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Mechanisms of androgen-independent prostate cancer

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

[1] *Samuel Lunenfeld-Tanenbaum Research Institute, 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*

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Author for correspondence
E.P. Diamandis, M.D. PhD
Mount Sinai Hospital, Joseph & Wolf Lebovic Ctr.,
60 Murray St [Box 32]; Flr 6 – Rm L6-201
Toronto, ON, M5T 3L9, Canada
Tel: 416-586-8443; Fax: 416-619-5521;
e-mail: ediamandis@mtsinai.on.ca

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ABSTRACT

Prostate cancer is the second leading cause of cancer-related deaths among men in North America. Almost all prostate cancers begin in an androgen-dependent state, so androgen deprivation therapy is administered and results in improved clinical outcomes. However, over time, some cancerous cells are able to survive and grow during this treatment, resulting in androgen-independent prostate cancer. At this point, the disease is fatal, as there are no effective targeted therapies available. Most prostate cancer tumors require androgen receptor (AR) signalling for survival. During the progression to androgen-independence, this signalling cascade has been found to be altered at many levels within prostate cancers. Mechanisms that enhance AR signalling during androgen deprivation include: AR gene amplifications, AR gene mutations, changes in expression of AR co-regulatory proteins, changes in expression of steroid-generating enzymes, ligand-independent activation of AR via 'outlaw' pathways, and AR-independent pathways that

become activated, termed 'bypass' pathways. One or more of these aforementioned changes can lead to prostate cancer cells to gain androgen-independent properties. Understanding the molecular alterations that occur during this process will allow for improved therapeutic strategies to target key molecules and pathways important for this progression.

Introduction

Prostate cancer is the most commonly diagnosed and second leading cause of cancer-related deaths among men in North America [1]. Statistically, one in six men will develop some form of prostate cancer in their lifetime, and interestingly, almost 50% of men have tumors within their prostate upon autopsy. This indicates that prostate cancer is a slow growing cancer that may not directly lead to morbidity. However, there are aggressive forms of the disease that ultimately lead to fatal outcomes. Prostate cancer is initially diagnosed with a physical digital rectal examination followed by a serum prostate-specific antigen (PSA) test [2, 3]. PSA is one of the best known cancer biomarkers available, however, has its own limitations as well. PSA is also elevated in other pathological conditions of the prostate including benign prostate hyperplasia and prostatitis. In addition, PSA does not provide powerful prognostic potential, as it is unable to discriminate between indolent and aggressive forms of prostate cancer [2, 3]. Patients presenting with positive PSA tests undergo a prostatic biopsy, where histological assessment of prostatic tissue is analyzed to determine whether cancer is present or not [2, 3]. Not surprisingly, 75% of positive PSA cases do not present with cancer, indicating the lack of specificity of the marker. It is for these reasons, that active research is being pursued to identify additional biomarkers that either complement serum PSA and/or discriminate between indolent and aggressive forms of the disease. One of the best prognostic indicators for prostate cancer is Gleason score (GS), which characterizes the glandular architecture of the prostate based on a histological score that represents the level of 'de-differentiation' of the cancer [4, 5]. Briefly, GS is comprised of two numbers that represent the common Gleason patterns ranging from 1 to 5, where 1 represents well differentiated cellular architecture and 5 represents an aggressive un-differentiated one. It is now well accepted that the transition from a pattern 3 to 4 represents the development of aggressive prostate cancer [6].

Androgen receptor AR signalling

The AR is a protein that is able to bind to androgens and act a transcription factor to regulate a diverse array of genes. Most endogenous androgens are generated via the hypothalamus-pituitary-Leydig cell axis [17]. There is also a very small amount of androgens generated by the adrenal glands. The hypothalamus releases LHRH which is in turn promotes the pituitary gland to release LH, which is able to bind to Leydig cells of the testes and promote testosterone production (the most common androgen) [17]. Once generated, testosterone is able to enter the bloodstream and localize to

