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ANDROGEN METABOLIC GENES IN PROSTATE CANCER PREDISPOSITION AND PROGRESSION

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1. ABSTRACT

Significant evidence implicates androgens in prostate cancer etiology. We review recent data with regard to the association between several allelic variants of specific androgen-metabolic genes and the predisposition to prostate cancer. We also review the emerging evidence regarding the role of genetic variants of these genes as well as the androgen receptor in prostate cancer progression. Based on the prostate cancer paradigm, we propose that a multidisciplinary attack on the problem—involving biochemistry, genetics, pharmacogenetics, endocrinology and molecular epidemiology—may be important for the understanding and successful treatment of complex (in terms of etiology) human diseases.

2. PROSTATE CANCER

It is estimated that 232,090 U.S. men will be newly diagnosed with prostate cancer in 2005 and 30,350 will die of this disease (1). Prostate cancer is the most common malignancy among men in the United States (1). Prostate cancer also represents a substantial public health problem in other industrialized nations, for example those of the European Union (2) This disease is rare in younger men before the age of 40, but the rate of increase thereafter is greater than for any other cancer(3). There is a large variation in prostate cancer rates between racial/ethnic groups. For example, in Los Angeles, African-Americans, who also have the highest prostate cancer rate in the world, have a 70% higher rate than Caucasian Americans, who

have a substantially higher rate than Hispanic Americans (Latinos), while Chinese- and Japanese-Americans (i.e. Asian-Americans) have roughly one-half the rate of Caucasians (4). It is noteworthy that Chinese and Japanese men have among the lowest prostate cancer rates in the world, they are about 1/8th to 1/20th the rates of their U.S. counterparts(4). These data strongly suggest a substantial genetic component to prostate cancer risk, although environmental factors are also known to play an important role. Finally, there is significant evidence of more aggressive disease and a less favorable outcome (e.g., lower survival) for African-American prostate cancer patients than their Caucasian-American counterparts (5), which may be at least partially due to differences in socioeconomic status.

There is a significant familial and, therefore, most likely genetic component to prostate cancer risk. In younger men, the familial form is most consistent with an autosomal dominant way of inheritance (6). The location and action of putative familial loci for prostate cancer, however, remain controversial (reviewed in reference 7). Here we examine recent findings of both constitutional SNPs (single nucleotide polymorphisms) and somatic mutations in androgen-metabolic genes and the conclusions that can be drawn for prostate cancer predisposition and/or progression.

3. ANDROGENS AND PROSTATE CANCER

Androgens play a critical role in both normal and abnormal prostate development (Figure 1). Studies of androgens and prostate cancer go back over 60 years for which Charles Huggins won the Nobel Prize for his discoveries concerning the hormonal treatment of prostate cancer in 1966 (8, 9). In men, testosterone is synthesized in large amounts, primarily by the Leydig cells of the testes (Figure 1) (10). Testosterone is transported into the prostate where it is irreversibly metabolized intracellularly to dihydrotestosterone (DHT; Figure 1) (10). DHT (or, much less efficiently, testosterone) is bound by the intracellular androgen receptor (AR; Figure 1) (10). This complex then translocates to the cell nucleus where it activates the transcription of various genes with androgen-responsive elements (AREs) in their promoters (Figure 1) (10). DHT can also be inactivated in the prostate by further reduction to 3α- or 3β-androstanediol by the 3α- and 3β-hydroxysteroid dehydrogenase enzymes (10).

Numerous epidemiologic studies have examined the association between serum androgen levels and prostate cancer risk (reviewed in reference 11). In this review we will instead focus on the association between androgen metabolic genotypes and prostate cancer.

4. STEROID 5A-REDUCTASE

Steroid 5α-reductase (or testosterone 5α-reductase; 10, 12) catalyzes the irreversible conversion of testosterone to DHT, the most potent androgen. Two isozymes exist: the type I steroid 5α-reductase which is encoded by the SRD5A1 gene and the type II isozyme, which in turn is encoded by the SRD5A2 gene (Table 1)(10, 12). The type I isozyme is primarily expressed in liver and in skin cells, whereas type II plays a major role in prostate development and disease predisposition. The SRD5A2 gene is located on chromosome 2 (band 2p23; Table 1) and spans over 40 kb of genomic DNA, with 5 exons and four introns (10). Many mutations in the SRD5A2 gene that cause a rare form of male pseudohermaphroditism have been identified and characterized by functional studies (for examples, see references 10 and 12).

