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Review

Androgen Receptor Gene and Hormonal Therapy Failure of Prostate Cancer

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Androgen receptor (AR) is a nuclear transcription factor that binds male sex steroids and mediates the biological effects of these hormones to the target cells, such as the epithelial cells of the prostate gland, by activating transcription of androgen-dependent genes. Withdrawal of androgens or the peripheral blockade of androgen action remain the critical therapeutic options for the treatment of advanced prostate cancer. However, after initial regression, many prostate cancers become hormone refractory and progress further with eventual fatal outcome. Understanding the mechanisms of tumor progression and endocrine therapy failure is an important goal. A large number of different molecular mechanisms may be responsible for development of hormone-refractory recurrent tumors. Many of these involve the AR gene and its complex downstream signaling pathways. The role of AR mutations and altered transactivational properties of the receptor have received the most attention as causative factors for progression. However, other mechanisms, such as AR gene amplification and overexpression or increased local bioconversion of androgens, may contribute to the development of progression by mechanisms that involve androgen-dependent cell growth. Here we review the role of the AR gene and its putative downstream effector pathways during human prostate cancer progression and endocrine therapy failure. ***(Am J Pathol 1998, 152:1–9)***

Androgens play a significant role in regulation of prostate cancer growth as already suggested more than 50 years ago by Huggins and Hodges.[1] This association of androgens with prostate cancer is important in all aspects of prostate cancer research, from carcinogenesis and risk factors for prostate cancer to the therapeutic management of cancer. For example, risk factors to prostate cancer may include increased androgen availability[2,3] or altered cellular responsiveness to androgens.[4,5] On the other hand, hormonal therapy remains the treatment of choice for patients who present with advanced, inoperable prostate cancer.[6] However, in most cases, the therapy eventually fails. Many unanswered questions remain regarding the molecular mechanisms underlying this common clinical problem, failure of endocrine therapy, and the development of hormone-refractory prostate cancer.

Endocrine therapy, administered either by androgen deprivation or by blockade of androgens at the level of the androgen receptor, usually results in a favorable clinical response and a dramatic regression of prostate cancer as a result of the apoptotic cell death.[6,7] Although complete disappearance of symptoms is achieved in many cases, the response does not imply cure. Tumor progression and therapy failures are very common when the treatment is continued for many months or years.[6,8,9] This represents a serious clinical problem, as no effective alternative therapies are currently available for patients with hormone-refractory tumors. Understanding the molecular mechanisms of prostate cancer progression during androgen deprivation is therefore critical and needs to be addressed to improve the care of patients with advanced prostate cancer.

Androgen receptor (AR) binds androgens and mediates their effects on target cells.[10] Much research activity has focused on the role of the AR in tumor recurrence and progression.[11] The role of AR gene mutations in tumor progression has been studied in the vast majority of these studies.[12–24] Such mutations can lead to impaired steroid binding specificity and to altered transactivational prop-

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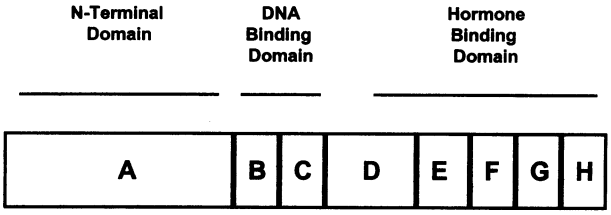


Figure 1. Schematic picture of human AR structure. Amino acid positions of each exon are noted above the bar.

erties of the AR protein so that it retains its activity even when bound to other steroids than androgens.

Recent studies suggest that the amplification[19,20,25] and increased transcriptional activity of a wild-type AR gene[20,26] may also contribute to the treatment-related progression of many prostate cancers. These and several other molecular mechanisms can be envisioned to lead to improper activation of downstream genes and signaling pathways that ultimately induce reactivation of cell proliferation. Furthermore, the survival- and growth-promoting role of androgens can be overtaken by other signaling and growth factor pathways.[4,27,28]

The involvement of the AR gene in the prostate cancer has been studied from human tumor specimens with the tools of modern molecular pathology. At the same time, basic research has uncovered numerous downstream targets of androgen signaling. As reviewed here, together these advances may in the future help to uncover the mysteries of the molecular mechanisms of therapy resistance and recurrence of prostate cancer.