effector tissues including the prostate. Free circulating testosterone is able to enter prostate cells, where it is converted to its more active metabolite, dihydrotestosterone (DHT), by the 5-alpha reductase enzyme [17]. DHT within the prostate cell is then able to bind to cytosolic AR, which then undergoes a conformational change and translocates into the nucleus [18]. In the nucleus, AR acts as a transcription factor, binding to specific DNA sequences known as androgen responsive elements (ARE), leading to the expression of a variety of genes [19]. The AR protein consists of three major domains: ligand-binding domain, DNA-binding domain, and the N-terminal domain. The ligand binding domain is integral for the binding of DHT and testosterone to AR. The DNA-binding domain, as its name suggests, is responsible for the interaction of AR with specific ARE within the DNA in the nucleus. The N-terminal domain has also shown to be very important for AR signalling, as inhibition of this domain results in decreased AR transcriptional activity [20]. Many genes including PSA, are regulated by AR signalling.

AR signalling is absolutely critical for normal prostate cell function, so it's not surprising that prostate cancer cells also require its signalling for survival. Almost all prostate cancers begin in an androgen-dependent state, where AR signalling is predominant for cancerous growth and proliferation. When ADT is administered, many of the cancerous as well as normal cells undergo cell death due to the reduction of a crucial signalling cascade [21]. However, over time, some cancerous cells are able to manifest specific molecular and cellular changes in order to activate AR signalling, irrespective to whether there is a blockade of androgens. Many mechanisms as to how this is achieved has been studied, including amplification and mutation of the AR gene, changes in expression of co-regulatory proteins, alterations in steroidogenic producing pathways, and activation of the AR via ligand-independent manners known as 'outlaw' pathways [14, 22-25]. In addition, recent interest has shifted outside of focusing particularly on the AR pathway, where the much active research is looking at identifying novel 'bypass' pathways (AR-independent pathways) for the development of AIPC (Figure 1). Many of these 'outlaw' and 'bypass' pathways will be further discussed.

AR gene amplifications:

A common way for cells to compensate for the loss of a key cellular pathway is the over-activation or expression of an integral protein within that pathway. In the case of AR signalling and prostate cancer, cancerous cells have been shown to over-express AR at both the mRNA and protein level in vitro and in vivo models [26-28]. Studies have shown that almost 25-30% of AIPC contain AR genetic amplifications [28]. Such genetic amplifications have not been observed in cases where ADT was not administered, providing further evidence that AR gene amplification is a common by-product of hormone therapy. Elevated AR expression at both the mRNA and protein expression has been shown to sensitize cancer cells to lower-than-normal concentrations of androgens [29]. Although ADT is

efficient at reducing endogenous androgen production, it does not completely stop its production; so theoretically, any minimal amount of androgens still present can activate the AR. With excessive AR expression via genetic amplification, even small amounts of androgens can activate the protein resulting in downstream signalling. Interestingly, AR overexpression at the protein and mRNA level has also been observed in the absence of AR gene amplifications, indicating there could be other modes of regulation of AR including epigenetic factors and miRNAs [19]. It is clearly evident based on clinical studies that AR overexpression is a common event that occurs during the development of AIPC, and therefore therapies able to focus on particularly blocking its expression or signalling cascade could potentially be utilized for clinical use.

