RASSF1A*, Ras association (RalGDS/AF-6) domain family member 1; *ABCB1*, ATP-binding cassette, sub-family B (MDR/TAP), member 1; *APC*, adenomatous polyposis coli; *GSTP1*, glutathione S-transferase pi 1; *BCL2*, B-cell CLL/lymphoma 2; *PTEN*, phosphatase and tensin homolog.

Fig. 3. Mechanisms of androgen independence in prostate cancers.

AIPC can arise through many cellular changes. The AR-signaling pathway is by far the most commonly studied pathway in the context of AIPC. This pathway has been shown to be aberrantly regulated at various levels, including gene amplifications, mutations, and changes in AR coregulators or steroidogenic enzymes. The AR protein has also been shown to be activated in a ligand-independent manner via outlaw pathways by a number of different proteins. Various AR-independent bypass mechanisms/pathways have also been implicated in the development of AIPC. PCa, prostate cancer; CR, coregulator; T, testosterone; *BCL2*, B-cell CLL/lymphoma 2; *PTEN*, phosphatase and tensin homolog.

product of hormone therapy leading to AIPC. Gene amplifications of the AR loci have also been found in many clinical prostate cancer samples that were in an androgen-independent state, indicating that gene amplification may lead to AR protein overexpression, and subsequently to increased AR signaling. Recently, it was found that increased AR expression sensitized prostate cancer cells to lower-than-normal concentrations of androgens *(23)*. High AR protein expression was found to help cancer cells survive and continue proliferating in environments with minimal androgen concentrations, a finding that may explain the evolution of AIPC during androgen deprivation *(23)*. Furthermore, AR overexpression at the mRNA and protein level has also been observed in the absence of *AR* gene amplification, which suggests the existence of gene amplification–independent regulators such as epigenetic and miRNA factors *(14)*. It appears that tumors have selective pressures for continued AR signaling to allow for survival and further evolution, and therefore therapies that are more efficient at blocking this crucial signaling pathway are potentially promising approaches to prevent cancer progression.

AR MUTATIONS

AR mutations are another means for prostate cancer cells to gain androgen-independent properties. The *AR* gene is located on the X chromosome, and a loss of function of the gene results in androgen-insensitivity syndrome. The frequencies of genetic mutations in the AR loci are typically rare in early stage prostate tumors (0%–4%) *(24)*, but are more frequent in advanced and recurrent tumors *(25)*. *AR* mutations have been reported in 10%–20% of patients with androgen-independent tumors, strengthening the model that particular mutations in the *AR* gene help cells to survive and proliferate in androgen-deprived conditions *(25)*. The McGill androgen receptor gene mutation database (http://androgendb.mcgill.ca/) website provides an extensive list of all reported *AR* mutations, as well as the specific domains within the protein that have been altered. In this review we focus only on the most common *AR* mutations present in the context of AIDP.

The first reported *AR* mutation was described in the hormone-dependent LNCaP human prostate cancer cell line derived from lymph node metastasis *(26)*. This cell line contains a missense mutation at codon

877 of the *AR* mRNA, resulting in an amino acid substitution of threonine to alanine *(26)*. This mutation occurs in the ligand-binding domain, and results in decreased ligand specificity, whereby other hormones such as progesterone, estrogens, and many antiandrogens can also activate the protein. During androgen ablation, such a mutation becomes beneficial for the cancer cells because it broadens the specificity of the AR's reliance on androgens for activation, leading to growth advantage and survival over cells that lack this mutation. In an early study, Gaddipati et al. *(27)* found that 6 of 24 metastatic tumor samples harbored this T877A mutation, indicating that the mutation is commonly found in patients with AIPC. Furthermore, the frequency and nature of *AR* mutations in prostate cancers appears to depend on the stage and selective pressures exerted on the cancers. Localized cancers appear to have less frequent mutations, whereas tumors that have metastasized have an increased incidence of *AR* mutations *(25)*. Results of a study by Marcelli et al. showed that point mutations were found in 8 of 38 patients with lymph node metastasis who were treated with hormone therapy, whereas no mutations were found in 99 prostatectomy-removed glands of patients who received no hormone therapy *(28)*.

As is the case with the H877A mutation, most of the clinically relevant *AR* mutations are found in the ligand-binding domain of the protein, resulting in either broadened ligand specificity or constitutive protein activity. These mutations also include H874Y, V715M, L701H+T877A, and Y741C *(25, 29–31)*. Loss-of-function mutations of the *AR* have also been observed in a small number of patients. Although loss-of-function mutations are poorly understood, it is speculated that these mutations can confer growth advantage to cells that have already surpassed the need for androgens.