Several investigations have examined the androgen metabolic gene SRD5A2 as a candidate gene for prostate cancer. In an extensive screen for SRD5A2 SNPs (single nucleotide polymorphisms;13) a nonsynonymous SNP was found, the A49T variant (Figure 2), which results in the replacement of the normal alanine in codon 49 with threonine (Table 1). The A49T missense substitution has been reported to significantly increase the risk of prostate cancer in different populations (14-16) and increases the apparent maximal steroid 5α-reductase activity (V_{max}) 5-fold *in vitro* (14). Therefore, the kinetic data provide a likely explanation for the increased risk associated with the A49T mutation. Other epidemiologic studies, however, have reported a non-significant association between the A49T variant and prostate cancer predisposition (17-19). Another SRD5A2 SNP, the V89L missense substitution (replacing the valine at codon 89 with the polymorphic leucine; Table 1; Figure 2) has also been associated with an increased prostate cancer risk (20). Other studies however, have found no association between the V89L SNP and prostate cancer (21). These ambiguities may perhaps be explained by the distinct racial/ethnic make-up of the samples and the modest biochemical effect of the V89L SNP discussed below.

Functional *in vitro* studies of SRD5A2 variants have provided critical mechanistic insights and often parallel *in vivo* findings (6,22,23). As noted above, the A49T substitution results in a significant increase in V_{max}, suggesting that the substantially increased risk associated with this mutation may be the result of higher intraprostatic DHT levels caused by the A49T variant. The V89L variant in contrast results in a very modest decrease in V_{max} (23). Functional data may be important in choosing candidate SNPs suitable for epidemiologic studies (24) since an almost 200-fold variation has been demonstrated in the V_{max} of various SRD5A2 variants (23). Therefore, variants with substantially higher (or lower) activity may be more suitable markers for molecular-epidemiologic association investigations than neutral polymorphisms, because mechanistically important variants may be directly responsible for the observed associations (24).

The SRD5A2 locus also encodes significant (~ 60-fold) pharmacogenetic variation for the competitive steroid 5α-reductase inhibitor finasteride (22, 23, 25). Interestingly, finasteride has a reduced affinity for the A49T mutant enzyme *in vitro* (22, 23, 25). Similar variation was identified for many other SRD5A2 enzyme variants and for two other 5α-reductase inhibitors (23, 25).

