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Androgen Receptor Gene Polymorphisms and Alterations in Prostate Cancer: Of Humanized Mice and Men

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Abstract

Germline polymorphisms and somatic mutations of the androgen receptor (AR) have been intensely investigated in prostate cancer but even with genomic approaches their impact remains controversial. To assess the functional significance of AR genetic variation, we converted the mouse gene to the human sequence by germline recombination and engineered alleles to query the role of a polymorphic glutamine (Q) tract implicated in cancer risk. In a prostate cancer model, AR Q tract length influences progression and castration response. Mutation profiling in mice provides direct evidence that somatic AR variants are selected by therapy, a finding validated in human metastases from distinct treatment groups. Mutant ARs exploit multiple mechanisms to resist hormone ablation, including alterations in ligand specificity, target gene selectivity, chaperone interaction and nuclear localization. Regardless of their frequency, these variants permute normal function to reveal novel means to target wild type AR and its key interacting partners.

Keywords

androgen receptor; polyglutamine tract; AR gain-of-function mutations; humanized mice; mouse prostate cancer models; therapy selection

1. Introduction

The androgen receptor (AR) is a crucial regulator of male physiology and orchestrates the development and function of the prostate gland. Cancer of the prostate is a major health problem, confounded in etiology by numerous genetic and environmental factors (Nelson et al., 2003). Despite the marked complexity and heterogeneity in the origin and course of prostate cancer, early stages of tumor growth depend on androgen and, despite therapies aimed at depleting hormone and blocking receptor transactivation, recurrent disease depends on reactivation of AR (Scher and Sawyers, 2005). Just as cancer cells evolve over time due to both intrinsic and extrinsic forces, so AR function transitions from maintaining homeostasis to promoting oncogenesis (Litvinov et al., 2003). Given AR's key role in these processes, variation in the structure or expression of the *Ar* gene is logically suspected of influencing initiation or progression of disease. Extensive investigation of *Ar* germline

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polymorphism and somatic mutation has produced inconsistent results on the importance of AR genetic variation in prostate cancer. Despite the controversies, many insights into wild type receptor function have been derived from analysis of genetic variants implicated in disease.

In order to use experimental tools to test the role of AR variants in prostate cancer, and to avoid the confounding heterogeneity of man, we established a mouse model in which the murine *Ar* gene was converted to the human sequence by germline recombination (Albertelli et al., 2006). The DNA and ligand binding domains (DBD, LBD) of human and mouse ARs are identical, with a few amino acid differences in the hinge region (Fig. 1). However the large amino termini (NTD) encompassing the major transactivation domains differ by 15% in protein sequence as well as in position and extent of polymorphic polyglutamine (Q) and polyglycine (G) tracts. Most of the NTD is encompassed within *Ar*'s large first exon, allowing mouse and human sequences to be swapped by knock-in technology resulting in a locus in which hAR is expressed under murine regulatory control. This "humanized" model allows us to assess effects of germline polymorphisms on disease phenotypes (Albertelli et al., 2008) and to evaluate relationships between somatic mutation and treatment regimen (O'Mahony et al., 2008). Comparison to patient data directly substantiates the relevance (Steinkamp et al., 2009).

Here we present findings pertaining to controversies in the role of the AR polyQ tract and *Ar* gene mutations in prostate cancer. We summarize recent key studies in the literature but focus primarily on results from our laboratory, including data from both mouse and human prostate cancer patients. More comprehensive reviews and discussion of implicated mechanisms have recently been published (Buchanan et al., 2009; Robins, 2009).