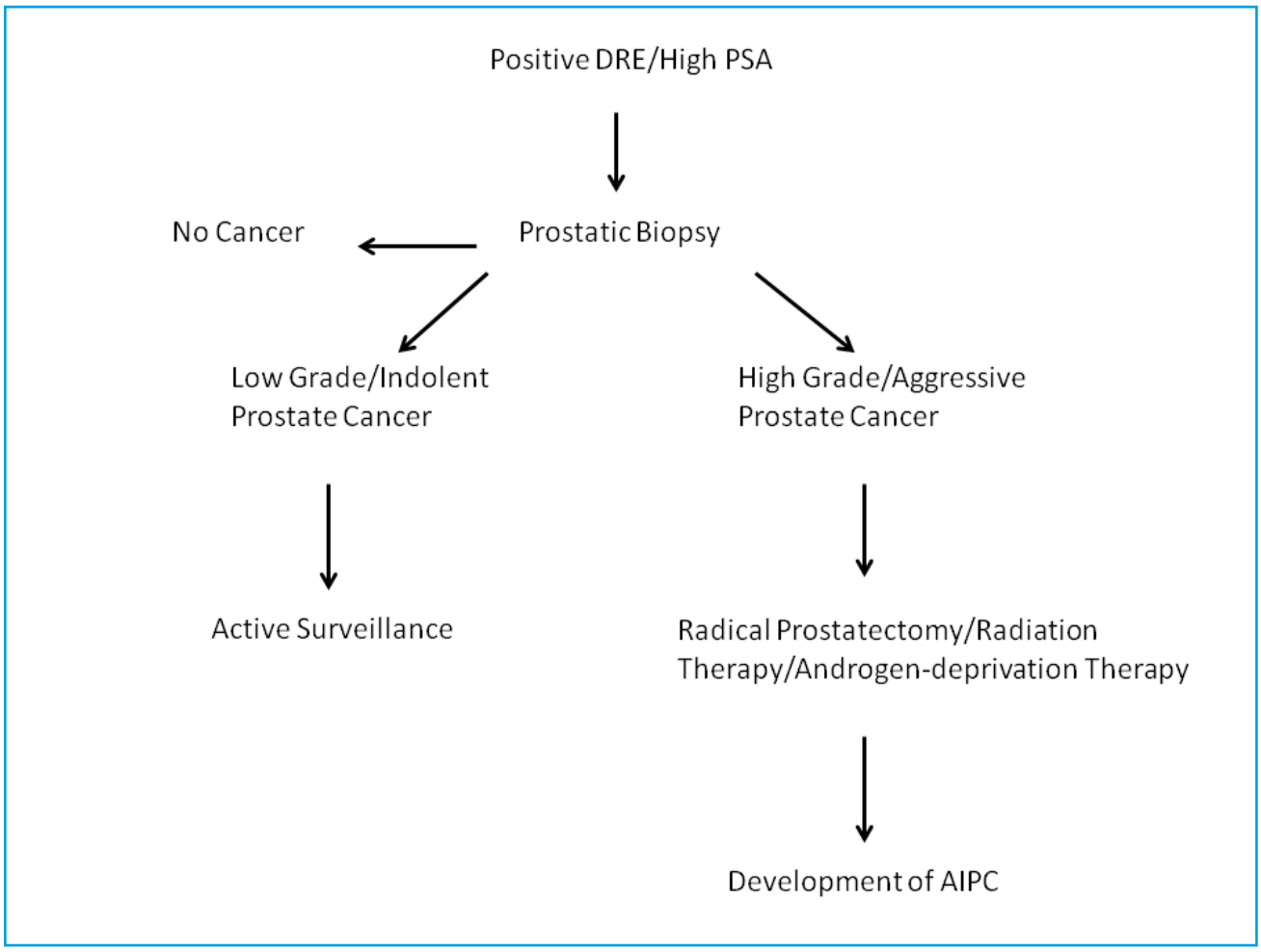

Figure 1. Prostate cancer diagnosis and treatment.

After an initial physical digital rectal examination followed by a positive PSA test, a prostatic biopsy is examined. Based on histology, the biopsy will either confirm no cancer or cancer, and based on the Gleason scoring system, prostatic cancerous cells will be assessed a Gleason score. Gleason score 6 and less cancers do not require any curative treatments and undergo active surveillance, whereas Gleason 7 or higher cancers are normally treated with radical prostatectomy and androgen deprivation therapy. Patients often regress to androgen-independent prostate cancer, where there are no effective targeted therapies available.

AR gene mutations:

Along with genetic amplifications, another mode of aberrant AR signalling could result due to genetic mutations of the AR gene itself. As previously mentioned, AR consists of three major domains, and specific mutations in each of these domains could have a large impact on the function of the protein. The AR is a gene located on the X chromosome, and loss of function results in a condition known as androgen-insensitivity. Over the years, many novel mutations have been identified within the AR gene. The McGill androgen receptor database (http://androgendb.mcgill.ca), has compiled a list of all the AR mutations identified to date, as well as the specific domains they occur within. We will only focus on the most frequent mutations found in AIPC patients.