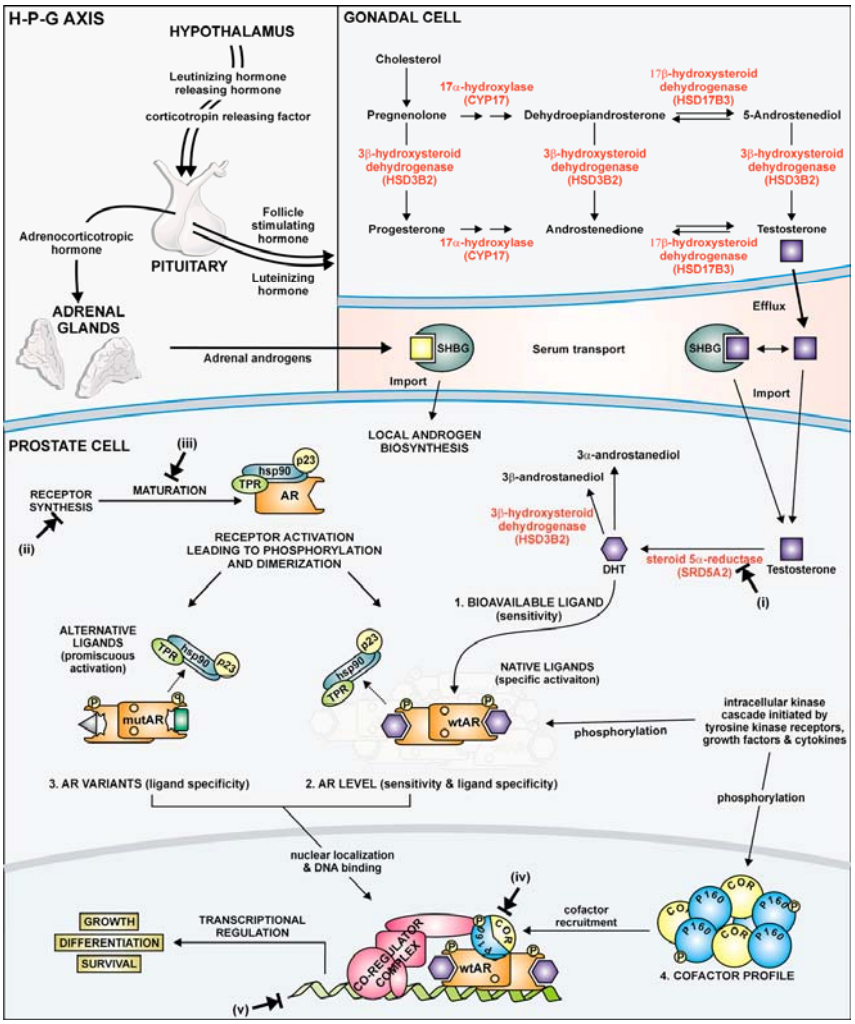

Figure 1. The Androgen signaling axis with particular emphasis on the biochemical and signaling pathways discussed in this review. Androgen signaling initiates in the hypothalamic-pituitary-gonadal (H-P-G) axis. Through a cascade of hormonal stimuli, the adrenal glands and gonadal organs are stimulated to produce androgens. In gonadal cells, the major biosynthetic steps in the conversion of cholesterol to testosterone (T) are illustrated. In the serum, up to 95% of the testosterone is bound to steroid hormone binding globulin (SHBG). It is predominantly the proportion of free testosterone that is taken up by prostate cells, where it is rapidly converted by steroid 5α-reductase to the more bioactive androgen, dihydrotestosterone (DHT). The contribution to androgen signaling in the prostate of enzymes that catabolize DHT is still being elucidated. The vast majority of androgen signaling in the normal prostate is then mediated by the androgen receptor (AR) in response to increasing levels of DHT. However as detailed below, prostate cancer cells often evolve alternative means of activating the AR in a castrate environment. Following synthesis, the AR exists in dynamic equilibrium between an immature state and an active form capable of binding high affinity androgenic ligands via association/dissociation with a complex that includes heat-shock proteins, p23 and a tetratricopeptide (TPR) containing protein. Ligand binding results in the dissociation of this complex, receptor dimerization and phosphorylation, nuclear transport, DNA binding, the recruitment of components of the transcription machinery and other cofactor molecules, such as the p160 coactivators, and ultimately, the activation of particular gene pathways. **1**-**4**. Mechanisms of continued androgen signaling implicated in maintaining prostate cancer growth in a castrate environment following androgen ablation therapy. **1**. Tumor cells may acquire mechanisms to accumulate androgens, such as sequestration by steroid hormone binding globulin or altered regulation of enzymes involved in the synthesis and metabolism of androgens. **2**. Castrate-resistant clinical prostate cancer samples often exhibit increased AR levels compared to early stage tumors or normal prostate cells. This may result from amplification or overexpression of the AR gene. **3**. AR gene mutations can allow promiscuous activation of the AR by alternative ligands, such as glucocorticoids, estrogens, adrenal androgens, progestins and traditional receptor antagonists such as hydroxyflutamide. Other mutations may alter the recruitment of cofactors. **4**. An altered profile of AR coregulators (coactivators and corepressors) may facilitate ligand-independent AR signaling, or enhance AR activation by low levels of ligand. (i-v). Potential points of therapeutic intervention: (i) 5a-reductase inhibitors (eg. finasteride; dutasteride); (ii) antisense AR oligonucleotides; (iii) Hsp90 inhibitors (eg. 17-allylaminogeldanamycin); (iv) ARis, antibodies, histone acetylase and deacetylase inhibitors (eg. SAHA); (v) specific response element blockers (eg. polyamides).

