Collocation of Androgen Receptor Gene Mutations in Prostate Cancer

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ABSTRACT

Consistent with both the development of the normal prostate gland and prostate tumorigenesis being dependent on testicular androgens, targeting the androgen-signaling axis (i.e., androgen ablation therapy) remains the predominant treatment regime for patients with metastatic prostate cancer. Although there is a very good initial response to androgen ablation, these treatments are essentially palliative. Recent evidence suggests that treatment failure may not result from a loss of androgen signaling but, rather, from the acquisition of genetic changes that lead to aberrant activation of the androgen-signaling axis. A consistent finding is that androgen receptor (AR) gene mutations, present in metastatic prostate cancer and in human prostate cancer cell lines as well as in xenograft and other animal models, result in decreased specificity of ligand-binding and inappropriate receptor activation by estrogens, progestins, adrenal androgens, glucocorticoids and/or AR antagonists. Because a significant proportion of missense mutations in the AR gene reported in prostate cancer collocate to the signature sequence and AF-2, two discrete regions of the ligand-binding domain critical for androgen signaling, we recently proposed that collocation of mutations identified in prostate cancer would identify additional regions of the AR important in receptor function. This approach led to the identification of a four-amino acid region at the boundary of the hinge and ligand-binding domains of the receptor that forms half of a potential protein-protein binding site. AR gene mutations have also been identified that collocate to areas in the DNA-binding domain, to the NH$_2$-terminal transactivation domain, and to the hinge region in prostate tumors. In nearly every case, missense mutations in the AR gene identified in prostate cancer that collocate to discrete regions of the receptor contribute to altered androgen signaling and provide a potential mechanism to explain the reemergence of tumor growth during the course of hormone ablation therapies.

Introduction

The development and maintenance of the normal prostate gland requires a functional androgen-signaling axis (1, 2). The primary components of the androgen-signaling axis include the biosynthesis of androgens in the testes; the transport of androgens to target tissues; the conversion of testosterone to its more active metabolite, DHT; the binding of ligand to the AR, a nuclear transcription factor; and the transactivation of target genes (1, 2). Similarly, prostate tumorigenesis requires a functional androgen-signaling axis, and its components form the principal targets of hormonal therapies that inhibit the growth of prostate cancer. These therapies include reduction in the bioavailability of androgens by surgery (orchidectomy) or the administration of luteinizing hormone-releasing hormone agonists or antagonists (3, 4). In addition, AR antagonists such as hydroxyflutamide, bicalutamide, or nilutamide are used either alone, to block the actions of androgens in tumor cells, or in combination with luteinizing hormone-releasing hormone agonists (3, 4). Although these approaches result in tumor regression and a decline in PSA levels, they are not curative, and the majority of patients succumb to disease (4, 5).

Mechanism of Therapy Failure

Studies using prostate cancer cell lines and the Dunning animal model suggested initially that loss of AR gene expression could be a mechanism for loss of androgen sensitivity in vitro and failure of hormonal therapy in vivo (6, 7). Subsequent immunohistochemical studies of clinical prostate cancer demonstrated that AR is expressed in essentially all metastatic tumors, including those that continue to grow after androgen ablation (8, 9). Moreover, recent studies suggest that amplification or mutation of the AR gene could contribute to disease progression (reviewed in Ref. 9). Although amplification of the
AR gene has been reported in approximately 22% of prostate cancer metastases (10), and in 23–28% of primary tumors after androgen deprivation (11, 12), this mechanism alone is not sufficient to explain the failure of hormonal therapy to prevent progression of the disease. Identification of an AR gene mutation in the human prostate cancer cell line LNCaP, which results in an AR variant (Thr-Ala877)⁴ that is inappropriately activated by progestins, estrogen, adrenal androgens, and the antiandrogen hydroxyflutamide (13), provided insight into a molecular mechanism that could contribute to the failure of androgen ablation therapies. In particular, somatic mutations in the AR gene resulting in a similar phenotype to that of the LNCaP AR could provide an explanation for the clinical syndrome of steroid-hormone and anti-antiandrogen withdrawal response. This syndrome is characterized by tumor regression and decreasing serum levels of PSA in hormone refractory patients when treatment with an antiandrogen, a progesteragenal agent, or estrogen is selectively discontinued at a time of clinical progression of disease (14–18). Activation of AR variants by classical antagonists could promote the survival of cells expressing these variants, whereas withdrawal of these antagonists would lead to tumor regression. Somatic missense mutations in the AR gene have been detected in primary and metastatic forms of prostate cancer at frequencies of up to 44% and 50% of cases, respectively (9, 19–21), as well as in human prostate cancer cell lines and xenografts (13, 22, 23).