The frequency of AR mutations are very low (up to 4%) in patients with early stage tumors [30]. However, in late stage/aggressive tumors, the frequency is elevated to 10-20% in cases of AIPC [31]. This further supports the notion that AR mutations are a common mechanism that prostate cells may utilize to gain androgen-independent properties. The first reported AR gene mutation was in the hormone-dependent LNCaP human prostate cancer cell line derived from a lymph node metastasis [32]. The LNCaP cell line contains a unique missense mutation at codon 877, resulting in the amino acid threonine being substituted to an alanine [32]. Interestingly, this mutation occurs within the ligand-binding domain of the AR protein, and has been shown to reduce the ligand specificity of the protein, whereby other molecular such as progesterone, estrogens and many antiandrogens can also activate the protein. Such a mutation would be highly beneficial for a cancerous cell, as during ADT, they no longer require androgens, but instead, can utilize other common circulating hormones or molecules to activate the AR protein and its downstream signalling cascade. Studies have shown that this specific T877A is very common during AIPC [33]. Localized androgen-dependent cancers have been shown to have less AR gene mutations, whereas tumors that have metastasized and become more aggressive harbour greater number of mutations [30, 31, 34]. An interesting study by Marcelli et al., showed that mutations were found in 8 of 38 patients with lymph node metastasis who were treated with ADT, whereas no such mutations were observed in patients that did not undergo therapy [35]. Other common AR mutations include H874Y, V715M, L701H+T877A and Y741C [31, 34, 36, 37]. All these mutations are also within the ligand-binding domain of the protein, resulting in either broadened ligand specificity or constitutive protein activity.

In addition to AR gene mutations, recent interest in AR splice variants has also been observed in AIPC. In a study by Guo et al., three novel AR splice variants were identified in AIPC, all lacking the ligand-binding domain [38]. Subsequent studies assessing the exact role of these splice variants and their activity need to be further addressed, however, they present another interesting mechanism which prostate cancer cells can potentially utilize to gain androgen-independent properties. Potential drugs that could inhibit such splice variants could represent a novel area of therapeutic intervention.

Alteration in AR co-regulators:

As mentioned, the AR is a transcription factor, capable of binding to specific DNA sequences (AREs) to induce or inhibit the transcription of a variety of genes. As a result, there are many co-regulatory proteins that are able to bind to AR and either activate (co-activators) or suppress (co-repressors) gene expression of downstream target genes. Alterations in the expression of any of these co-regulatory enzymes could have an impact on AR signalling, and be a possible mechanism for cells to gain androgen-independence. There have been over 170 documented proteins that have been shown to act as coregulators with AR [39]. Any shift in the balance of these proteins can have a drastic effect on the overall expression of AR regulated genes. Some of the more well studied coactivators of AR signalling include TIF2, GRIP1, SRC1, and a broad group known as AR-associated (ARA) proteins [40, 41]. Gregory et al., found that levels of TIF2 and SCR1 were elevated in AIPC samples that also had increased AR expression [42]. On the other hand, two of the most common AR co-repressors include NCoR and SMRT [43]. Both of these proteins are able to recruit histone deacetylases, resulting in chromatin condensing and reduced transcriptional activity [43]. During the development of AIPC, both these co-repressors have been shown to be down-regulated, resulting in increased AR-mediated transcriptional activity [44].

Aberrant androgen-generating enzyme pathways:

The main purpose of ADT is to block/reduce endogenous androgen activity. This can either be achieved via blocking the androgen production pathways or by directly inhibiting androgen affinity towards the AR. Many AIPC patients have aberrant signalling in the precursor pathways that generate androgens, usually in the form of over-production to compensate during ADT [24]. Many of the current androgen-blocking agents are directed towards inhibiting the hypothalamus/pituitary/Leydig axis, and are very efficient as this is the major androgen generating mechanism of the body. However, the adrenal glands are also capable of generating low concentrations of androgens, and blockade of this pathway may also be required for efficient androgen deprivation [45]. In addition, recent studies have shown that tumor cells themselves are capable of generating their own androgens via de novo synthesis [46]. Such a mechanism is very intriguing, as cells that are undergoing ADT can activate certain cellular enzymes and pathways to produce their own endogenous testosterone to active AR. In particular, many enzymes within the cholesterol biogenesis pathway, a precursor to androgen production, have been shown to be elevated in tumor cells [47]. Essentially, prostate cancer cells may be utilizing various alternate pathways to produce endogenous androgens to activate the AR signalling cascade, during times of androgen deprivation.

