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The role of molecular genetics in chemoprevention studies of prostate cancer

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Research into the molecular genetics of prostate cancer to date has largely focused on the possible existence of one or several single-locus high-penetrance susceptibility genes and several candidate regions have been identified, but confirmatory studies of these regions have been inconclusive. Increasingly, attention has turned to identification of candidate genes which may increase prostate cancer risk because their products play an important role in possible etiological pathways for prostate cancer. Of various such pathways which have been suggested for prostate cancer, the best studied in terms of molecular genetics is the androgen signalling pathway. Two genes in this pathway, the androgen receptor (*AR*) gene and the steroid 5-alpha reductase type II (*SRD5A2*) gene, have been under particular scrutiny and polymorphic markers in each of these genes that reproducibly predict prostate cancer risk have been identified. Such studies may have important implications for prostate cancer chemoprevention trials. As etiological pathways become better understood at the molecular level, piecing together multiple genetic variants in a pathway will allow identification of high-risk individuals and potential targets for chemopreventive interventions. Moreover, understanding the role of these genes in prostate cancer etiology may help in defining heterogeneity in response to such interventions. Finally, these genes or their products may themselves be legitimate targets for building a chemoprevention strategy.

Introduction

The molecular genetic epidemiology of prostate cancer is an evolving field (Ross *et al.*, 1998, 2000). Much of the work in this area has until recently focused on the identification of one or several single-locus high-penetrance susceptibility genes that might carry with them, for individuals with mutated forms, very high lifetime risk of developing prostate cancer. Interest in this type of susceptibility has been stimulated by the highly reproducible finding that prostate cancer is a strongly familial disease. Men with a first-degree relative with prostate cancer have a 2–3-fold increased risk relative to the population as a whole (Monroe *et al.*, 1995). This strong familial risk has been found in populations with both a high and low risk of the disease. Having several such relatives and/or a relative with prostate cancer at a relatively young age are associated with further increases in risk (Carter *et al.*, 1992). Prostate cancer has an unusual family risk pattern in that if an individual has a brother with prostate cancer, risk is roughly twice as high as if the father had the disease (Monroe *et al.*, 1995). This pattern of risk contrasts with risk associated with other familial cancers, such as breast cancer, for which risk is roughly the same whether a sister or a mother is affected. This pattern of risk has provided leads as to the mode of transmission of the purported susceptibility gene(s). Linkage analyses in multiplex families have led to the identification of several candidate regions for susceptibility loci, but so far, confirmatory studies have generally been inconclusive for each of these regions and final identification and cloning of a major locus gene for prostate cancer is unlikely to be forthcoming (Smith *et al.*, 1996; Xu *et al.*, 1998).

Increasingly, molecular genetic epidemiological research on prostate cancer has focused on the identification of candidate genes which increase prostate cancer risk because their protein product

plays a role in an etiological pathway of disease development. Although many etiological/prevention pathways have been suggested for human prostate cancer, currently the strongest epidemiological and experimental evidence supports four: androgen signalling; antioxidation; vitamin D signalling; and insulin-like growth factor (IGF) signalling. Although susceptibility genes may modify individual risk through any of these pathways, evaluation of such genetic influences is not well understood for any of them and has not even begun for some. Thus although there is substantial epidemiological evidence that the antioxidant carotenoid lycopene may lower risk of prostate cancer (Giovannucci *et al.*, 1995), both epidemiological and randomized clinical trial evidence that selenium lowers risk (either as an antioxidant or through other anticarcinogenic effects) (Clark *et al.*, 1998; Yoshizawa *et al.*, 1998) and experimental evidence that the antioxidant vitamin tocopherol lowers risk (Heinonen *et al.*, 1998), no attempt has yet been made to determine if any genetic factors might modify any chemopreventive efficacy. Similarly, there is prospective epidemiological evidence that IGF-I levels are predictive of prostate cancer occurrence (Chan *et al.*, 1998). As polymorphic markers in genes for IGF and its binding proteins have been identified (Rosen *et al.*, 1998), these are potential future targets for determining individual susceptibility related to IGF-induced carcinogenesis, but no research in this area has yet been reported and validation of genotype/phenotype relationships has not been completed for all such markers.

However, there is a steadily increasing number of reports on polymorphic variants of low-penetrance genes in the androgen signalling pathway and in the vitamin D signalling pathway in relation to prostate cancer risk. The remainder of this chapter reviews the current state of knowledge in these areas, summarizes how current knowledge can affect existing or planned chemopreventive activities and provides some general thoughts about the future of this field as it pertains to chemoprevention of prostate cancer. Various strategies already available to assess chemopreventive efficacy in human studies in terms of biochemical and histological parameters or of prostate cancer risk *per se* are described.