**Collocation of AR Gene Mutations in the LBD**

In contrast to the inherited syndrome of androgen insensitivity, where germ-line mutations in the AR gene result in the loss of receptor function and cause abnormal male sexual development (24–26), somatic missense mutations in the LBD of the AR gene identified to date in clinical prostate cancer result in receptor variants with either reduced discrimination for ligand-dependent activation of the receptor or a gain in functional activity in response to native and/or nonclassical ligands (9, 20, 22, 27, 28). Moreover, the majority (79%) of missense AR gene mutations identified in the LBD of the AR in clinical prostate cancer cluster to three discrete regions that influence AR activity and, collectively, span only 8% of the receptor coding sequence. These regions are (a) codons 701–730, which encompass the “signature sequence,” the highly conserved loop between helices 3 and 4 of nuclear receptors (29); (b) codons 874–910, which flank AF-2, the primary binding site for the p160 accessory proteins (coactivators) essential for AR activity; and (c) codons 670–678, which are located at the boundary of the hinge and LBD and may define a protein-protein interaction site (Refs. 26, 30; Fig. 1, A and B). Two additional mutations have been identified by independent studies (31, 32) in adjacent codons in the hinge region (Fig. 1A).

The most characterized group of mutations in the LBD of the AR identified in clinical prostate cancer are those located in the signature sequence, a 20-amino acid region of nuclear receptors that contains most of the conserved amino acids involved in ligand recognition and specificity (29). In vitro characterization has demonstrated that mutations identified in this region of the AR in clinical prostate cancer do not abrogate ligand-binding, but result in receptors that exhibit an altered response to estrogens, progestins, adrenal androgens, and other nonclassical ligands (28, 33–35). Two recent reports defining the AR crystal structure⁵ confirm these observations, because none of the amino acids that are known to be mutated in prostate cancer correspond to those that are absolutely essential for direct contact with the ligand (36). This contrasts with the complete form of androgen insensitivity (CAIS), where loss of ligand-binding is commonly associated with mutations that collocate to codons 688–710 (26), which are adjacent but NH₂-terminal to the signature sequence. Mutations in this region of the AR that result in CAIS occur in amino acids that make direct contact with the ligand (36, 37).