Function of Androgen Receptor in Transcriptional Regulation and Androgen Signaling

The effects of androgens on target cells are pleiotropic and involve the activation and down-regulation of a large number of different genes.[29] The AR has a central role in mediating the biological effects of androgens to different downstream genes. The AR protein is a member of a steroid receptor family that belongs to a larger family of nuclear receptors.[30] The gene is composed of eight exons (Figure 1), of which A encodes the transactivation domain, B and C encode one each of the zinc-finger elements responsible for DNA binding, and exons D to H encode the ligand-binding domain.[31,32]

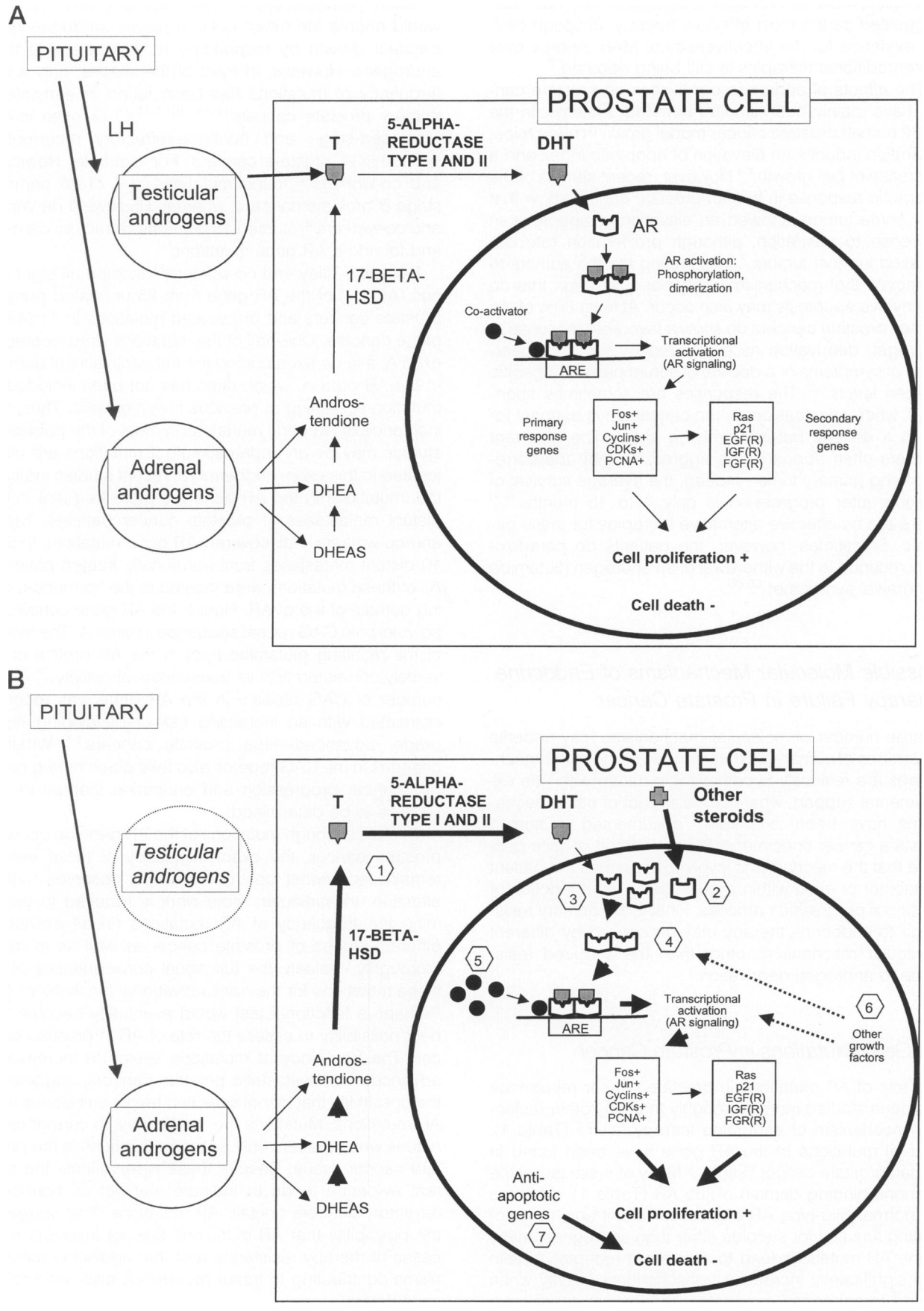
After binding the androgen, AR is phosphorylated, dimerized, and translocated into the cell nucleus where it binds to androgen-responsive elements (AREs) at the promoter regions of target genes[33,34] (Figure 2). Transcriptional activation involves formation of a multiprotein transcription complex, which includes proto-oncogenes Jun and Fos[35–37] and RelA,[38] a member of the NF-κB family. These factors can modulate and repress AR-mediated transactivation.[38,39] Recently, a specific co-activator of the AR, ARA-70, was found to have the ability to enhance the transcriptional activity of AR 10-fold.[40] Numerous other nuclear receptor co-activators may also modulate the tissue-specific transactivation activity of AR.[41] Finally, emerging evidence suggests that AR may directly regulate genes that do not contain AREs.[42]