2. AR polymorphisms and prostate cancer

AR's NTD coordinates interactions with coregulators and nonreceptor transcription factors that are critical for both ligand-dependent and -independent transactivation (Robins, 2004; Shen and Coetzee, 2005). The N-terminal polyQ tract, encoded by a CAG repeat, was first linked to pathology in Kennedy disease. Abnormal expansion of this tract underlies late-onset atrophy of spinal and bulbar motor neurons due to misfolding and aggregation of the mutant AR, compounded by partial loss of function (Lieberman and Robins, 2008). Transfection assays ascribe the latter to an inverse correlation between Q tract length and AR transcriptional strength (Mhatre et al., 1993). Sensitivity to Q tract length difference within a "normal" range (9 – 37 CAGs) is likely due to effects on overall AR structure rather than to site-specific interactions (Irvine et al., 2000). Shorter Q tracts enhance AR's critical intramolecular N-C interaction, allowing response to lower androgen concentrations and more effective coactivation by p160 proteins (Buchanan et al., 2004; Wang et al., 2004). Because Q tract length affects AR activity in transfection, associations between such variation and male traits have been sought. Substantial evidence links AR Q tract length to male fertility (Davis-Dao et al., 2007) and in hypogonadal men to response to testosterone replacement (Zitzmann et al., 2004). Association of Q tract length with prostate cancer, where a more active AR might promote oncogenesis, remains controversial.

2.1. Human genetic and genomic studies of the CAG repeat and prostate cancer

That short Q tract ARs increase prostate cancer risk was first conjectured based on their greater prevalence in the high-risk African-America population (Coetzee and Ross, 1994), and gained credence from their increased transcriptional activity *in vitro* and evidence of Q tract contraction in malignant but not benign prostate cells (Schoenberg et al., 1994; Alvarado et al., 2005). Epidemiological studies initially found association between fewer CAG repeats and increased risk or more aggressive disease (e.g., Irvine et al., 1995;

Giovannucci et al., 1997; Stanford et al., 1997)). However results vary with ethnic group and most recent studies find no association with risk (e.g., (Lindstrom et al., 2010; Price et al., 2010)). Conflicting conclusions stem from many aspects of study design, including small sample sizes, differences in environment, genetic admixture, ascertainment bias (including diagnosis before or after the age of PSA testing) and differences in tract length cut-off points. Regardless of these variables, modern genotyping technology applied to large case-controlled groups fails to substantiate *Ar* CAG tract length as a predictor of prostate cancer (Price et al., 2010). *Ar* may be more comparable to a quantitative trait, the effect of which depends on variation at other loci and on environmental interactions. In support of this, allelic differences in genes participating with *Ar* in the androgen axis, including those encoding enzymes of testosterone synthesis (cytochrome P450c17) and conversion (steroid-5- α -reductase type 2), reveal haplotypes that do differ significantly in prostate cancer risk (Lindstrom et al., 2006).

While *Ar*'s CAG repeat may not impact risk of prostate cancer, it may affect other aspects of disease that are subject to AR function, such as progression or treatment response. Several studies support a relationship between Q tract length and serum levels of testosterone and estradiol, likely due to the effect of differential AR strength in systemic feedback control (Huhtaniemi et al., 2009; Lindstrom et al., 2010). Whether Q tract variation might also impact intratumoral hormone levels, or their effect, is of interest in light of increasing evidence of prostatic androgen synthesis (Montgomery et al., 2008). Furthermore, CAG repeats are inherently unstable and Q tract contraction is found in malignant prostate cells but not in adjacent normal tissue (Alvarado et al., 2005). Heterogeneous somatic variation in Q tract length may play a role in the increased AR activity that occurs in prostate cancer recurrence. Given the complex and contradictory data on the role of the Q tract in prostate cancer, we turned to a mouse model to gain biological insight.

2.2. Effects of polyQ tract length on AR biology: insights from genetically engineered mice

To investigate the effect of AR Q tract length on prostate cancer, we developed knock-in strains with *hAr* alleles containing 12, 21 or 48 CAG repeats (Albertelli et al., 2006). All mice in this allelic series (referred to as AR12Q, AR21Q, or AR48Q mice) are grossly normal and have similar fertility, with no neurological problems evident in the AR48Q mice (much longer CAG repeats are needed to model neurodegeneration) (Lieberman and Robins, 2008). Transactivational differences due to Q tract length are however detectable in expression of prostatic AR target genes. Moreover, allele-dependent responses following hormonal perturbation corroborate a role of AR Q tract length in systemic androgen sensitivity (*ms. in press*). Therefore these mice model human AR Q tract length variation within a "normal" range.