Table 1. Characteristics of androgen-metabolic genes analyzed in prostate cancer

Gene	Gene Product	Chromosomal Location	Constitutional Mutations	Somatic Mutations
AR	Androgen receptor	Xq11-12	30,31,117	32,33,117
SRD5A2	Steroid 5α-reductase	2p23	17, 24, 25	27
HSD3B2	3B-hydroxysteroid dehydrogenase	1p13	31	None reported
CYP17	Cytochrome p450c17	10q24.3	32, 33	None reported
HSD17B3	17B-hydroxysteroid dehydrogenase	9q22	42	None reported

The numbers in this table indicate individual references. The presence of constitutional and/or somatic mutations refers to DNA from either patients' lymphocytes or their affected tumor tissue. Only polymorphic variants (31) have been reported in the HSD3B2 gene.

This significant pharmacogenetic variation should be considered when prescribing steroid 5α-reductase inhibitors for prostatic diseases. Recently, the PCPT (prostate cancer prevention trial) reported that treatment of men 55 years of age or older with finasteride resulted in a significant decrease in prostate cancer incidence measured over a seven-year period (26), suggesting that finasteride prevents or delays the appearance of prostate cancer. However, finasteride treatment also resulted in a significant increase in the incidence of prostate tumors of high grade (7 or higher) Gleason score (26). This unexpected finding may at least partially be explained by the presence of specific SRD5A2 variants (like A49T; Figure 2) that are not efficiently inhibited by finasteride, in a subset of the study population. For individuals carrying the A49T allele, the competitive steroid 5α-reductase inhibitor dutasteride might have been a better choice (23, 25). Thus, pharmacogenetic considerations need to play an important role in future trials and treatment regimens.

In addition to affecting predisposition, the SRD5A2 gene may also play an important role in prostate cancer progression. First, common somatic genetic alterations in a $(TA)_n$ dinucleotide repeat polymorphism located in the 3'-untranslated region (UTR; Figure 2) of the SRD5A2 gene were reported in prostate tumors(27). Subsequently, the same prostate tumors were reported to contain both somatic missense mutations and other *de novo* somatic mutations (perhaps best called somatic polymorphisms) that do not alter enzyme activity, in the coding region of the SRD5A2 gene (28). Most of the recurrent somatic SRD5A2 mutations identified increased 5α-reductase activity (28), suggesting that higher intraprostatic DHT steady state levels resulting from these mutations may play a role in prostate cancer progression (Figure 2). Thus, the SRD5A2 gene may be significantly involved in both predisposition and progression of prostate cancer. This dual involvement is very interesting and may open new avenues of research that may, in turn, result in more rational and/or personalized treatment of patients and even chemoprevention. Finally, the involvement of this androgen-metabolic gene in prostate cancer progression may lead to new strategies for treating androgen-independent tumors by considering both SRD5A2 and AR gene mutations (23, 25, 27, 29-33).

5. ANDROGEN RECEPTOR

The androgen receptor (AR), which is the pivotal component of the androgen metabolic cascade / androgen signaling axis, is a member of the superfamily of nuclear transcription factors that regulate a diverse range of cellular functions by providing a direct link between signaling molecules and gene transcription (34-36). The AR gene contains a polymorphic trinucleotide $(CAG)_n$ microsatellite (which is often simply referred to as CAG) normally distributed in the population between 6-39 repeats with an average of 21 (37, 38). The $(CAG)_n$ microsatellite encodes a polyglutamine (poly-Q) tract in the receptor, the length of which is inverse related to AR transactivation activity (39-41). The observation that the allelic frequency distribution of AR-$(CAG)_n$ repeat length paralleled the different incidence of prostate cancer amongst different U.S. racial-ethnic populations (42) led to several pilot and then case-controlled studies demonstrating an approximate 2-fold increased prostate cancer risk, decreased age of onset and/or increased risk of advanced disease for reduced AR-$(CAG)_n$ repeat length (38, 43-47). However, this association has not been consistently observed, possibly due to small sample sizes, population differences and/or failure to appropriately match cases and controls (48- 57). Importantly, recent biochemical evidence suggests that the effect of polyQ tract length on AR function may be more complex than a simple linear relationship with AR activity (39). Reassessment of cancer risk and $(CAG)_n$ repeat length using this new model is therefore clearly warranted.