More than 100 androgen-regulated genes are known today, and it is likely that many more still remain to be discovered. Although the exact mechanisms and primary mediators of AR-dependent growth promotion remain very poorly understood, the androgen-regulated genes include several candidates that directly function in cell growth regulation. These include cyclins A,[43–45] D1 to D3,[45–48] and E[45,49,50] and cyclin-dependent kinases[51–53] that regulate cell cycle progression, early-response genes Fos and Jun,[35–37] intracellular signal transduction genes, such as Ha-ras and p21,[54–57] as well as a large number of peptide growth factors[58–63] and their receptors.[60,62,63] Overall, there appears to be substantial cross-talk between the AR pathway and that of the other growth factor pathways. This cross-talk may be significant for the emergence of endocrine therapy failure in that other pathways may increasingly contribute to growth signal transduction when androgens are deprived.

Response of Prostate Cancer to Androgen Deprivation

Endocrine therapy can be accomplished by blocking the synthesis of androgens in the testicular tissue, which leads to a subsequent decrease in their concentration in the circulation. Luteinizing hormone releasing hormone (LHRH) agonists, administration of estrogen, or surgical castration induce such androgen deprivation. The functions of androgens can also be blocked in the peripheral tissues by anti-androgens, such as flutamide, nilutamide, and bicalutamide. The combination of the two therapy

Figure 2. Androgenic regulation of growth in normal prostate epithelial cells and possible mechanisms by which hormone-refractory recurrent prostate cancer may arise. **A:** In a physiological state, the testicular glands secrete testosterone, which is converted into more potent androgen DHT by 5-α-reductase in the prostate tissue. The adrenal glands secrete large amounts of androgen precursors dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), and androstenedione, which are converted locally into bioactive androgens. In the target cells, androgens bind to the ligand-binding domain of AR protein, leading to phosphorylation and dimerization of the hormone-receptor complex, binding to AREs in the promoter regions of androgen target genes. There are both primary response genes and genes with a delayed response to androgens. Both of these downstream genes mediate the mitogenic effects of androgens to the prostate cells. **B:** Androgen deprivation by surgical or chemical castration eliminates androgens originating from the testes. Recurrent, hormone-refractory prostate cancer may arise as a result of a number of possible mechanisms: 1) conversion of bioactive androgens from the inactive adrenal precursor steroids, 2) AR gene mutations leading to an AR protein with altered transactivational properties, such as activation by other steroids, 3) increased expression of androgen receptors as a result of AR gene amplification, 4) auto-activation of the AR protein taking place (even in the absence of androgens) by protein kinase activity or via various other androgen-independent growth factors, 5) increased activation of downstream genes, which may result from an excess of co-activators (such as ARA-70 or AIB1) of AR, 6) several downstream genes possibly activated both by AR signaling as well as by other growth factor pathways (cross-talk) and growth factors possibly also sharing with AR the same downstream mechanisms for enhancing cell proliferation, and 7) anti-apoptotic genes possibly blocking programmed cell death normally induced by androgen deprivation. ACTH, adrenocorticotropic hormone; HSD, hydroxysteroid dehydrogenase; LH, luteinizing hormone; T, testosterone.