Introducing a prostate-targeted SV40 T-antigen (Tag) oncogene by crossing to TRAMP mice reveals differential effects of the humanized *Ar* alleles on cancer initiation, progression and response to androgen ablation (Albertelli et al., 2008; Robins et al., 2008) (Fig. 2). Mice with short Q tract ARs develop prostatic intraepithelial neoplasia (PIN) earlier than mice with median (21Q) or long (48Q) tracts. However, the resulting AR12Q tumors are well differentiated, progress slowly and allow longer survival of AR12Q than AR21Q mice. Remarkably, following androgen ablation Q tract length effects are in directions opposite to those in hormone intact mice. Castrated AR12Q mice develop tumors later than the other strains and live longer than their intact littermates, indicating good response to treatment. In contrast, AR48Q-TRAMP mice fail to benefit from androgen ablation. Detection of these divergent responses due to a modest variation in AR activity is more evident on a homogeneous genetic background. This suggests that *Ar* variation at the extremes of the normal range may also impact certain cases of human disease.

Effects in TRAMP mice are complicated by the AR dependence of the oncogenic transgene, but that is unlikely to account for all differences noted. In some regards this complexity mimics the androgen-responsiveness of *TMPRSS2-ERG* fusion genes that are critical in progression (Tomlins et al., 2007). Some of the tumor characteristics may reflect varying AR functions at different disease stages and in distinct cell types (e.g., stroma vs. epithelia). For example, it is reasonable to predict that weak AR action in TRAMP would enhance castration response due to loss of the androgen-driven Tag oncogene. Instead, AR48Q castrates fare worse after castration, perhaps because weak stromal AR fails to repress growth of pre-existing androgen-independent cells. Similarly, the hypersensitive AR12Q may more ably maintain epithelial differentiation despite hormone ablation and thereby slow tumor growth. Results from these mice supports further investigation of an influence of AR Q tract variation in later stage human disease. In addition these mice provide a unique experimental model in which to define downstream events that predict response to therapy. Our future studies will elucidate mechanisms by which Q tract length impacts ligand-independent AR function and define corresponding pathophysiology in models that more accurately reflect human disease.

3. AR alterations in prostate cancer

Somatic mutation is a hallmark of cancer, whereby uncontrolled growth stems from loss of tumor suppressor genes and aberrant function of oncogenes. Coincident genomic instability leads to a mutator phenotype, in which each cancer cell may come to harbor hundreds of mutations (Bielas et al., 2006). Most mutations likely have little phenotypic effect and are considered “passengers”, but some may be “drivers” that provide a growth advantage or promote metastasis (Greenman et al., 2007; Jones et al., 2008). Recent studies using genomic tools provide quantitative data reinforcing this view. A high-throughput interrogation of 1,000 tumors for known mutations indicates that very few mutations occur frequently but mutations in several oncogenes co-occur (e.g., *PIK3CA* mutations coincide with mutations in *KRAS*), highlighting sensitive pathways rather than individual genes as potential therapeutic targets (Thomas et al., 2007). Genome-wide characterization shows that mutation rates and genes mutated vary substantially across tumor types and subtypes (Kan et al., 2010). Interestingly, mutation rates appear relatively low in prostate cancer, where ~75% of tumors carry *TMPRSS2-ERG* gene fusions (Tomlins et al., 2007). In contrast, in a lung tumor next-generation sequencing revealed a wide variety of somatic mutations, with differing patterns in expressed vs. nonexpressed genes (Lee et al., 2010). While most of these mutations are likely passengers some, such as those occurring in kinase genes, appear to be drivers because they evidence distinct selective pressures within the tumor environment. For prostate cancer, numerous mutations have been identified in *Ar* and many are gain of function (Buchanan et al., 2009). However, most aberrant ARs have been identified in small studies and the effects of these mutations have largely been determined *in vitro* (a notable exception is AR-E231G – see below). Therefore *Ar* mutation frequency and the functional significance of mutant ARs in disease remain unclear. As with the Q tract controversy, genomic approaches and mouse models are producing new insights.