AR status and a functional androgen signaling axis are particularly important for patients who are either diagnosed with, or subsequently develop, metastatic prostate cancer, as they determine the success of androgen ablation (i.e. orchidectomy, treatment with LHRH agoniss/antagonists and/or AR antagonists), the only treatment option in the advanced disease setting (58, 59). Despite an initial response to these therapies in 80-90% of patients with metastatic disease, treatment is essentially palliative and disease progression eventually ensues (59, 60). Paradoxically, the failure of androgen ablation therapy in a clinical setting is accompanied by rising serum levels of the androgen regulated protein, PSA (prostate specific antigen). This observation provided the impetus to further examine the androgen signaling pathway in prostate cancer, with the result that it is now well accepted that the cellular mediator of androgen action, the AR (Table 1; Figs. 1 and 3), remains a major determinant of progression and survival in both early stage prostate cancer and in a castrate environment following androgen ablation (33, 61).

Many studies have addressed the role of AR in prostate cancer using xenograft mouse models of the human disease. Analogous to the clinical setting, prostate cancer xenograft lines typically grow only in an androgen-dependent state and are sensitive to androgen ablation, but frequently regrow in a low androgen environment

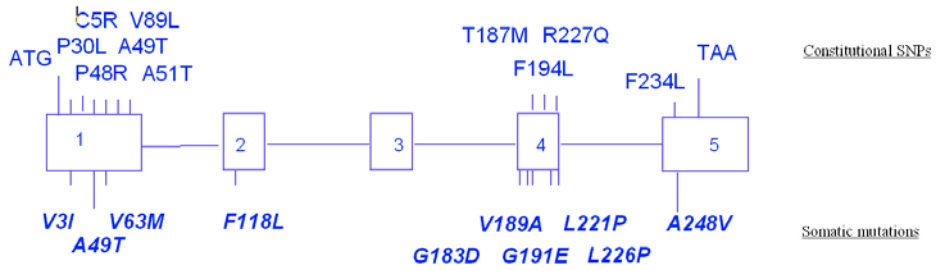

Figure 2. Steroid 5α-reductase type II missense SNPs (in constitutional DNA) and somatic mutations shown on the SRD5A2 exons. The translation start and stop codons are marked above the respective exons. SNPs in constitutional (or "germline") and somatic mutations are indicated separately.