A
PITUITARY
LH
Testicular androgens
Adrenal androgens
PROSTATE CELL
T
5-ALPHA-REDUCTASE TYPE I AND II
DHT
AR
AR activation: Phosphorylation, dimerization
Co-activator
ARE
Transcriptional activation (AR signaling)
17-BETA-HSD
Andros-tendione
DHEA
DHEAS
Primary response genes
Fos+ Jun+ Cyclins+ CDKs+ PCNA+
Ras p21 EGF(R) IGF(R) FGF(R)
Secondary response genes
Cell proliferation +
Cell death -
B
PITUITARY
Testicular androgens
Adrenal androgens
PROSTATE CELL
T
5-ALPHA-REDUCTASE TYPE I AND II
DHT
Other steroids
1
2
3
4
5
6
7
17-BETA-HSD
Andros-tendione
DHEA
DHEAS
ARE
Transcriptional activation (AR signaling)
Other growth factors
Fos+ Jun+ Cyclins+ CDKs+ PCNA+
Ras p21 EGF(R) IGF(R) FGR(R)
Anti-apoptotic genes
Cell proliferation +
Cell death -

strategies, maximal androgen blockage (MAB), has been suggested as the most effective therapy, although clinical evidence for the effectiveness of MAB therapy over more traditional therapies is still being debated.[2]

The effects of androgen deprivation on prostate cancer have mainly been studied in model systems. In the PC82 human prostate cancer model grown in nude mice, castration induces an elevation of apoptotic index and a decrease of cell growth.[64] However, recent studies of the apoptotic response in human prostate cancer show that only some tumors showed an elevation of apoptosis in response to castration, although proliferation rate decreased in most tumors.[7] This finding led the authors to speculate that mechanisms of tumor regression that do not involve apoptosis may also occur. At least 80% of the human prostate cancers do show a favorable response to androgen deprivation as evidenced by the disappearance of symptoms or a decline of serum prostate-specific antigen levels.[6,9] The responses are sometimes short-lived, whereas occasionally the cancer stays dormant for up to a decade before surfacing again. The recurrent cancers often appear more aggressive than the corresponding primary tumor. Indeed, the average survival of patients after progression is only 4 to 15 months.[6–9] There are no effective alternative therapies for these patients. Sometimes, however, the patients do paradoxically respond to the withdrawal of an androgen (flutamide withdrawal syndrome).[65,66]

Possible Molecular Mechanisms of Endocrine Therapy Failure in Prostate Cancer

A large number of molecular mechanisms may underlie recurrence of human prostate cancer. Some of the mechanisms are relatively hypothetical in nature with little experimental support, whereas the impact of other mechanisms have been extensively documented in human prostate cancer specimens. Furthermore, it is quite possible that the mechanisms involved vary from one patient to another or even within a given tumor as a function of the tumor progression process. Finally, the primary resistance to endocrine therapy may be caused by different molecular mechanisms other than the acquired resistance to androgen deprivation.

AR Gene Mutations in Prostate Cancer

The role of AR mutations in prostate cancer recurrence has been studied more thoroughly than any other molecular mechanism of endocrine therapy failure (Table 1). Several mutations of the AR gene have been found in human prostate cancer tissues.[5] Many of these target the hormone-binding domain of the AR (Table 1). Whereas the normal wild-type AR expresses little, if any, transactivating function for steroids other than androgens, many of the AR mutations lead to an altered receptor protein with significantly increased transcriptional activity when bound to estrogens, progesterone, adrenal androgens, or even paradoxically anti-androgens (Table 1). This would enable the tumor cells to regain androgen-independent growth by responding to other steroids than androgens. However, in most of the studies, only a low frequency of mutations has been found in early-stage primary prostate cancers[12,14,15,17,21–24] or even in the advanced-stage and hormone-refractory recurrent[13–16,18–20,22–24] prostate cancers. For example, Newmark and co-workers[12] found mutations in 1 of 26 primary, stage B prostate cancers, whereas Ruizeweld de Winter and co-workers[16] studied 18 hormone-refractory cancers and found no AR gene mutations.