AR splice variants have also been identified recently, and were found to be overexpressed in AIPC. Guo et al. discovered 3 novel variants (AR3, AR4, and AR5) in AIPC, all lacking the ligand-binding domain *(32)*. Although studies on these variants are still premature, they nevertheless present a very interesting manner through which cancer cells can survive androgen ablation. Cells harboring these variants, which do not have a ligand-binding domain, can bypass the need for androgens, because the AR can become constitutively active. These isoforms are not inhibited by currently available antiandrogens, so the development of new drugs targeting these isoforms may provide another effective treatment for AIPC.

CHANGES IN EXPRESSION OF AR COREGULATORS

The AR is a nuclear transcription factor that relies on many interactions with various coregulatory proteins to assemble a productive transcriptional complex. These coregulators can either enhance (coactivators) or reduce (corepressors) AR transactivation, resulting in altered transcription rates. To date, approximately 170 proteins have been identified as AR coregulators *(33)*. Alterations in the balance of these coregulatory proteins can perhaps provide growth advantages to prostate cancer cells; thus, these coregulators are becoming very interesting targets for therapeutic intervention. Given the sheer number of AR coregulator proteins that have been identified, it is not plausible to discuss each one. Therefore, we focus on some of the main coregulators that have been thoroughly studied in the context of AIPC.

Coactivators. AR coactivators can be classified into distinct groups. SRC/p160 coactivators such as TIF2, GRIP1, and SRC1 share common structural components and are able to recruit other transcription factors to initiate transactivation of AR-regulated genes *(34)*. These coactivators are also able to recruit other coactivators that contain histone acetyl-transferase activity such as Tip60, CBP/p300, and p/CAF, which are involved in the acetylation of specific histone residues resulting in chromatin remodeling *(35)*. Gregory et al. found that concentrations of TIF2 and SCR1 increased in AIPC samples that had increased AR expression *(36)*. In another study, Tip60 was also found to be overexpressed in AIPC *(37)*. Another broad group of AR-associated (ARA) proteins have been described as being potent AR coactivators. These ARA proteins (ARA24, ARA 54, ARA55, ARA70, ARA170, and ARA267) do not share structural or functional similarities, but are all potent coactivators of the AR *(38)*.

AR corepressors. Unlike coactivators, AR corepressor proteins can form complexes with AR and inhibit the transcription of AR-regulated genes. Any alterations in the expression of these corepressor proteins can also play an important role in the development of AIPC. Two well-characterized AR corepressors are NCoR (nuclear receptor corepressor) and its homolog SMRT (silencing mediator for retinoid and thyroid hormone receptors) *(39)*. Both of these corepressors can recruit histone deacetylases, which promote chromatin packing, resulting in reduced transcriptional activity.

During androgen deprivation, changes in the concentrations of these coactivators and corepressors can promote the expression of androgen-regulated genes *(14–17)*. Increases in the expression of various coactivators, or decreases in expression of corepressors, can provide a means for increased AR activity, promoting prostate cancer cell survival in androgen-deprived conditions.

INCREASED STEROIDOGENIC SIGNALING PATHWAYS

As has become apparent, androgens are the mediators for AR activation. Following androgen binding to the AR, a conformational change occurs whereby the AR protein dissociates from heat-shock protein in the cytosol, translocating to the nucleus. Therefore, any alterations in the production of these androgens or their precursors could have an effect on the development of AIPC.

Although luteinzing-hormone–releasing hormone (LHRH) agonists are effective at blocking androgen production by the testes at concentrations much higher than the upper limit of the reference interval, the adrenal glands are also capable of producing circulating androgens, albeit at much lower concentrations. Along with endogenous production from the adrenal glands, recent studies have shown that de novo synthesis of androgens within the tumor may also play a critical role in maintaining concentrations that can activate AR (18). Furthermore, overexpression of enzymes involved in the steroid biosynthetic pathway has been observed in AIPC samples (18).