3.1 AR mutations – driving in heavy traffic

Somatic gain-of-function mutations in AR are compelling because their actions implicate mechanisms of disease recurrence in castration resistant prostate cancer (CRPC). Mutations have been identified in tumors that alter ligand specificity, increase sensitivity to low androgen levels or allow ligand-independent activation, all proposed as mechanisms of therapy resistance (Feldman and Feldman, 2001). The paradigm of a mutant that could evade treatment is AR-T877A, first identified in the LNCaP cell line and subsequently in several advanced prostate cancers (Taplin et al., 1995). The subtle shift in LBD structure due to T877A permits various noncanonical ligands, including the antiandrogen

hydroxyflutamide, to confer an active conformation (Hur et al., 2004). Other LBD mutations that result in promiscuous ARs, such as H875Y, also harbor clear potential for hormone resistance. Mutations in the NTD could lead to androgen-independent AR activation but fewer examples have been identified since sequencing this region is impeded by its high GC content and polymorphic repeats. Moreover, most studies have examined small numbers of patients using methods that do not detect low frequency *Ar* mutations, thus underestimating the heterogeneity of prostate cancer between individuals as well as within the tumor. Nevertheless, *Ar* mutations have been reported in up to one-third of tumor samples and most commonly following treatment, suggesting they may be selected by therapy (Taplin et al., 1999). The use of microdissection and laser capture has greatly improved the ability to detect *Ar* mutations (Marcelli et al., 2000; Alvarado et al., 2005).

Recent comprehensive surveys with large sample sizes support the common occurrence of somatic AR alterations in prostate cancer progression, with the most frequent event being gene amplification (Holcomb et al., 2009; Liu et al., 2009; Taylor et al., 2010; Robbins et al., 2011). Targeted exon sequencing of genes of high interest to prostate cancer identified promiscuous AR mutants, including AR-T877A and AR-H875Y, in 5-10% of the samples (Taylor et al., 2010; Robbins et al., 2011). The methodology however would not have detected variants present in a subpopulation of cells or prematurely truncated ARs due to nonsense mutation or alternative splicing. The latter has become a focus of recent attention (Jagla et al., 2007; Dehm et al., 2008; Hu et al., 2009). There is surprising diversity in these splice variants, many of which lack C-terminal sequences and thereby could encode constitutive ARs that drive castration resistance. However, most variants in tumor cells are low in abundance relative to correctly processed *Ar*, appear in response to hormone withdrawal and some may require wild type receptor to function, making it unclear whether they play a dominant role in resistance to therapy (Watson et al., 2010). Recently in a cell-based model, truncated ARs due to intragenic rearrangements have been shown to promote prostate cancer progression (Li et al., 2011). As with mutant ARs, further study of alternatively spliced forms may shed critical light on how wild type receptor drives progression despite antiandrogen therapy.