Recently, Tilley and co-workers[21] studied all eight exons (A to H) of the AR gene from 25 untreated primary prostate cancers and discovered mutations in 11 (44%) of the cancers. One-half of the mutations were located in exon A, a large exon coding the transactivational domain of the AR protein, which often has not been included in mutation screening in previous investigations. Thus, the incidence of AR gene mutations in most of the published studies may be an underestimate if mutations are often located in this exon. Furthermore, recent studies indicate that mutations in the AR gene are more frequent in the distant metastases of prostate cancer patients. Taplin and co-workers[18] discovered AR gene mutations in 5 of 10 distant metastases from hormonally treated patients. All of these mutations were located in the hormone-binding domain of the of AR. Finally, the AR gene contains a polymorphic CAG repeat sequence in exon A. The length of the resulting glutamine tract in the AR protein is inversely correlated with its transcriptional activity.[11] A low number of CAG repeats in the AR gene was recently correlated with an increased risk of developing high-grade, advanced-stage prostate cancers.[67] Whether changes in the CAG repeats also take place during prostate cancer progression and endocrine therapy failure remains to be determined.

Overall, although mutations of the AR gene do occur in prostate cancer, the exact frequency of these events remains somewhat controversial and deserves further attention. In particular, more work is required to determine the frequency of AR mutations (in all exons) at different stages of prostate cancer as well as to more thoroughly evaluate the functional consequences of all these mutations for the transactivational functions of AR. Perhaps a functional test would eventually become the best possibility to assess the role of AR in prostate cancer. The frequency of mutations seems to increase in advanced and metastatic prostate cancers, suggesting the possibility that clonal selection favors cell clones with AR mutations. Mutations are not found with current techniques when present only as subpopulations in the prostate cancer tissue. Despite these reservations, the current evidence tends to indicate that not all hormone-refractory cancers contain AR mutations. This suggests the possibility that AR mutations are not involved in all cases of therapy resistance and that additional mechanisms contributing to tumor recurrence also need to be considered.

Table 1. Androgen Receptor Gene Mutations Detected in Clinical Prostate Cancers

Tumor characteristics	n	Exons studied	Mutations found	Exons	Comments	Ref.
Untreated stage B prostate cancers	26	Exons E–H	1/26 (4%)	Exon E	No data from activational properties	12
Metastatic hormone-refractory prostate cancers	7	Exons B–H	1/7 (14%)	Exon D	Activated by adrenal androgens and progesterone	13
Untreated stage B primary tumors (7), endocrine-therapy-resistance autopsy samples (8)	15	Exons B–H	1/8 (13%) hormone-therapy-resistant patients showed one mutation in 1) prostate and one in the corresponding 2) metastasis	1) Exon D 2) Exon H	No data from activational properties	14
Transurethral resection specimens from advanced prostate cancers	24	Exons D–H	6/24 (25%)	Exon H	No data from activational properties	15
Hormone-refractory local recurrences	18	Exons B–H	No mutations			16
Untreated prostate cancers	40	CAG repeats	1/40 (3%)	Exon A	Activated by flutamide	17
Hormone refractory metastases: bone marrow (8), pleural fluid (1), and skin nodule (1)	10	Exons B–H	Mutation in 5/10 (50%) of cases	1) Exon H 2) Exon H 3) Exon H 4) Exon D 5) Exon D Exon E Exon H Exon H	Mutations in cases 1 and 2 were activated by estrogen and progesterone	18
Hormone-refractory local recurrences	7	Exon H (codon 877)	No mutations			19
Hormone-refractory local recurrences	13	Exons B–H	1/13 (8%)	Exon D	No abnormal activation	20
Untreated primary tumors	25	Exons A–H	11/25 (44%)	1) Exon A 2) Exon B 3) Exon D 4) Exon F 5) Exon G 6) Exon H	No data from activational properties	21
Untreated primary tumors (23), hormone-refractory local recurrences (6)	29	Exons B–H	1/29 (3%) germ-line mutation	Exon E	Mutated receptor was activated by estradiol	22
Untreated primary tumors (31), hormone-refractory recurrences (13)	44	Exons A–H	1/44 (2%) primary tumours	Exon F	No data from activational properties	23
Untreated primary stage B and C tumors (30), hormone-refractory recurrences obtained from autopsy (22)	52	Exons B–H	0/30 3/22 (14%)	1) Exon H 2) Exon H 3) Exons D and H	No data from activational properties	24