AR gene mutations that collocate to the region flanking AF-2 in prostate cancer also exhibit altered responses to a variety of androgenic and nonandrogenic ligands (reviewed in Ref. 9). The LNCaP AR variant, which exhibits increased activity in response to progesterone, 17β-estradiol, adrenal androgens, and hydroxyflutamide compared with wtAR, is the prototypical example of how a mutation in this region could result in a selective growth advantage for the tumor cells in which it is expressed (38). Matias et al. (36) have shown that 5 of 6 of the mutations identified in prostate cancer that are located in or adjacent to the ligand-binding pocket cluster around the Thr877 residue. The AR crystal structure presented in the study by Matias et al. shows that Thr877 is involved in the orientation of the ligand into the pocket but is not essential for ligand binding (36). Moreover, using a homology model of the AR, we recently demonstrated that the Thr877 residue comprises a large portion of the ligand-binding pocket surface, and that substitution of this residue for alanine results in changes to the shape and volume of the pocket such that bulkier ligands like progesterone can be accommodated (39). This observation was recently confirmed by crystallization of the AR-LBD containing the Ala877 mutation complexed with the native ligand, DHT. Together, these observations provide a structural justification for the altered response to nonclassical ligands observed for receptors containing mutations in the region of AF-2. However, other mechanisms, such as cofactor recruitment, may be important. For example, binding of the receptor antagonist, hydroxyflutamide to the LNCaP AR variant results in the release of putative repressor proteins that are normally only dissociated after the binding of agonists to the wtAR, thereby resulting in activation of the mutant receptor (40). In addition, it is possible that mutations in the AR gene adjacent to AF-2, as have been identified in clinical prostate cancer, could create subtle changes to the receptor-p160 binding surface, resulting in a gain of receptor function. Of note is that none of the mutations identified in this region in prostate cancer are located within the AF-2 core region (amino acids 891–897) where missense mutations, as identified in CAIS, abolish p160 binding and result in an inactive receptor (41).

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⁴ Numbering of amino acids is in accordance with that used in the AR Gene Mutations Database (26).

⁵ J.S. Sack, Bristol-Myers Squibb, Princeton, NJ, in press; and personal communication.
We have recently defined another region of the AR, located at the boundary of the hinge and LBD (i.e., 670-QPIF-673), where mutations identified in clinical prostate tumors collocate (30). AR variants arising from mutations in the 670-QPIF-673 tetrapeptide exhibit a 2- to 4-fold greater transactivation activity in response to DHT, nonclassical ligands, and hydroxyflutamide compared with
the wild-type receptor (30). An AR gene mutation identified in the TRAMP model (42, 43) also collocated with mutations identified in clinical prostate cancer to the 670QPIF673 tetrapeptide and exhibited a similar 2- to 4-fold greater transactivation activity compared with wtAR. None of the mutations identified in 670QPIF673 residues result in altered ligand-binding kinetics, receptor levels, or DNA-binding capacity compared with wtAR (30). Homology modeling revealed that 670QPIF673 residues are the sole components of a ridge bordering a hydrophobic cleft that forms a potential protein-protein interaction surface present in both the apo- and holo receptor conformations (30). Although the nature of the protein(s) that bind to this site is not known, several lines of evidence suggest that the region of the AR containing 670QPIF673 residues may be involved in an interaction of the receptor with repressor proteins that impair ligand-dependent activity directed by AF-2 (40, 44, 45). Disruption of the binding of a repressor protein by mutation of 670QPIF673 residues would therefore be consistent with the observed phenotype of increased activity that is independent of the nature of the bound ligand. Alternatively, it is conceivable that these mutations could enhance the binding of a coactivator, thereby resulting in similar functional consequences. Consistent with the observation that mutations in this region result in increased receptor activity, no mutations have been identified in the 670QPIF673 motif in CAIS.

Collectively, the observations described above suggest that mutations located in key functional subdomains of the AR could confer a survival advantage to prostate cancer cells, the extent of this being dependent on the hormonal environment of the tumor cells. This hypothesis is supported by the identification of AR variants in metastatic prostate tumors that are activated by hydroxyflutamide but not by the related AR antagonist, bicalutamide, after adjuvant treatment with hydroxyflutamide (35). To date, all AR gene mutations identified in human prostate cancer cell lines collocate to the same regions in the LBD as do mutations identified in clinical tumors and result in receptors with similar aberrant responses to nonclassical ligands (13, 46–48). A double mutation was recently reported in the AR gene in the MDA PCa 2b prostate cancer cell line (47) that results in two substitutions, Thr-Ala877 (identical to the LNCaP ARA70, and increased response to R1881 but not 17β-estradiol) only in the presence of the coactivator, ARA70, and increased response to R1881 but not 17β-estradiol in the presence of the coactivator, ARA160 (39). This suggests that mutations in the NH2-terminal transactivation domain of the AR may have an activity that could promote prostate tumor growth in vivo, but that the phenotype for some of these mutations may be manifest only in the presence of the appropriate cofactor. Therefore, it is possible that alterations in the profile of cofactors in prostate cancer cells attributable to altered expression or amplification, as has been observed in sporadic breast tumors (59), could be an important determinant of the selection of AR gene mutations in prostate cancer.