Androgen-related susceptibility genes

Although a number of genes in the androgen signalling pathway have been suggested as candidates for investigation (e.g., *CYP17* and *HSD17B3* as genes involved in testosterone biosynthesis; *SHBG* as a gene involved in testosterone transport and *HSD3a* and *HSD3b* as genes involved in androgen degradation in the prostate), for only three genes has there been any direct investigation of a particular marker in relation to prostate cancer risk (the androgen receptor (*AR*) gene, the steroid 5-alpha reductase type II (*SRD5A2*) gene and the *CYP3A4* genes) and confirmatory findings have been obtained for only two of these markers, the CAG trinucleotide repeat polymorphic marker in the androgen receptor gene and the A49T polymorphic marker in the *SRD5A2* gene (Ross *et al.*, 1998).

The majority of testosterone biosynthesis in males occurs in the testis under regulation of luteinizing hormone stimulation (Coffey, 1979). Testosterone is transported to target cells in the circulation either as its free form, weakly bound to albumin or more tightly bound to sex-hormone-binding globulin, the latter thought to be non-bioavailable to target tissues. Free testosterone diffuses freely into prostate cells, where it is irreversibly converted to its reduced, more bioactive form dihydrotestosterone. Dihydrotestosterone, and also testosterone with lower binding affinity, bind to the androgen receptor and this complex of ligand and receptor translocates to the nucleus for DNA binding and transactivation of genes with androgen response elements in their promoter regions (Ross *et al.*, 1998). Most of these "downstream" genes transactivated by the androgen receptor have not yet been characterized, but they are thought to include the major genes regulating cell division.

Cell division is thought to be a prerequisite for the development of much if not most human cancer, as cell division is thought to be necessary for cells to accumulate the genetic changes required for transformation to a malignant phenotype (Preston-Martin *et al.*, 1990). As cell division is largely controlled by androgen activity, any gene in the transactivation pathway for androgens becomes a legitimate candidate gene in relation to prostate carcinogenesis. However, there are several additional lines of evidence that androgens and,

hence, the genes which regulate androgen activity, are involved in prostate carcinogenesis. These have been previously reviewed (Ross *et al.*, 1998), but include the importance of androgen in induction or progression of prostate cancer in the few experimental models that mimic the human disease (Noble, 1977), the apparent absence of prostate cancer in men with constitutional underdeveloped prostate glands due to androgen deficiency (Ross *et al.*, 1998), the importance of androgen deprivation as an effective initial therapy in men with early advanced prostate cancer (Ross & Schottenfeld, 1996), the predictive value of circulating testosterone levels for subsequent prostate cancer development (Gann *et al.*, 1996a), and the differences in the hormonal environment in men of different racial/ethnic backgrounds with markedly different patterns of prostate cancer incidence (Ross *et al.*, 1986, 1992).

The androgen receptor (AR) is a transcription factor encoded by the *AR* gene on the X chromosome (Coetzee & Ross, 1994). The *AR* gene encodes three distinct regions of the AR molecule: a hormone (androgen)-binding domain, a DNA-binding domain and a transcription modulatory domain. The latter is completely encoded by a very large exon 1 which contains two well characterized polymorphic trinucleotide repeat sequences. The length of one of these sequences, a CAG repeat encoding a polyglutamine tract, was hypothesized by Coetzee and Ross (1994) to be related to androgen transactivation activity and to prostate cancer risk. The hypothesis was based initially on the observations that an expansion of this repeat is the cause of an X-linked adult-onset motor neuron disease, spinal and bulbar muscular atrophy or Kennedy's disease (La Spada *et al.*, 1991) and that men with this disorder transactivate androgens suboptimally and have evidence of hypoandrogenicity (Arbizu *et al.*, 1983). The hypothesis stated that men with longer CAG repeats within the normal range (9–33) will have progressively lower androgen transactivation activity (despite normal DNA-binding by the AR) and correspondingly lower prostate cancer risk (Coetzee & Ross, 1994). This hypothesis has received some support from *in vitro* studies demonstrating that there is a linear inverse relationship between CAG repeat length and transactivation activity as measured by reporter genes in transfection assays (Chamberlain *et al.*, 1994). Indirect support has also come from observations that average CAG length varies by race/ethnicity, with African Americans having shorter repeats on average, followed by Caucasians, with Oriental populations having, on average, the longest, as predicted from their respective prostate cancer incidence rates (Coetzee & Ross, 1994). In a population-based case–control study in Los Angeles, white men with less than the average number of CAG repeats in the control population had twice the risk of prostate cancer compared with men having more than the average number (2.5 times the risk of advanced disease) (Ingles *et al.*, 1997). This relationship has been confirmed by other studies (Stanford *et al.*, 1997; Giovannucci *et al.*, 1997).