Outlaw pathways:

The AR protein is preferentially activated by endogenous androgen ligands. However, like other steroid hormone receptors, AR has also been shown to be activated in ligand-independent mechanisms referred to as outlaw pathways. Cytosolic AR has been shown to interact with many molecules in a nongenomic role, and activate various pathways. Various growth factors, cytokines, kinases and other proteins have been shown to interact with and activate AR in a ligand-independent manner.

Some of the most common growth factor proteins that interact with AR include IGF1 and EGF. IGF1 has been extensively studied with respect to AR signalling, as it has been shown to prolong it, even in the absence of androgens [36]. Interestingly, in the presence of antiandrogens, AR signalling is abrogated, indicating that IGF1 and AR have a direct interaction with one another [36]. IGF1 has also been shown to induce the expression of AR co-activator TIF2, indicating another indirect way to potentiate AR signalling [48]. EGF is another growth factor able to induce AR signalling in a ligand-independent way [36]. The EGF-regulated gene, SPINK1, has been shown to be elevated in cases of aggressive prostate cancer, indicating the importance of this growth factor with respect to prostate cancer pathogenesis [49, 50].

In addition to growth factors, various cytokines have also been shown to interact with AR. Specifically, (NF)-kB signalling, which activates the cytokines IL-6 and IL-8, has been found to be elevated in many cases of AIPC [51]. Increased (NF)-kB signalling was shown to increase AR signalling in the LNCaP prostate cancer line, and this activation was halted after inhibition of (NF)-kB signalling [51]. In addition, both IL-6 and IL-8, like IGF1, were shown to directly bind and activate AR, as inhibition via antiandrogen treatment abolished this activation [51].

Receptor tyrosine kinases (RTK) are important signalling molecules that have been shown to be altered in various pathological conditions, especially cancers. One highly studied RTK that has been found elevated in AIPC is HER2/ERBB2 [52, 53]. This protein is overexpressed in many AIPC cell lines in vitro, as well as in many xenograft models of androgen-independence. The overexpression of HER2 in prostate cancer cells can directly activate AR signalling, and unlike IGF1, IL6 and IL8, in the presence of antiandrogens, this signalling is not disrupted [52]. This potentially indicates that activation of AR signalling via HER2 may be independent of the ligand binding domain. Other RTKs that have been implicated to the development of AIPC are the IGF and EGF receptors [10]. These receptors activate essential downstream survival pathways including AKT, MAPK, and STAT, many of which are also dysregulated in AIPC [10].

Bypass pathways:

Thus far we have discussed mechanisms of AIPC progression through AR signalling. Although alterations to various aspects of AR signalling are integral during the progression to androgen-independence, it is also important to mention other pathways, the AR-independent ones, which also become altered during prostate cancer progression. Such pathways are referred to as bypass pathways. Many of the outlaw pathways mentioned earlier can also be classified as bypass pathways, as signalling through various RTKs and receptors are able to activate a diverse and unique signalling cascade that is independent of AR signalling. For example, the IGF1 ligand once bound to its receptor, IGF receptor, is able to transduce a signalling cascade that can activate the expression of genes that are able to promote cellular growth and proliferation, allowing cancer cells another mechanism for enhanced survival. Many bypass pathways act through RTKs that activate a diverse range of kinases including MAPK/Ras/Raf, which in turn can activate various transcription factors such as (NF)-kB and c-MYC, resulting in changes that influence cell cycle regulation and cellular proliferative properties [54, 55].

One major signalling cascade that is altered during AIPC is the Akt pathway [56]. Akt signalling can act both in an outlaw mechanism via activation of AR, or independently through other intermediate proteins that affects major cellular processes including apoptosis and proliferation in prostate cancer cells [56]. Another highly studied molecule in prostate cancer pathogenesis, PTEN, which is a proapoptotic protein that inhibits Akt signalling, has been found to be decreased in expression in many cases of AIPC, further indicating the importance of Akt signalling [57].