3.2. AR mutation profiling in prostate tumors of mice and men

In mice, genetic background, environment and treatment can be controlled, allowing better assessment of the prevalence of *Ar* mutations in prostate cancer and the extent to which variant ARs reflect tumor biology. Prior study of intact (untreated) versus castrated (androgen ablated) TRAMP mice found that missense *Ar* mutations in primary tumors vary with hormonal status (Han et al., 2001). To obtain more direct evidence that this is due to the selection pressure of therapy, we used the humanized AR mice to ask whether distinct types of mutations arise with different treatments. hAR21Q-TRAMP mice were either untreated, castrated at 12 weeks (when PIN but not overt cancer is present), or treated with different antiandrogens upon detection of a palpable tumor. Comparison of bicalutamide to flutamide is particularly informative – the bulkier bicalutamide more significantly distorts the AR LBD and so unlike flutamide is less likely to impose an agonist conformation from a single amino acid change (e.g., AR-T877A). To identify potentially functional AR mutants that might be present in only some cells, the equivalent of 20 *Ar* mRNAs per tumor were sequenced from reverse-transcribed, PCR-amplified and subcloned cDNAs (O'Mahony et al., 2008). *Ar* sequences from testes of these mice provide an estimate of technical error rate; sequence differences in tumor samples were twice as frequent as in testes. To highlight changes more likely to be actual mutations, only those that recurred within a tumor or occurred in multiple tumors were studied. Since only one codon change recurred in testis this conservative view minimizes methodological errors. By this approach, missense

mutations in *Ar* that repeat within or between tumors occur at a rate of ~0.5/10 Kbp, with most having an overall tumor frequency of less than 10% of the cells.

Recurring missense AR mutations are least frequent in tumors from untreated mice, evidencing effects of treatment and corroborating clinical observations (Taplin et al., 1999). Mutations following castration occur mostly in the NTD, whereas flutamide-treated mice have more mutations in the C-terminal portion, including some premature stop codons that could lead to constitutively active ARs. Moreover, flutamide-treated mice have the most treatment-specific mutations. Bicalutamide treatment, as expected, led to fewer mutations that primarily occurred in the NTD. Many of the AR mutations in mouse prostate tumors cluster to regions found previously mutated in patients and are generally distinct from loss-of-function mutations identified in Androgen Insensitivity Syndrome (AIS) (Buchanan et al., 2009). Many mutations cluster near the most highly conserved segment of the LBD that affects ligand specificity (Fenton et al., 1997). Some of these that are known to be loss of function as germline mutations are surprisingly active in transfection assays. This suggests that mutations that are null in development may display partial or altered function in other contexts, such as the tumor environment (see below).

To compare directly in man whether mutations differ in number or type after treatment, and whether distinct antagonists select for different mutations, we performed a similar analysis with a set of high quality samples of metastases from either hormone-naïve patients or those treated with only one antagonist (flutamide or bicalutamide) (Steinkamp et al., 2009). As with the mouse samples, the equivalent of 20 *Ar* mRNAs were sequenced and similar base change rates were found. Interestingly, despite having fewer human than mouse tumors, more mutations recurred at higher rates (both in more tumors and at higher levels per tumor), providing stronger evidence of treatment selection. In general, mutations occurring at low frequency but in multiple tumors are located in the NTD. In contrast, mutations present in several clones from a single patient tend to be case-specific, not restricted by AR domain and reflective of treatment differences (Fig. 3). Few mutations recur in hormone-naïve samples. The “mutator phenotype” is not equal between samples, with less than half of the tumors accounting for most of the mutations.

Several similarities emerge from our mouse and human *Ar* mutation profiling, including comparable overall mutation frequencies and fewer mutations in hormone-naïve tumors. The variety of recurring mutations suggests that selective value may stem from numerous rare mutations rather than a few common ones, with detection of rare events dependent on sequencing methodology. A significant difference between the mouse and human studies is that more mutations were present at higher frequencies in man. This may reflect the longer time with disease in man allowing a greater period for selection to operate (Steinkamp et al., 2009). Furthermore, human metastases are more clonal in nature than heterogeneous mouse primary tumors. In fact, recent analyses using next generation sequencing suggest somatic genetic variation and selection play a greater role in cancer ontogeny than previously suspected (Gottlieb et al., 2010). Even silent base changes are not random, suggesting selection for greater AR activity may operate at the level of efficiency of translation or co-translational protein folding. Below specific examples illustrate how *Ar* mutations may be functionally relevant to prostate cancer progression.