The other gene in the androgen signalling pathway that has been a subject of fairly detailed study as a candidate gene for prostate cancer is the steroid 5-alpha reductase type II (*SRD5A2*) gene. *SRD5A2* is one of two 5-alpha reductase isozymes, but is the most active in prostate tissue (Thigpen *et al.*, 1992). The *SRD5A2* gene is located on chromosome 2. In addition to silent single nucleotide polymorphisms, seven substitution single nucleotide polymorphisms (SNPs) have been described by Reichardt *et al.* (1995) and a TA repeat polymorphism has been described in the 3′-untranslated region (3′UTR) region of the gene. Although unique allelic variants have been described for both African Americans and Asians for the TA repeat, no clear functional relevance has yet been ascribed to this marker (Ross *et al.*, 1995).

For the seven substitution polymorphisms, Makridakis *et al.* (1999) have conducted transfection assays in which they compared the pharmacokinetic properties of the mutant enzymes *in vitro* with those of the wild-type enzyme. These assays suggested that some of these substitution changes represented true polymorphisms, in that despite an amino acid change, the mutant enzyme kinetic properties were identical to those of the wild-type enzyme. However, others resulted in substantial increases in enzyme activity (in particular an alanine to threonine substitution at codon 49 (A49T)), while others resulted in decreased enzyme activity (e.g., a valine to leucine substitution at codon 89 (V89L)). The results in this artificial system have been validated by the demonstration that the A49T mutation, despite being quite uncommon in the general population, with a

variant allele frequency of < 1.0%, was strongly associated with prostate cancer, especially advanced disease, in two populations in Los Angeles, Latinos (RR for advanced disease in men with at least one T allele = 3.6, $p = 0.04$) and African Americans (RR for advanced disease in men with at least one T allele = 7.1, $p = 0.001$) (Makridakis *et al.*, 1999). Although the V89L allele has not yet been reported to be inversely associated with prostate cancer risk, as predicted by the *in vitro* assays, it has been demonstrated to be correlated with low circulating androstanediol glucuronide levels, an index of whole-body 5-alpha reductase activity (Reichardt *et al.*, 1995).

A third gene in the androgen signalling pathway that has undergone preliminary epidemiological evaluation is the *CYP3A4* gene, whose product is involved in oxidation of testosterone in the prostate to a series of biologically inert metabolites. Rebbeck *et al.* (1998) compared the allele distribution of an SNP in a series of high-grade advanced versus low-grade localized prostate cancer. Among men in the advanced prostate cancer group, 46% carried a variant allele compared with only 5% of men in the low-grade/early-stage group (OR = 9.5, $p < 0.001$).

Vitamin D signalling pathways

Vitamin D (or, more accurately, 1,25-dihydroxyvitamin D, the bioactive vitamin D metabolite) is a potent antiproliferative agent in the prostate as well as a prodifferentiation agent for prostate cells *in vitro* (Peehl *et al.*, 1994). As prostate cells themselves metabolize vitamin D precursor compounds to 1,25-dihydroxyvitamin D, vitamin D stimulation of the prostate is under both local and systemic control. There are some, although not totally consistent, epidemiological data indicating that circulating levels of 1,25-dihydroxyvitamin D are inversely associated with prostate cancer development (Corder *et al.*, 1995; Gann *et al.*, 1996b). Vitamin D has inhibitory effects on prostate growth in experimental models, further supporting a possible chemopreventive role in prostate cancer. Vitamin D signalling is mediated by the vitamin D receptor. A number of polymorphisms in the vitamin D receptor (*VDR*) gene with common allele variants have been identified (Morrison *et al.*, 1992). Several of these have been shown to have biological correlates in relation to bone mineral metabolism, in which vitamin D plays an important role, and have been extensively studied in relation to fractures or other health outcomes caused by altered bone mineral metabolism. A few of these polymorphic markers have also been studied in relation to prostate cancer risk. Ingles *et al.* (1997) reported that a polyA microsatellite with a bimodal polymorphic distribution was strongly related to prostate cancer risk in whites; men with at least one 'long' A allele of this marker, which is located in the 3´UTR of the gene, had a 4.6-fold higher prostate cancer risk than men homozygous for 'short' polyA alleles. Similar results have been obtained for other markers in linkage disequilibrium with the polyA microsatellite in whites (Taylor *et al.*, 1996). Studies in African Americans have also supported the notion that polymorphic markers in and around the 3´UTR of the *VDR* gene are associated with increased risk of prostate cancer (Ingles *et al.*, 1998).