In the prostate, free testosterone is converted to its more active metabolite, DHT, and this reaction is catalyzed by the enzymes 5α-reductase types 1 and 2. Results of comparative analysis of testosterone and DHT in the serum and prostate tumor samples showed higher DHT concentrations in the tumors than the serum, suggesting that the cancer cells might be upregulating the 5α-reductase enzymes to provide sufficient DHT for AR signaling (40). By quantifying the transcripts encoding enzymes involved in steroid biosynthesis, Montgomery et al. aimed to determine whether prostate cancer metastases were capable of synthesizing androgen de novo (41). They found that castration-resistant metastases expressed higher levels of many enzymes responsible for the synthesis of adrenal androgens. They also observed high concentrations of intratumoral androgens in castrate-resistant xenografts lacking the adrenal cytochrome P450, family 17, subfamily A, polypeptide 1 (*CYP17A1*) gene, which is critical for the production of androgens by the adrenal glands.

More recently, Hofland et al. conducted a study showing that intratumoral steroid biosynthesis contributes less androgen to the tumors than circulating adrenal androgens, suggesting the blockade of *CYP17* as an interesting therapeutic target in AIPC patients (42). Further studies using abiraterone acetate, a potent inhibitor of *CYP17*, have also been shown to have a positive effect on survival.

OUTLAW PATHWAYS

So far we have discussed the various ways AR signaling can be altered in the presence of its androgen ligands. However, various steroid hormone receptors, including AR, can also be activated through ligand-independent mechanisms referred to as outlaw pathways. The AR also has nongenomic roles where it can interact with various other signaling pathways and alter them. Many growth factors, cytokines, kinases and other proteins have been demonstrated to activate the AR at low or even near-zero concentrations of androgens.

Growth factor–induced activation. Growth factors such as insulin-like growth factor-1 (IGF1), keratinocyte growth factor, and epidermal growth factor (EGF) can activate the AR, allowing the induction of the transactivation of AR target genes in low androgen conditions (29). IGF1 has been the most widely studied of the growth factors, and has been shown to potentiate the signaling of AR, even in the absence of androgens. Interestingly, when prostate cancer cells are subjected to antiandrogen treatment, IGF1 is no longer able to activate the AR, suggesting a direct interaction between these proteins (29). IGF1 has also been shown to promote AR signaling by increasing the expression of various AR coactivators such as TIF2 and insulin-degrading enzyme (43). Likewise, EGF signaling activates the AR in a ligand-independent manner and induces the transcription of AR-regulated genes (29). Recent studies have shown that the EGF-regulated protein SPINK1/TATI is overexpressed in aggressive prostate cancers, specifically in ETS-rearrangement–negative cancers (44, 45). These growth factors are all ligands for receptor tyrosine kinases (RTK), which illustrates the importance of RTK signaling in prostate cancer progression.

Cytokine activation. Along with growth factors, various cytokines can activate the AR. The nuclear factor (NF)-κB signaling pathway, which has been shown to be upregulated in many AIDC, regulates the cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8). Increased NF-κB signaling results in increased AR activation in LNCaP cells, and AR activation is inhibited by blocking this signaling pathway (46). Likewise, IL-6 and IL-8 are known to stimulate AR activity and enhance the expression of AR-regulated genes and, much like IGF1, when cells are treated with antiandrogens, AR signaling is inhibited even in the presence of these cytokines, suggesting a direct interaction.

RTK-induced activation. RTKs are important signaling molecules that are aberrantly expressed in a wide variety of pathologies, especially cancers. One of the major RTKs that has been implicated with AIPC is HER-2/*neu* (also known as ERBB2). HER-2/*neu* has been shown to be overexpressed in androgen-dependent cell lines that have been converted to androgen-

independent cell lines, as well as sublines that have been xenografted into castrated mice (47, 48). Overexpression of HER-2/*neu* in prostate cancer cells can activate AR-induced genes in the absence of androgens, but unlike IGF1, IL-6, and IL-8, treatment with antiandrogens does not block the AR-signaling pathway, indicating that this pathway is independent of the AR ligand-binding domain (47). Along with HER-2/*neu*, other RTK pathways that have been implicated in AR signaling activation are IGF receptor and EGF receptor, the receptors for IGF1 and EGF, respectively. These receptors are known to induce downstream activation of essential growth and survival pathways, including AKT, mitogen-activated protein kinase (MAPK), and STAT (signal transducer and activator of transcription) pathways, many of which are also aberrantly expressed during AIPC.

Wnt-signaling activation. The canonical Wnt-signaling pathway has also been implicated with AIPC because β-catenin, a main downstream effector in this pathway, has been shown to interact with the AR and modulate its function (49, 50). Interestingly, during AIPC, Wnt has been shown to activate the AR, which can then act as coactivator for β-catenin activation of Wnt-related genes as well as AR-induced genes. Studies in vivo have also shown that β-catenin and AR both co-localize and interact in castrate-resistant tumors in mice, but surprisingly not in tumors harvested from noncastrated mice (51). Taken together, current data indicate that Wnt/AR crosstalk is another important interaction that can promote AIPC in a ligand-independent manner.