3.3 Mutant ARs exploit multiple mechanisms to evade therapy

Mutations found in numerous studies cumulatively highlight AR domains within which different mutations may affect a common function and produce a similar phenotype (Buchanan et al., 2009). Further evidence that these mutant ARs may not be simply bystanders is inherent in the means they may use to evade therapy and is reinforced by finding fewer additional mutations in tumors with prevalent variant ARs. For example, the

human tumor in which we identified the promiscuous AR-V716M (below) had only one other missense mutation (Steinkamp et al., 2009). Similarly, few AR mutations recur in xenograft tumors originating from VCaP cells that have amplified *Ar* – additional variation may have little value when high levels of wild type AR are attained (*O'Mahony and Robins, unpublished*).

3.3.1 Promiscuity is priceless: AR-V716M—A mutation most clearly supporting treatment selection is V716M, an alteration known to create a permissive receptor responsive to a wide array of hormones and antagonists (Culig et al., 1993). This mutation was present in all 20 *Ar* clones sequenced from the lung metastasis of a flutamide-treated patient whose normal tissue DNA was wild type (Steinkamp et al., 2009). Two additional metastases from this patient also yielded only the mutant sequence, indicating a clonal population carrying AR-V716M accounted for these three metastases and therefore arose either early in metastatic invasion or within the primary tumor itself. The absence of other *Ar* mutations recurring in this patient suggests this variant was effective enough to reduce selective value of other mutations. While fixation of a mutation like V716M may be relatively rare, many cancers may have subsets of cells with different mutations that together provide a similar growth advantage. Interestingly, the patient with AR-V716M survived 19 years beyond diagnosis. It is tempting to speculate that in this case residual activity of the promiscuous AR fostered slower tumor growth than a poorly differentiated tumor lacking AR altogether.

3.3.2 Unchaperoned oncogenic behavior: AR-E255K—The AR NTD has been less examined for somatic mutation, but in the eleven human samples we studied 19 mutations recurred in the NTD and 14 of these fell into known functional motifs. The most highly conserved region of the AR NTD encodes a domain that interacts with CHIP (COOH-terminus of **H**SP70-**I**nteracting **P**rotein), an E3-ubiquitin ligase that controls steady-state levels of AR by promoting its degradation (He et al., 2004). *Ar* mutations in this region have been identified in both mouse and human prostate tumors and may abrogate cell checks on AR activity. Importantly, the mouse mutant, AR-E231G, is sufficient for oncogenesis as a prostate-targeted transgene (Han et al., 2005). A similar human mutant, AR-E255K, from a flutamide-treated patient prolongs the half-life of AR in both the presence and absence of ligand (Steinkamp et al., 2009). Moreover, a substantial proportion of AR-E255K localizes to the nucleus in the absence of hormone. CHIP interaction domain mutants are not only unfettered from ligand control but also exhibit increased and differential transactivation (Buchanan et al., 2009; Steinkamp et al., 2009). For AR-E255K this is more notable in benign RWPE cells than in malignant PC-3 cells and varies with target promoter; differences in specificity as well as activity could reflect differences in the array of host coregulators. Varied transactivation by mutant ARs demonstrate how they not only drive persistent gene expression in the face of androgen ablation but that they may do so differentially, promoting distinct pathways of tumor progression that may differ in selective value dependent on disease stage.

3.3.3 Avenues of differential gene expression: AR-W435L, AR-R753Q—

Mutations in almost any receptor domain may influence target gene selection via conformational changes that impact recognition or affinity for the response element or that alter intra- or intermolecular interactions. Two mutations from our studies exemplify the multiple ways transcriptional selectivity may be affected. AR-W435L, found in two antiandrogen-treated patients, alters the WxxLF motif that plays a role in AR's critical N-C interaction. While this motif is secondary to the more N-terminal FxxLF motif essential for ligand-dependent activity, WxxLF may be more important in ligand-independent activation (Dehm et al., 2007). W435L changes the motif to LxxLF, which bears greater similarity to

the LxxLL interaction motif of coactivators. The effect of this is to strengthen N-C interaction, as demonstrated in a mammalian two-hybrid assay, and to confer stronger transactivation of a reporter gene (Steinkamp et al., 2009). Interestingly, AR-W435L activity varies with the host cell and target promoter tested, suggesting differential promoter recognition as well as broadly increased AR activity may have selective value and may change over the course of disease.