Analogous to the observations for the hinge and LBD, AR gene mutations identified in clinical prostate cancer also collocate to discrete regions of the NH2-terminal transactivation domain of the receptor that are implicated in mediating receptor activity. In a recent study, we examined the complete coding sequence of the AR gene for mutations in metastatic tissue biopsies from 12 patients who exhibited the clinical syndrome of steroid-hormone and antiandrogen withdrawal response to hydroxyflutamide. Four of 7 mutations identified in the tumor samples collocated to a small COOH-terminal portion (codons 502–535) of the NH2-terminal domain of the AR. An additional mutation (Asp-Gly526) identified previously in our studies of primary prostate tumors (21) and contains a number of overlapping, strong constitutive transactivation functions that modulate AR activity in a cell- and promoter-dependent manner (55–57). Although this domain accounts for approximately 60% of the coding region, it is extremely GC rich, which has limited the ability of PCR-based approaches to examine the NH2-terminal transactivation domain of the receptor for sequence alterations in prostate cancer. Nevertheless, in the only two studies to date to examine the complete AR coding sequence in advanced prostate cancer, approximately one-half of the AR gene mutations identified were located in the NH2-terminal domain of the receptor (21, 58). Similarly, in a recent study more than one-half of the missense mutations identified in the AR gene in tumors derived from TRAMP mice at 24–28 weeks of age were located in the NH2-terminal transactivation domain of the receptor (39). Intriguingly, in that study 7 of 9 of the mutations identified in TRAMP mice that were castrated at 12 weeks of age were located in the NH2-terminal transactivation domain, whereas in intact animals, 6 of 6 of the mutations identified were in the LBD. These observations provide additional evidence that the hormonal environment may drive the selection of AR gene mutations conferring a particular phenotype. In support of this hypothesis, 4 of 9 of the mutations identified in castrated TRAMP mice resulted in receptors with increased transactivation function in the absence of ligand, and none resulted in the loss of receptor activity (39). More detailed characterization of the mutations in TRAMP mice revealed that one NH2-terminal substitution (Glu-Gly231) resulted in a receptor with increased transactivation function compared with wtAR in response to R1881 and 17β-estradiol only in the presence of the coactivator, ARA70, and increased response to R1881 but not 17β-estradiol in the presence of the coactivator, ARA160 (39). This suggests that mutations in the NH2-terminal transactivation domain of the AR may have an activity that could promote prostate tumor growth in vivo, but that the phenotype for some of these mutations may be manifest only in the presence of the appropriate cofactor. Therefore, it is possible that alterations in the profile of cofactors in prostate cancer cells attributable to altered expression or amplification, as has been observed in sporadic breast tumors (59), could be an important determinant of the selection of AR gene mutations in prostate cancer.

NH2-terminal Transactivation Domain

The large NH2-terminal domain of the AR interacts with both cofactors (51–53) and the LBD of the receptor (54) and

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another identified in the TRAMP model (39) collocate to this region of the AR. This COOH-terminal region of the NH2-terminal domain of the AR is known to modulate the transactivation capacity of the receptor in both a ligand-dependent and ligand-independent manner (60, 61) and has recently been shown to be involved in direct interactions with the p160 coactivators and the transcription regulator p300/CBP (51, 62, 63). This suggests that mutations in this region could alter the ability of the receptor to respond to these and other cofactors, thereby altering the transactivation capacity of the AR in a manner that could provide a growth advantage.