Apoptosis is an important mechanism that cells utilize to undergo cell death in order to ensure stability. During androgen deprivation, many prostate cancer cells undergo apoptosis, so a mechanism a cancerous cell could utilize to ensure survival is the activation of proteins that inhibit this process, known as anti-apoptotic proteins. Once such protein, Bcl-2, has been found elevated in many cases of aggressive AIPC [58, 59]. A study conducted by Liu et al., demonstrated that when Bcl-2 expression was blocked in AIPC xenografts, the resulting tumors were smaller than ones that did not have Bcl-2 expression inhibited [59].

Recent progress:

Extensive research has been conducted on the development of AIPC with regards to aberrations in various signaling pathways, most notably AR signalling and others already mentioned (Table 1). In addition to abnormal signalling pathways, other factors including epigenetic alterations and miRNA regulation are also being studied to understand the progression of AIPC.

Epigenetics is an important mode of regulation that cells use to ensure proper gene expression. Changes in cellular epigenetic signatures are common developments during cancer development.

Some common epigenetic alterations during AIPC development include changes in genes involved in cell cycle control, cell invasion, cellular architecture, DNA damage repair, tumor-suppressors and oncogenes. The most notable epigenetic alteration in AIPC is GSTP1 promoter methylation, with a frequency of 70-100% in prostate cancer DNA samples [60]. Recently, RGS2 promoter hypermethylation was also observed in AIPC, as it allows cells to gain a more aggressive androgen-independent phenotype [61].

TABLE 1. Pathways Activated during Androgen-independent Prostate Cancer

Signalling Pathway	Receptors Involved	Consequence of Pathway	Reference
Akt Pathway	Various Receptor Tyrosine Kinases	Decreased Apoptosis and Increased Survival	10, 54
IGF Pathway	IGF Receptor	Increased Cell Growth and Proliferation	10, 36, 48
EGF Pathway	EGF Receptor	Increased Cell Growth and Proliferation	10, 36
AR Pathway	Androgen Receptor	Increased Survival and Growth	54
JAK/STAT Pathway	IL6 Receptor	Increased Survival and Growth	10. 51, 54
MAPK Pathway	Various Receptor Tyrosine Kinases	Increased Proliferation and Decreased Apoptosis	10,54
PKC Pathway	TGFβ Receptor	Increased Proliferation and Decreased Apoptosis	54

Another mode of regulation that has recently been studied for the progression of AIPC is via miRNAs. Various miRNAs have been shown to promote this transition, most notably miR-221, miR-222, mir-125b and miR-146 [62]. Interestingly, miR-221, miR-222 and miR-125b have been found to be over-expressed in AIPC, whereas miR146 has been shown to be down-regulated [63-66].

Further investigation is currently being pursued in several of these fields to identify aberrantly expressed genes that are involved in AIPC progression, in the hopes of generating potential useful clinical biomarkers and treatments.

Conclusion:

Prostate cancer is a curable disease if detected early in an indolent form (ie. radical prostatectomy); however, aggressive forms require ADT which ultimately results in the development of AIPC. Once at

this stage, there are no targeted therapies, and cells will likely have metastasized to distal sites and eventually results in fatal outcomes. Understanding the molecular alterations during the progression of prostate cancer to an androgen-independent state is of utmost importance in to order to first understand the disease, and second, to generate effective targeted treatments to enhance patient care. Much research has heavily focused on AR signalling, a definite key player in the process, however, further work identifying other novel molecules and pathways are currently being pursued. Of the aforementioned mechanisms of androgen-independence, AR gene amplification and mutations still remains one of the better accepted modes for this transition. For this reason, many more sensitive inhibitors of AR are being developed and tested in patients with the hopes of alleviating symptoms. In addition, recent interest in blocking circulating adrenal androgens has also provided an interesting avenue of therapeutic intervention for this disease. However, further studies are being conducted to assess the potential of such therapies. Once we are able to fully understand the molecular pathogenesis of this disease, the next steps will be to target key proteins such as AR and other important pathways in order to provide specific therapeutic intervention that can result in decreased morbidity.

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