Strategies to evaluate chemopreventive agents for prostate cancer in human populations

There are three strategies currently in use in human populations to evaluate chemopreventive efficacy either against prostate cancer *per se* or in the context of biomarkers of prostate cancer risk or histological precursors of prostate cancer. Each of these strategies has its own particular strengths and limitations.

One strategy is to give a potential chemopreventive agent to a patient with biopsy-proven prostate cancer who is awaiting definitive prostate surgery. This strategy has the advantage of pre- and post-intervention tissue availability and large quantities of tissue to evaluate post-intervention. As the individual undergoing therapy has prostate cancer, there is a low likelihood of causing harm through administration of the agent. Disadvantages of this strategy include the short duration of the intervention (usually a matter of weeks), our current general lack of knowledge regarding changes in biomarkers suggesting possible efficacy, and concern that the response of patients with prostate cancer to a chemopreventive intervention might be different to that of healthy individuals. [*This strategy would also fall outside the definition of chemoprevention adopted by the participants in the workshop – Ed.*]

The second strategy takes advantage of the fact that men with elevated prostate-specific antigen

(PSA) who are sextant biopsy-negative for prostate cancer require a second biopsy as part of routine clinical management, typically one year later. This creates a one-year window for evaluating a chemopreventive intervention with small amounts of tissue available pre- and post-treatment. This strategy has the advantage of allowing a longer-term evaluation of the agent but, like the pre-prostatectomy approach, it is unclear what biomarkers should be evaluated to provide evidence of efficacy as a chemopreventive agent. As many of these men have high-grade prostatic intraepithelial neoplasia (PIN) lesions, a probable histological precursor of prostate cancer, alteration in these lesions has been suggested as a possible target of efficacy. Availability of tissue after the intervention as part of the routine clinical management of these patients is an enormous advantage of this approach. This second strategy has been tested in a small randomized study of the 5-alpha reductase inhibitor finasteride (Cote *et al.*, 1998).

The final strategy is a full-fledged prevention trial in healthy individuals. This is a long-term strategy and involves large numbers of participants. The duration and size of such trials yield advantages of contributing to our understanding of the efficacy of an intervention over a long period and permitting evaluation of interesting subgroups. On the other hand, these same factors contribute to the enormous cost of such studies and to their logistic complexity. Unlike the other two strategies, prostate cancer is, by definition, the outcome being assessed. However, to assess the development of histological, as opposed to clinical prostate cancer, using this design requires an invasive procedure (i.e., a prostate biopsy) outside the course of routine clinical care.

Implications of prostate cancer molecular genetics for chemoprevention trials

While chemoprevention trials are typically designed to have adequate statistical power to measure the overall impact of an intervention against a placebo or non-intervention group (or one intervention against another), one should not expect homogeneity in response. A strategy to help identify those individuals who will derive the greatest (or least) benefit from the intervention is highly desirable. A full understanding of etiological pathways at the molecular level would undoubtedly help achieve these goals. Recent work on the *SRD5A2* gene illustrates the potential importance of such an understanding in a chemoprevention setting. Studies of polymorphic variants of the *SRD5A2* gene *in vitro* have not only demonstrated huge variability in the pharmacogenetic properties of the mutant enzymes versus the wild type (as much as 150-fold variability for some parameters), but also substantially variability in response to the 5-alpha reductase inhibitor finasteride (Makridakis & Reichardt, 2000). Thus, in the current national chemoprevention trial of finasteride intervention in the United States, it is highly probable that any chemopreventive efficacy, or lack of it, will be modified by underlying genetic susceptibility related to the *SRD5A2* gene. Determining *SRD5A2* genotype should become an important component of that large study.

Another potential value of including candidate gene studies in chemoprevention trials is the possibility of identifying appropriate target groups for the intervention. It is generally considered that, although polymorphic variants of candidate genes which alter cancer risk may be relatively common in the population, these variants have only a modest influence on risk, but there are notable exceptions (Makridakis *et al.*, 1999). Full elucidation of molecular etiological pathways, as is being achieved in the area of androgen signalling in the prostate, can potentially allow the development of a polygenic etiological model of the disease (Ross *et al.*, 1998). Such a model, in turn, can allow identification of groups of individuals at very high risk of prostate cancer development by virtue of multiple high-risk allelic variants in candidate genes involved in these pathways. These individuals can then become targets for chemoprevention interventions, hopefully increasing the efficiency in terms of number of patients if not time for the completion of such a study.

Finally, understanding the relationship between candidate genes and disease risk opens up the possibility that a particular gene or its product may itself become a target for chemopreventive interventions. As understanding these relationships may also allow a fuller understanding of complex molecular etiological pathways, this approach can also potentially contribute to the development of new chemopreventive agents (Ross *et al.*, 1998).

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