Additional evidence in support of this region being important in AR transactivation has been provided by preliminary analysis demonstrating that one of these AR variants, Ser-Gly515, exhibits increased transactivation compared with wtAR in response to DHT in a promoter-dependent manner.\(^8\)

Collocation of AR gene mutations in clinical prostate cancer in the NH2-terminal transactivation domain also occurs within or adjacent to the polymorphic CAG repeat (codons 54–78) that encodes a glutamine tract of variable length (48, 64, 65). The majority of epidemiological studies to date have reported an association between a reduction in CAG repeat number and an increased risk of developing prostate cancer (48, 65–68), earlier age of onset (69), and more aggressive disease (48). Initial studies investigating the effect of reduced CAG repeat length on receptor activity were inconclusive, demonstrating either an increase in transactivation capacity (70–74) or no significant effect (61, 75, 76). A more recent study demonstrated that increased AR activity associated with a shorter CAG repeat length is dependent on cell context (57), possibly accounting for the discrepancy between some reports in the literature. In vitro analyses suggest that increased AR activity associated with shorter CAG repeat length could be attributable to alterations in receptor mRNA stability, interaction with cofactors that bind to the NH2-terminus of the receptor, or interaction between the NH2- and COOH-terminal domains of the AR (52, 57, 62, 75, 77). An effect of CAG repeat length on AR activity could explain the association between CAG repeat length and prostate cancer risk. Somatic contractions in the CAG repeat of the AR gene, which potentially increase AR activity in a subpopulation of cells and thereby contribute to disease progression, have been identified in three independent studies of clinical prostate tumors (50, 78, 79). In addition, recently we have identified a somatic mutation within the CAG repeat of the AR gene in a primary prostate tumor that results in interruption of the polyglutamine repeat by two leucine residues. Corrected for protein levels, this AR variant has a 2- to 4-fold greater ability to transactivate target genes compared with wtAR in vitro (80).\(^8\)

Four additional somatic mutations have been identified in or adjacent to the CAG repeat region of the AR gene in human prostate cancer (21) but have not yet been characterized. Additional analysis of inherited and somatic alterations in this region of the AR gene in prostate cancer is warranted to determine the contribution of this motif to AR activity and its potential to influence the development and/or progression of the disease.

DBD

Five somatic missense mutations have been identified in clinical prostate tumors that collocate to a 14-amino acid region at the COOH-terminal end of the first zinc finger motif in the DBD of the AR (21, 81). The effect of each of these mutations is unknown, but none of the codons in which they occur have been reported to contain mutations that cause receptor inactivation in CAIS. Androgen action is mediated by ligand-activated AR homodimers that either bind directly to androgen response elements at the promoter region of target genes or interact with other transcription factors such as AP-1 to up- or down-regulate genes involved in proliferation, differentiation, apoptosis, and metastasis (82–92). Mutations in the AR-DBD have been shown to selectively affect transactivation and transrepression functions of the AR on different promoters, despite a reduced DNA-binding ability (93, 94), and may represent a predisposing factor for male breast cancer (95). Because of the high homology of the DBD across members of the nuclear receptor superfamily, the cell and promoter specificity of different receptors is, in part, mediated by only a few changes in the DBD sequence (96). It has been speculated that mutations in the DBD could result in AR variants that bind to response elements normally specific for other nuclear receptors (95), leading to inappropriate activation or repression of growth regulatory pathways. In an analogous manner, mutations in AR response elements have been shown to increase the sensitivity of the enhancer for the glucocorticoid receptor (97). Modeling experiments suggest that residues in the AR-DBD could form a protein interaction surface (95), and several AR coactivators that interact with the DBD in a ligand dependent manner (98–100) are predicted to alter receptor activity via local chromatin remodeling (98), interaction with components of the transcriptional machinery (99), or inhibiting nuclear export (101). It is also possible that mutations in the DBD of the AR gene identified in prostate cancer could alter the affinity of receptor binding to response elements, resulting in altered expression of a range of target genes regulated by the AR.