AR Amplification in Prostate Cancer

Studies with a recently developed whole-genome screening technique, comparative genomic hybridization (CGH), indicated that a common genetic alteration in hormone-refractory, locally recurrent prostate cancers was the amplification of the chromosomal region Xq11–12.[68] This coincides with the chromosomal location of the AR gene and raised the question of whether the AR gene would be amplified in response to androgen deprivation. Analogous examples of gene amplifications leading to resistance of cancers to chemotherapeutic drugs have been described in the literature.[69,70]

Fluorescence *in situ* hybridization (FISH) was performed on tumor interphase nuclei, from both primary and recurrent cancers, using a large-insert genomic P1 probe for the AR gene. A 2.7 to 28-fold amplification of the AR gene was found in 15 (28%) of 54 locally recurrent tumors, whereas none of the primary tumors showed any amplification.[20] Thus, AR gene amplification appeared not to be involved in the development of primary prostate cancer, but the results strongly suggested a role for

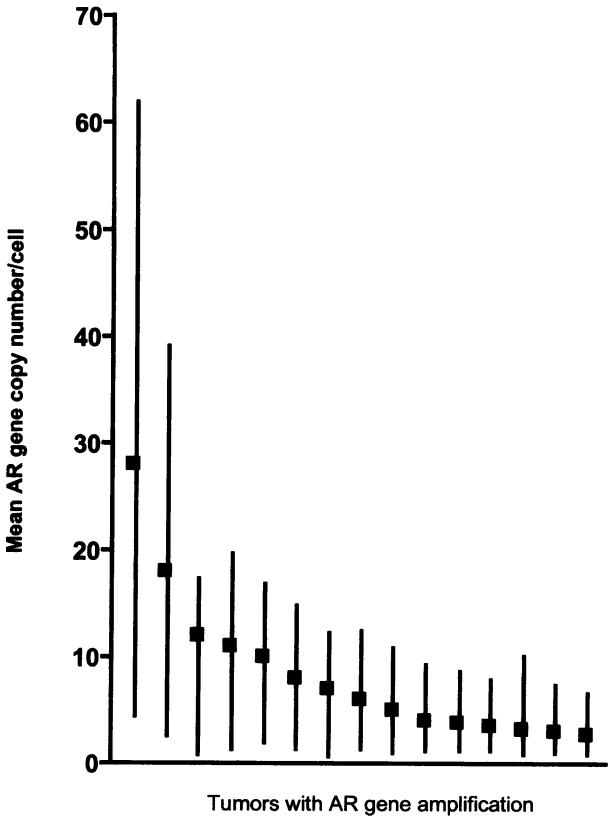


Figure 3. The AR gene copy numbers per cell showed substantial intra-tumor variability in cases with AR amplification. The AR copy numbers ranged from 1 to 62 in individual tumor cells from AR amplified tumors.

amplification in the development of tumor progression and failure of androgen deprivation therapy.

The absence of functionally significant mutations in exons B to H in the AR gene of AR-amplified tumors further supports the fact that the amplified AR gene is structurally normal and has retained specificity to androgens. Studies by mRNA *in situ* hybridization suggested that AR amplification leads to increased expression of the AR gene, as would be expected from an amplification target gene. The AR protein expression detected by immunohistochemistry was also high in all cases of hormone-refractory prostate cancers studied in this and other series.[16,19,71,72] Finally, the tumors that relapsed with AR amplification were the ones that initially had responded most favorably to androgen deprivation, suggesting that the most androgen-dependent tumors are the ones that may recur through a mechanism involving AR gene amplification. The results on AR amplification raise the hypothesis that many recurrent tumors may not be androgen independent as usually thought but that the tumors may have acquired increased sensitivity and capacity to utilize the residual androgens remaining after hormonal therapy for supporting their growth.

FISH analyses also indicated a great intra-tumor heterogeneity of the AR gene copy number distribution in tumors with AR amplification (Figure 3). Gene amplification can only arise in genetically unstable cells. Thus, tumors that recur through AR amplification must be genetically particularly unstable. Such instability may lead to intra-tumor heterogeneity. This is supported by findings of frequent chromosomal copy number variability as well as aneuploidy by FISH and flow cytometry[20,73] in the recurrent AR-amplified tumors. Variability in AR copy number may not be the only characteristic showing intra-tumor variability in therapy-resistant tumors. One could hypothesize that even within a given recurrent tumor there may be tumor cells that have acquired growth advantage by different, perhaps multiple, mechanisms.