Some AR alterations that arise in prostate cancer are presumed to be null mutations, particularly if they were previously identified in AIS. Yet some have partial or altered function that could be significant in cancer. AR-R753Q is especially intriguing since as a germline mutation it underlies rat testicular feminization as well as cases of human AIS. The molecular basis for this is reduced androgen binding capacity resulting in impaired N-C interaction. In transfection into prostate cells, AR-R753Q proves to be as potent as wild type AR at activating a subset of promoters. AR-R753Q fails to activate selective AREs that bind AR with greater specificity but show weak activation (O'Mahony et al., 2008). A similar inability to activate AR selective elements occurs in a mouse with an AR DBD mutation that leads to reduced fertility (Schauwaers et al., 2007). Thus although germline AR-R753Q cannot activate genes critical for male development, somatic AR-R753Q may induce a subset of genes, perhaps those involved in oncogenic rather than differentiative pathways. Numerous other mutations may produce a similar loss of function in the context of development but a gain of function in the context of cancer.

3.3.4 Alternative splicing as a path of resistance: AR23—Variant proteins that arise from alternative splicing (distinct from actual mutation) are widespread in cancer (David and Manley, 2010) and may be particularly significant in CRPC where constitutive ARs can be produced from misspliced transcripts that omit the LBD (Dehm et al., 2008; Hu et al., 2009). The mechanism by which these variants arise is unclear, but the fact that their occurrence increases rapidly when androgen is withdrawn and recedes when hormone is readministered suggests AR may be involved, directly or indirectly, in processing its own transcript. AR, like other steroid receptors, is known to participate in splicing (Auboeuf et al., 2002; Dong et al., 2007) and disease conditions or aberrant ARs may foster aberrant splicing events (Yu et al., 2009). Many misspliced ARs terminate prematurely, destroying their ability to bind ligand and freeing them from cytoplasmic tethering by HSP90, thus permitting their localization to the nucleus where they are constitutively active. These variant forms are usually a small fraction of the total *Ar* mRNA in the cell and it remains unclear whether they provide a major means of treatment resistance (Watson et al., 2010; Li et al., 2011).

A distinct misspliced *Ar* called AR23 differs from the constitutive variants in lacking intrinsic activity but promoting full-length AR function. We found this variant in the majority of antiandrogen-treated patient samples (Steinkamp et al., 2009). Use of a cryptic splice site in intron 2 inserts 69 bps in frame to create a 23 amino acid extension between the two zinc fingers of the DBD. AR23 was previously identified in an AIS patient due to a mutation upstream of exon 3 and also in a bicalutamide-treated prostate cancer patient (Bruggenwirth et al., 1997; Jagla et al., 2007). Beyond being incapable of binding DNA, AR23 forms cytoplasmic speckles in response to hormone. While this receptor cannot participate directly in gene regulation, cotransfection of AR23 with wild type AR leads to greater activation of a reporter gene, even in the presence of antagonist. This action is not specific to AR since some other transcription factors are also enhanced in their activity in the presence of AR23. AR23 likely exists in an unfolded state, as suggested by its cytoplasmic aggregation, which may compromise the cell chaperone system critical for normal protein activity. In this manner AR23 may sustain wild type AR activity in the presence of antagonists, as supported by its prevalence in treated but not untreated patients.

4. Concluding Remarks

The genomic era has added significant depth to our understanding of prostate cancer. Some discoveries are remarkable, such as the unanticipated and broad role of *TMPRSS2-ERG* gene fusions. These findings only serve to substantiate AR as a central player in all stages of disease and, as yet, the key target in therapy. Studies with genetically engineered mice allow functional analyses not possible in man, using homogeneous genetic backgrounds and uniform environments to highlight distinct disease modalities. Results from our lab and others confirm the relevance of *Ar* genetic variation to disease progression, and derive novel insights into mechanisms of treatment resistance from specific variations.