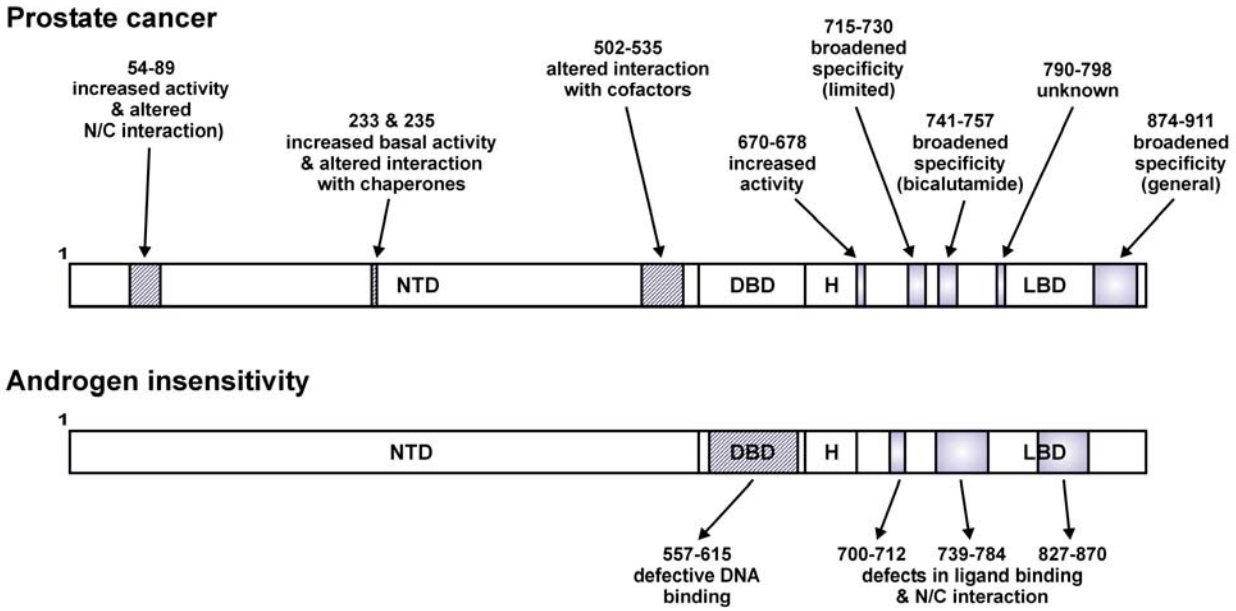

Figure 3. Collocation of androgen receptor mutations in prostate cancer and the inherited form of androgen insensitivity. The regions of the AR ligand binding domain (LBD) in which 80% of the missense AR mutations identified in prostate cancer and AIS collocate are indicated (grey boxes[32,33]). Regions in the AR DNA binding (DBD) and amino-terminal transactivation (NTD) domains where missense mutations have been shown to exert altered receptor function in prostate cancer[33,39,71,78,79,118,119] and AIS[117] are also designated (hatched boxes). Broadly defined effects of naturally occurring mutations detected in prostate cancer and AIS within regions of collocation are indicated.

following castration and continue to secrete PSA. In support of the hypothesis that AR continues to play an important role in growth of castrate-resistant prostate cancer, several studies have demonstrated an increase in the level of AR and/or AR regulated genes in xenograft tumors following castration in comparison to untreated tumors (62-65). In particular, comprehensive gene expression profiling determined that the AR was the only gene upregulated in the progression from androgen sensitive to castration resistant growth in all seven human prostate cancer xenograft models analyzed (63). In clinical disease, recent studies have demonstrated an increase in both AR mRNA and protein levels during progression from localized disease to metastatic castrate-resistant prostate cancer (33). Moreover, increased AR levels in clinically localized disease have been associated with relapse following radical prostatectomy, suggesting that the AR is a determinant of metastatic spread (66, 67).

Several mechanisms have been proposed to contribute to increased AR levels in castrate resistant tumors, including increased expression of the AR and AR gene amplification, the latter being demonstrated in up to 29% of castrate resistant tumors along with a concomitant increase in androgen regulated genes (68- 70).

The AR has a modular structure, consisting primarily of a large amino-terminal transactivation domain (NTD), a centrally located DNA-binding domain (DBD), and a carboxy-terminal ligand binding domain (LBD) that confers high affinity and specificity for androgen binding (Figure 3). AR gene mutations have been reported in prostate cancer at a frequency of 5-50%, with a higher frequency reported in more advanced disease and following androgen ablation therapy (33). In contrast to the inherited syndrome of androgen insensitivity, where AR gene mutations result in a loss of receptor function, those

detected in prostate cancer occur in distinctly different regions of the receptor, and generally confer promiscuity or increased sensitivity for receptor activation by other steroid hormones and/or by the specific antiandrogen used in clinical management of the disease (Figure 3) (32, 33, 71). These observations led to the concept of "therapy-mediated selection pressure", which is exemplified by the detection of AR gene mutations which confer enhanced receptor activity in response to flutamide but not to other anti-androgens in tumors from patients treated with hydroxyflutamide plus orchidectomy or LHRH agonists/antagonists, as part of a combined androgen blockade strategy (72, 73). Clinically, patients who progress during treatment with one antiandrogen often respond to another (74, 75). Studies of the autochthonous TRAMP model of prostate cancer (76, 77) demonstrate that therapy mediated selection of AR mutations is not limited to antiandrogen treatment. Whereas non-castrate mice develop mutations in the AR LBD, mice castrated at 12 weeks of age developed mutations in the NTD (78). Enforced expression of the AR-NTD variant, E231G, in the mouse prostate confers rapid development of prostatic intra-epithelial neoplasia that progresses to invasive and metastatic disease in 100% of mice (79). This finding suggests that the AR can also act in an appropriate environment as a proto-oncogene to promote tumor formation.

Extended culture of LNCaP human prostate cancer cells in androgen depleted media resulted in sensitization of the AR to 10-fold lower concentrations of androgens and the ability to respond to bicalutamide, without changes in AR structure or level (80). These effects may be explained by changes in the level or function of proteins that orchestrate specific aspects of AR signaling. For example, the transcriptional activity of the AR is mediated, in part, by the recruitment of coregulator proteins that enhance (coactivators) or repress (corepressors) receptor function (Figure 1). An increase in coactivator levels has been shown in prostate cancer compared to benign prostate tissues, and during the development of castrate resistant disease (81- 85). These coactivator proteins can selectively enhance the activity of the AR upon binding of alternative ligands, such as estradiol and hydroxyflutamide, sensitize the AR to lower concentrations of native and non-native ligands, or promote different modes of AR activation by altering conformational changes and homodimerisation following ligand binding (78, 82, 86-88). Conversely, decreased expression of corepressors such as NCoR and SMRT, which mediate, in part, the antagonist action of bicalutamide, flutamide and mifepristone, would facilitate increased agonist activity of these agents (89, 90). However, while more than 120 AR interacting proteins have been described, it is not currently known how the majority of these influence receptor function, or which are expressed in the prostate and have altered expression during disease etiology (Figure 1).