Conclusions and Implications

AR gene mutations have been detected at a frequency of up to 50% in advanced and metastatic prostate cancer (12, 20, 21, 27, 31, 34, 50, 81, 102–105). In localized disease, however, only a small number of AR gene mutations have been reported (31, 50, 81, 102–104, 106), which could be attributed to the number of tumor cells expressing a receptor variant, with a gain in functional activity being in the minority until an appropriate selection such as androgen ablation is applied. The potential clinical importance of a therapy-mediated selective pressure for particular AR variants exhibiting increased functional activity is illustrated by the syndrome of steroid-hormone and anti-androgen withdrawal, which is observed in up to 30% of patients with hormone-refractory disease after discontinuation of treatment with flutamide, nilutamide, bicalutamide, diethylstilbestrol, megestrol acetate, or chlorormadinone acetate (14, 16–18, 107–111). Moreover, AR gene mutations detected in patients who were treated with hydroxyflutamide in conjunction with hormone ablation therapy result in receptors exhibiting a marked increase in activity in response to hydroxyflutamide but

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8 G. A. Coetzee, G. Buchanan, and W. D. Tilley, unpublished observations.
not to DHT or other androgenic ligands (35). Withdrawal responses have been reported at a higher incidence after combined androgen blockade with an AR antagonist compared with antagonist alone, leading the authors to conclude that prolonged exposure to antagonists, rather than a low level of androgens, was the predominant factor in the withdrawal response (15, 16).

In one study, inhibition of adrenal steroid production with ketoconazole subsequent to discontinuation of androgen therapy resulted in a higher proportion of patients (55%) exhibiting a withdrawal response and an increased duration of response (112) than that reported for withdrawal of the antiandrogen alone (15). In the TRAMP model, it has been shown recently that different hormonal environments result in the selection of AR variants with mutations in distinctly different regions of the receptor (39). Together, these findings suggest that androgen ablation therapies provide a selective pressure that results in clonal selection of prostate cancer cells whose survival is prolonged by the therapy.

Additional evidence for the hypothesis that mutations in the AR gene identified in clinical prostate cancer provide a selective growth advantage derives from the comparison between mutations identified in prostate tumors and in CAIS. Similar to the collocation of AR gene mutations in clinical prostate cancer, the majority (80%) of inactivating missense mutations identified in the LBD of the AR gene in CAIS collocate to three discrete regions (amino acids 688–710, 749–780, and 831–866) that encompass only 10% of the AR coding sequence (Fig. 1, C and D). There is essentially no overlap between the regions where mutations collocate human prostate cancer and the corresponding regions in CAIS (Fig. 1), which is consistent with the different functional consequences of these two classes of mutations observed in vitro. In addition, many of the AR gene mutations identified in tumors derived from the TRAMP model also collocate with those identified in clinical prostate cancer to the signature sequence and regions in the LBD, and the CAG repeat and COOH-terminal regions of the NH₂-terminal transactivation domain. The parallels between the development and the progression of both clinical and TRAMP tumors, the similar response to hormonal manipulation, and the observation that AR variants in both human and TRAMP tumors exhibit increased functional activity, establish the TRAMP model as an excellent in vivo system to address the ontogeny and biological significance of AR gene mutations in prostate cancer.

The finding of AR gene mutations in clinical prostate cancer, including treatment-resistant or castrate-metastatic disease, that confer increased functional activity in the presence of native and nonclassical ligands in addition to AR gene amplification, implies that resistance to androgen ablation therapy is not necessarily attributable to acquisition of an androgen-independent growth state but, more likely, is attributable to increased sensitivity of the androgen-signaling axis. This represents a paradigm shift in our understanding of hormone-refractory prostate cancer and has important implications for the development of new treatment strategies. If the androgen-signaling axis remains a determinant of prostate tumor growth after the failure of conventional hormonal therapy, new treatments are required that are more effective irrespective of the structure of the AR.

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References