Increased Local Bioavailability of Androgens

Another molecular mechanism that could allow the cancers to relapse by an androgen-dependent mechanism during androgen deprivation therapy involves the increased conversion of adrenal androgen precursors to dihydrotestosterone (DHT). DHT originates predominantly from the testes, but adrenal precursor steroids can also be locally converted into testosterone, and testosterone into DHT.[2,3] Although castration-induced androgen deprivation causes a 95% reduction in serum testosterone levels, the concentration of DHT in the prostate cancer tissue is decreased by only approximately 60%.[2,3,74,75] It appears possible that, after androgen deprivation, a compensatory increase in the conversion of adrenal precursor steroids to active androgens takes place in the tumor or the stroma. This rescues the tumor cells from apoptotic death and provides a means to support cancer cell growth. This concept of increased local biosynthesis has been strongly supported by Labrie and co-workers[75] who indicate that this necessitates the use of MAB therapy for patients. Current clinical evidence gives only marginal support to the benefit of MAB therapy.[76] However, it is possible that this molecular mechanism of generating androgen-dependent recurrent tumors is important only in a subset of the patients.

Alternative Mechanisms for Activation of Androgen Signaling

Even in the absence of AR mutations or amplifications, one can envision that enhancers and co-activators of the transcriptional activity of AR could help to maintain the androgen-dependent growth promotion in an androgen-deficient environment. Steroid receptors interact with a large number of accessory proteins when binding to DNA and activating transcription.[10,30] The ARA-70 may increase AR-dependent transcriptional activity by a factor of 10.[40] Furthermore, the AIB1 gene is a recently cloned nuclear receptor co-activator that was found to be amplified in breast cancer and hypothesized to enhance estrogen-dependent growth promotion.[41] The role of such co-activators in the recurrence of human prostate cancer deserves additional study.

Furthermore, basic research into the androgen-dependent genes and the downstream growth-regulatory pathways of androgens raise interesting possibilities that should be investigated further in the clinical setting. Besides androgens, a number of other mechanisms may be

involved in the stimulation of AR-mediated gene transcription.[4,27,28] There is a substantial degree of physiological cross-talk between the different growth factor pathways. In the strong selection pressure induced by the withdrawal of androgens, activation of the alternative pathways for promoting cell survival and growth may take place. For example, fibroblast growth factor 7 (FGF-7) has been shown to activate transcription of several androgen-dependent genes.[61,63] Thus, FGF-7 (or other growth factors with similar effects) could take over some of the downstream effects of AR in tumor growth promotion. AR signaling pathway has also been shown to be activated by protein kinase A,[77] insulin-like growth factor-1, keratinocyte growth factor, and epidermal growth factor in the absence of androgens.[33,60] One can envision that, of the multitude of downstream effector molecules responsible for the maintenance of androgen-dependent cell growth, a large fraction could be autoactivated or activated by other pathways than those involving androgens (Figure 2).

Thus, studies of the molecular mechanisms of tumor recurrence should be expanded to include events both up- and downstream of the AR signaling pathway. Upstream events include the bioconversion of androgens in the prostate tissue and the role of the structural and copy number changes of the AR gene and that of the coactivators and repressors in AR-dependent transcription, whereas downstream events include the growth-signaling molecules that are either physiologically involved in androgen-dependent growth or that may assume novel functions when the androgen availability is compromised by therapy.

Conclusions

There are probably numerous mechanisms by which prostate cancer cells may become resistant to hormonal therapy. Many of these mechanisms directly involve either the AR gene or its numerous downstream effector molecules. The resulting recurrent tumors may not be exclusively androgen independent, but there may be a significant fraction of tumors that are highly dependent, and perhaps hypersensitized to the residual bioavailable androgens (Figure 2). The distinction between these two alternatives is important for developing more rational and effective therapies for patients with advanced prostate cancer. Future therapeutic interventions should be based on the detailed understanding of the molecular processes that lead to the cancer development and progression in an individual patient. Molecular pathology investigations involving not only the AR gene, but also several interconnected growth-regulatory pathways, in human prostate cancer progression will be instrumental in achieving this goal.

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