The evolving evidence that the AR is one of the key critical mediators of continued prostate cancer growth following hormone ablation makes a compelling argument for the development of new strategies that directly target the receptor (33). Such new therapies are necessary for significant advances in the management of patients with advanced prostate cancer who will ultimately fail androgen ablation. Several approaches to achieve this goal have been documented. Reducing the level of AR and suppression of human prostate cancer cell growth *in vitro* and *in vivo* has been achieved with a variety of methods, incl. double-stranded RNA interference, antisense oligonucleotides, hammerhead ribozymes and analogues of the ansamycin antibiotics such as 17-allylamino-17-demethoxygeldanamycin (91- 98). Inhibition of AR function has been achieved with dominant negative AR inhibitors, microinjection of AR antibodies, "decoy" double stranded DNA fragments containing specific AR response elements and histone deacetylase inhibitors including suberoylanilide hydroxamic acid and phenylbutyrate (94, 99-102). Providing issues such as the mode of delivery and the potential for disruption of multiple signaling pathways can be circumvented, these newer approaches that directly target the AR provide considerable promise as therapeutic options for castrate-resistant prostate cancer.

6. 3B-HYDROXYSTEROID DEHYDROGENASE

DHT, the most active intraprostatic androgen, is inactivated through two reductive reactions catalyzed by 3α- and 3β-hydroxysteroid dehydrogenase (Figure 1)(10). Therefore, 3β-hydroxysteroid dehydrogenase is critical for the regulation of intraprostatic DHT steady state levels by affecting its degradation rate(10). Human 3β-hydroxysteroid dehydrogenase can catalyze a number of steroid substrates, including DHT (103). Enzyme activity is encoded by two homologous and closely linked loci: the HSD3B1 and HSD3B2 genes, which are both located in chromosome band 1p13 (Table 1) (103, 104). The type II 3β-hydroxysteroid dehydrogenase enzyme encoded by the HSD3B2 gene is expressed in androgenic tissues (103) and may therefore regulate DHT levels by initiating the inactivation of this potent androgen in the prostate.

A complex $(TG)_n$ $(TA)_n$ $(CA)_n$ repeat (105) in the HSD3B2 gene has been reported to be very polymorphic, consisting of at least 25 different alleles (106). Racial/ethnic variation of this complex repeat in the HSD3B2 locus parallels prostate cancer risk (106), suggesting that this locus may also play a role in prostate cancer predisposition and/or progression (Table 1). No missense HSD3B2 mutations associated with either predisposition or progression of prostate cancer have yet been reported to date. However, SNPs that do not change amino acids have been associated with prostate cancer predisposition in both HSD3B2 and HSD3B1 genes, and these SNPs seem to have an additive effect on risk (107).

7. 17B-HYDROXYSTEROID DEHYDROGENASE

Another androgen metabolic locus that may play a role in prostate cancer predisposition is the HSD17B3 gene which encodes 17β-hydroxysteroid dehydrogenase (HSD) type III, also called testicular 17-ketoreductase (Figure 1) (108). This enzyme utilizes NADP(H) as a

cofactor and favors the reduction of androstenedione to testosterone, in the testis (109). Thus, mutations in this gene may increase the production of T in the testes, which can directly or indirectly (through DHT) activate the AR, potentially increasing prostate cancer risk.

The HSD17B3 gene is located in chromosomal band 9q22 (Table 1) and contains 11 exons (109). At least 18 mutations and one polymorphism that cause male pseudohermaphroditism have been functionally characterized in this gene (see e.g. reference 109). Another SNP, the G289S (glycine at codon 289 replaced by serine) missense substitution, has been reported to significantly increase risk for sporadic prostate cancer (110). Thus this androgen metabolic enzyme may also be involved in prostate cancer predisposition.