In mice, differences in AR Q tract length exert measurable effects on disease parameters including development of PIN and rate of cancer progression, both in the presence and absence of hormone. While Q tract length is not by itself a risk factor for prostate cancer in man, it contributes to variation in the androgen axis, in which many genes conspire to influence hormone sensitivity. Q tract lengths at the extremes of the normal range may have more detectable effects when hormone levels are changing, as occurs in development and aging, or clinically, as when hormone is replaced in hypogonadal men or ablated in prostate cancer treatment. Individual variation in efficacy of new drugs that more effectively inhibit androgen synthesis (e.g., abiraterone) may reveal an influence of AR Q tract length.

Somatic *Ar* mutations are generally low in frequency but provide valuable information about mechanisms of treatment resistance. Myriad mutations affect multiple distinct functions (specificity of hormone and DNA binding, coactivator and chaperone interactions, nuclear localization), with downstream gene expression programs varying with the receptor form. AR's enormous plasticity in evading treatment is similar to the complexity p53 demonstrates whereby many different mutations drive transition from tumor suppressor to oncogene. The mutability of AR is but one factor making it unlikely that single agents will universally abolish activity. Promising new drugs such as MDV3100 and EPI-001 provide new environments for selection (Sadar, 2011); cases of resistance may reveal additional novel AR alterations. Moreover, improved anti-AR therapies may impair differentiation and therefore be ineffective in redirecting cell growth in certain instances. The mutant ARs direct attention to upstream and downstream interacting partners that may provide new ways to combat disease in general or to fight resistance in specific cases. The broad landscape of low level *Ar* mutation suggests AR is more than a passenger, but not often a driver. Nevertheless the difficulty of silencing AR in prostate cancer suggests it acts as a backseat driver.

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Highlights

- Genomics and mouse models shed light on the role of AR gene variation in cancer
- AR's polymorphic glutamine tract influences prostate cancer progression in mice
- AR somatic mutations identify multiple mechanisms and pathways to evade therapy
- Targeting interacting partners identified by mutant ARs may combat resistance

Fig. 1.

Structural and functional domains of the AR. Diagrammed are the largely unstructured amino terminus (NTD), zinc-chelating fingers of the DNA binding domain (DBD), and the ligand binding domain (LBD) that is comprised of 12 alpha helices, with dihydrotestosterone (DHT) depicted in the pocket. Relative positions are indicated for the polymorphic Q tract, motifs involved in N-C interaction (FxxLF, WxxLF), and activation functions that depend on ligand binding (AF-2) or are ligand-independent (AF-1, AF-5).

Fig. 2.

Mouse prostate cancer parameters vary with AR Q tract length. At top is modeled the inverse correlation between Q tract length and AR transcriptional activity based on transfection assays. In 12 week old TRAMP mice, the percent of prostate dorsal lobe epithelium involved in PIN is greatest in 12Q and least in 48Q mice. Despite this apparently earlier initiation, tumors in 12Q mice progress slowly (leading to a longer time with disease) and have a well-differentiated phenotype; tumors in 48Q mice progress slowly after a long lag to initiation. Following androgen ablation, 12Q mice develop palpable tumors much later than intact littermates, whereas there is no difference in time to palpation for 48Q mice. This qualitative depiction summarizes data in Albertelli et al., 2008.

Fig. 3.

Recurring AR mutations from human prostate cancer metastases. Missense mutations found in more than one tumor or multiple times in a single tumor are shown; those occurring multiple times in a single patient are underlined. Color code for treatment groups is as follows: green – flutamide, purple – bicalutamide, orange – both antiandrogens, blue – no endocrine treatment, black – both treated and untreated.