Non–AR-Related Pathways: Bypass Pathways

The mechanisms discussed so far are dependent on AR and its signaling cascade for the development of AIPC. However, alternative pathways can also be involved in the progression to AIPC, regardless of AR signaling. These pathways have been deemed "bypass" pathways, because they are defined as pathways that are completely independent of AR signaling. It is interesting to note that many of the outlaw pathways we mentioned previously, which activate AR signaling in a ligand-independent manner, can also act as bypass pathways as well. Growth factors such as IGF1 and their respective tyrosine kinase receptors can activate a signal transduction cascade, inducing the expression of various genes responsible for promoting cell growth and survival. Many of these pathways activate critical kinases such as MAPK/Ras/Raf/protein kinase C, which influence cell-cycle regulation and increase cell proliferation by activating various transcription factors such as AP1, c-MYC, and NF-κB (52). For example, Weber

et al. have demonstrated that prostate cancer specimens recurring in castrated mice have increased MAPK expression and activity (53).

The Akt signaling cascade is also important for the development of AIPC in an AR-independent manner. As mentioned previously, Akt signaling can activate AR signaling via outlaw mechanisms; however, it also has key roles in the control of apoptosis and proliferation in prostate cancer cells (54). Interestingly, PTEN, a proapoptotic protein that inhibits the Akt signaling cascade, is frequently found to be mutated or functionally inactive in AIPC, further demonstrating the importance of Akt signaling in cancer cell survival (55). Akt signaling can also alter cell cycle regulation by decreasing protein expression of p27, an important cell-cycle regulator (56).

During androgen deprivation, apoptotic pathways are induced, indicating that antiapoptotic factors become an important way to circumvent programmed cell death due to the absence of AR signaling. Bcl-2 is a critical antiapoptotic protein that can help prostate cancer cells block apoptosis. In normal prostatic epithelial cells, Bcl-2 is not normally expressed. It is expressed, however, during prostatic intraepithelial neoplasia as well as AIPC (57). Liu et al. were able to detect Bcl-2–expressing prostate cancer cells in xenografts in castrated mice, and when these investigators blocked Bcl-2 using siRNA in LNCaP cells, they observed a delay in the onset of AIPC in xenograft models (58).

Both bypass and outlaw pathways have been shown to interact with one another, because they can induce AR signaling as well as other pathways. Further investigation is needed, however, to determine the exact molecular mechanisms of this occurrence. Upregulation of oncogenes such as Bcl-2 and decreases in tumor-suppressor genes can play prominent bypass roles in the development of AIPC.

Epigenetic Alterations

Epigenetic regulation plays a critical role in normal cellular development, so it is not surprising that many genes have aberrant epigenetic signatures during cancer development. A number of epigenetic alterations, the most prominent being methylation and histone modification, have been reported in prostate cancer progression and specifically AIPC. Unfortunately, a discussion of each alteration is beyond the scope of this review. Thus, we focus on the most relevant epigenetic alterations that occur during AIPC.

Hypermethylation has been linked to the AR gene in a subset of metastatic and AIPC cancers. We have reported that AR overexpression is a mechanism by which AIPC compensates for decreased androgen concentrations; however, 20%–30% of AIPC, including

the 2 highly studied cell lines DU145 and PC3, do not express AR *(59)*. The loss of AR is due to promoter hypermethylation, and it has been observed to be more prevalent in AIDP tumors than primary androgen-dependent cancers *(60)*. Gene silencing of the AR via DNA methylation suggests an alternative mechanism leading to androgen independence in a subset of patients with advanced prostate cancer.

Furthermore, epigenetic modulation of various genes involved in cell cycle control, cell invasion, cellular architecture, DNA damage repair, tumor suppressors, and oncogenes has been shown in many prostate cancer samples and cell lines *(61, 62)*. The major genes that have been shown to be epigenetically altered in prostate cancers include cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4) *(CDKN2A)*, cadherin 1, type 1, E-cadherin (epithelial) *(CDH1)*, CD44 molecule (Indian blood group) *(CD44)*, O-6-methylguanine-DNA methyltransferase *(MGMT)*, Ras association (RalGDS/AF-6) domain family member 1 *(RASSF1A)*, ATP-binding cassette, sub-family B (MDR/TAP), member 1 *(ABCB1)*, adenomatous polyposis coli *(APC)*, and glutathione S-transferase pi 1 *(GSTP1)*. *GSTP1* promoter methylation is the most frequently detected epigenetic alteration, with a frequency of 70%–100% in prostate cancer DNA samples *(63)*. Methylation of this gene has been detected in prostate cancer cells and in DNA isolated from blood, plasma, or serum; prostate secretions; voided urine; and prostate biopsy specimens *(61, 62)*. However, further investigation must be conducted in this field, because it shows promise as a way to identify aberrantly expressed genes involved in prostate cancer progression and to provide potential useful clinical biomarkers of aggressiveness based on specific epigenetic signatures.