8. 17A-HYDROXYLASE/17,20 LYASE

The enzyme cytochrome p450c17 (or steroid 17 α-hydroxylase/17,20 lyase) catalyzes two sequential reactions in the biosynthesis of testosterone in both the gonads and the adrenals; this enzyme is the product of the CYP17 gene (Fig. 1; Table 1; 10). A polymorphic T to C transition (A1/A2 allele) has been identified in the 5' untranslated region (UTR) of the CYP17 gene (111, 112). This polymorphism has been reported at increased frequency in Caucasian prostate cancer patients, compared with controls (20, 111, 112). Another study, however, reported no significant difference in the frequency of this SNP between Caucasian prostate cancer patients and controls except for patients with a family history of the disease (108). In addition, this SNP does not appear to play any role in early-onset prostate cancer (113).

9. CONCLUSIONS

In this review we presented the evidence and rationale supporting the involvement of a series of androgen-metabolic candidate genes in prostate cancer predisposition and progression. Selection of these genes was guided by endocrinologic, epidemiologic, biochemical and molecular criteria. We discuss that while some of the allelic variants examined were consistently shown to be associated with increased prostate cancer risk, many other variants show significant variability in risk. The reasons for this variability are unknown, but may include small sample size, distinct racial/ ethnic background of the samples, or a minor effect on the risk exerted by these variants. Deciphering the role of such variants may be difficult without functional studies that may support their role in disease etiology and/ or progression.

The molecular-epidemiologic approach presented here for the androgen-metabolic genes has general applicability. First, SNPs (13) are identified and then their individual contributions to the phenotype are measured, in concert with other allelic variants in the same or other genes. Second, molecular-epidemiologic investigations are supported by appropriate biochemical and pharmacologic/pharmacogenetic studies (*in vitro* and/or *in vivo*) that determine the functional significance of each allelic variant (15, 114). These multidisciplinary investigations are likely to involve epidemiologic, pharmacologic, molecular and biochemical methods. This convergence of various disciplines will probably result in an integrated molecular view of complex disease phenotypes, which can then be complemented by the analysis of environmental contributions.

In summary, the multidisciplinary analysis of androgen-metabolic genes in prostate cancer has proven useful for extending our knowledge of both the predisposition and the progression of this disease. Further investigations are likely to yield additional advances in understanding this significant public health problem. Extensions of this approach to other complex human diseases, such as benign prostatic hyperplasia (BPH), are also likely to lead to important insights into the etiology of these diseases (115).

The approach presented here focused exclusively on the sporadic form of prostate cancer. There are, however, two forms of the disease: a common, sporadic form and a much more uncommon familial form, a dichotomy typical of many complex human diseases (116). It is noteworthy in this context that none of the genes discussed in this review map to any of the regions that have been associated by linkage with the familial disease (Table 1 and data not shown). This finding suggests that the more common, sporadic form of prostate cancer and the infrequent familial form may have distinct etiologies. This hypothesis may also have significant implications for the investigation of other complex human phenotypes.

SNPs have come into widespread use already (13) Unfortunately, little attention is currently being paid to the examination of the functional significance of SNPs (24), perhaps due to the variability and hence difficulty of the assays required to functionally characterize each SNP (which makes it difficult to automate the functional analysis). The molecular epidemiologic data reviewed here have implications for association and linkage studies with SNP markers. Specifically, the epidemiologic and kinetic data on the rare A49T missense mutation in the SRD5A2 gene highlight the enormous importance of functional studies for SNP investigations. Therefore, extensive functional analyses should be pursued for all missense SNPs throughout the genome (and perhaps all SNPs), however time consuming this may be. In fact, we propose that SNPs should be classified as functionally neutral or functionally significant based on appropriate experimental data. The former may be best used in linkage studies, while the latter may be best used as "candidate alleles" (or "candidate SNPs") in candidate genes. In other words, functionally neutral SNPs are useful in identifying appropriate candidate genes for each phenotype, while functionally significant SNPs are useful for epidemiologic (i.e., association) and pharmacologic studies, because they are more likely to actually cause the phenotype (24). Therefore, the multidisciplinary analysis of prostate cancer may have resulted in the discovery of significant new strategies for human molecular genetic and epidemiologic research into multifactorial (complex) phenotypes.

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