miRNA Regulation

miRNA regulation is another mechanism that can have an impact on the progression to AIDP. These small noncoding RNAs can regulate gene expression in a posttranscriptional manner. They have critical functions during development, and the pathology of many diseases, including cancers, involves exploitation of miRNA regulation to promote tumor formation and progression. Just as various proteins can be classified as oncogenes and tumor-suppressor genes, miRNAs can act similarly. Upregulated miRNAs that inhibit tumor-suppressor genes in cancer cells have been termed oncogenic miRNAs or oncomirs. Conversely, miRNAs that are down-regulated to promote cancer progression are known as tumor-suppressor miRNAs. Many studies have been conducted with the aim of elucidating various miRNAs that are dysregulated during prostate cancer progression. At present, there is still controversy in the literature regarding miRNA regulation in prostate cancer, and study results are often contradictory. Nonetheless, specific miRNAs that have been studied in the context of AIPC include miR-221, miR-222, mir-125b, and miR-146. For a comprehensive analysis of many other miRNAs implicated in prostate cancer, several reviews are available *(64, 65)*.

Two of the most frequently overexpressed miRNAs in many cancers include miR-221 and miR-222, which have been identified as oncomirs because they target *p27*, an important cell cycle inhibitory gene *(66)*. Interestingly, these miRNAs have been found to be overexpressed in AIPC, but downregulated during androgen-dependent prostate cancers. Sun et al. determined that by overexpressing both miRNAs in the androgen-dependent LNCaP cell line there was amplified androgen-independent growth *(67)*. On the contrary, knocking down miR-221 and miR-222 in an androgen-independent LNCaP clone resulted in decreased growth rates of these cells and increased reliance and sensitivity to DHT.

Furthermore, unlike miR-221 and miR222, miR146 has been consistently shown to be downregulated in androgen-independent cell lines compared to androgen-dependent cell lines *(68)*. Androgen-independent PC3 cells exogenously overexpressing miR-146 have decreased cell proliferation and survival, which implies that miR-146 may act as a tumor suppressor that is downregulated during progression to AIPC.

miRNA-125b has also been shown to be overexpressed in AIPC. Androgen-independent LNCaP cells have increased miRNA-125b expression compared to parental cells *(69)*. The proapoptotic protein Bak1 is a target to miR-125b, and interestingly, this protein has been shown to be decreased in hormone-refractory tumors *(69)*. The overall role of miR125b as an oncomir in prostate cancer progression must be further investigated, considering that some studies have demonstrated its downregulation during AIPC *(70)*. In addition, miR-125b has been observed to be a tumor suppressor in breast cancers, where it can negatively regulate the expression of HER-2/*neu (71)*. This finding is particularly interesting because Her-2/*neu*, as previously described, has been found to be overexpressed during AIPC, supporting the idea that miR-125b may be decreased during AIPC to promote increased expression of HER-2. Further studies must be conducted to elucidate the exact roles of miR-125b during AIPC because it appears to act as both an oncomir and a tumor suppressor based on different cellular environments.

In a recent study by Narayanan et al., the identification of feedback loops between various miRNAs, the

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AR, and its corepressors was assessed *(72)*. These authors found that the AR could regulate gene expression through a 3-step pathway that includes miRNA activation, corepressor suppression, and DNA interaction *(72)*.

Summary and Future Directions

A number of changes occur in favor of the development of androgen independence during prostate cancer progression. Many important alterations take place during this process. Aberrant AR signaling at various levels provides evidence of the importance of this pathway for the development of AIPC. However, alternative pathways and other forms of regulation such as epigenetics and miRNAs make the pathology even more complicated. Future studies are needed to find additional key modulators involved in this process, because these will provide a more comprehensive understanding of the pathology. Once we understand the precise molecular mechanisms, targeted therapeutic strategies can be applied with a focus on key pathways and essential molecules, a prime example being the AR and its various interacting proteins.

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