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PROSPECTS

Prevalent Mutations in Prostate Cancer

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Abstract Quantitative and structural genetic alterations cause the development and progression of prostate cancer. A number of genes have been implicated in prostate cancer by genetic alterations and functional consequences of the genetic alterations. These include the *ELAC2* (*HPC2*), *MSR1*, and *RNASEL* (*HPC1*) genes that have germline mutations in familial prostate cancer; AR, ATBF1, EPHB2 (ERK), KLF6, mitochondria DNA, p53, PTEN, and RAS that have somatic mutations in sporadic prostate cancer; AR, BRCA1, BRCA2, CHEK2 (RAD53), CYP17, CYP1B1, CYP3A4, GSTM1, GSTP1, GSTT1, PON1, SRD5A2, and VDR that have germline genetic variants associated with either hereditary and/or sporadic prostate cancer; and ANXA7 (ANX7), KLF5, NKX3-1 (NKX3.1), CDKN1B (p27), and MYC that have genomic copy number changes affecting gene function. More genes relevant to prostate cancer remain to be identified in each of these gene groups. For the genes that have been identified, most need additional genetic, functional, and/or biochemical examination. Identification and characterization of these genes will be a key step for improving the detection and treatment of prostate cancer. J. Cell. Biochem. 97: 433–447, 2006. © 2005 Wiley-Liss, Inc.

Key words: prostate cancer; tumor suppressor gene; oncogene; mutation; deletion; amplification; haploinsufficiency

Prostate cancer is the most frequently diagnosed non-skin cancers and the second leading cause of cancer deaths in American men. PSA testing is an important screening modality for the increased detection of prostate cancer, but histological prostate cancer is common in aging men. An estimated 40% of men over age 50 have slow-growing and well-differentiated prostate cancer that can be diagnosed based on the current histology criteria, and the incidence increases with age. Most histological cancers, however, are indolent and pose little danger to the individuals affected. Only about 11% become clinically apparent, and 3% of them kill the patients [Scardino et al., 1992]. In the management of patients with prostate cancer, our uncertainty about the aggressiveness of the detected prostate cancers is a persistent dilemma. The current diagnostic methods, which are mainly based on histology and Gleason scoring, have proven effective in the diagnosis of prostate cancer and prediction of outcomes. However, the methods are quite limited for patients with an intermediate grade of cancer (Gleason 6 or 7), which can be either aggressive or indolent. For example, a prostate cancer with a Gleason score of 6 may or may not prove lethal. It has been recognized that analysis of molecular alterations can accurately predict the behavior of a cancer, and genetic analysis is the most powerful approach to identify the molecular alterations.

Pathway-based therapy can provide the most effective treatment for a cancer that has altered a specific molecular pathway. Therefore, in the cancer research field much effort has been devoted to the dissection of molecular pathways whose alterations lead to cancer development and progression. An excellent example is the development of rapamycin and its derivatives as promising therapeutic agents, which inhibit the mTOR protein kinase in an evolutionarily conserved signaling pathway that controls the cell cycle in response to changing nutrient levels. This signaling pathway contains a number of tumor suppressor genes, including

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PTEN, and a number of oncogenes, and is constitutively activated in many tumor types including prostate cancer. In this regard, the cancer molecules identified by genetic studies provide both molecular targets of investigation for dissecting cancer pathways and, in some cases, therapeutic targets that can be used to develop new cancer drugs.

Cancer results from structural and quantitative alterations in the molecules that control different aspects of cell behavior. Genetic alterations probably represent the most common mechanism for molecular alterations that cause the development and progression of cancer [Vogelstein and Kinzler, 1998]. Great efforts have been made to identify common genetic alterations and the underlying target genes. Genetic alterations can be inherited, as in hereditary cancers, or induced by endogenous and exogenous carcinogenic factors as in most sporadic cancers. Epigenetic mechanisms such as DNA methylation and imprinting can also cause molecular alterations during carcinogenesis. According to their roles in a cell, genes related to cancer can be categorized into two groups, tumor suppressor genes and oncogenes. The former refers to genes whose function is to restrain cells from uncontrolled growth and migration, and the latter refers to genes doing the opposite.

During the development and progression of cancer, tumor suppressor genes and oncogenes often undergo loss of function and gain of function, respectively, primarily through alterations in the genomes of cells. Genomic alterations not only include gene mutation, that is, changes in a gene's sequence that alter its function, but also include changes in the copy number or dosage of genes. With the development of approaches such as comparative genomic hybridization (CGH) and large-scale sequencing, a large number of cancer genomes have been examined. It appears that copy number change is more common than sequence change for a cancer gene. Although it is more difficult to confirm the role of gene copy number change in carcinogenesis, transgenic overexpression, and knockout of genes in mouse models have demonstrated that copy number change can clearly be a carcinogenic factor. In this review, mutation is more broadly defined as either sequence change or copy number change altering a gene's function at the genomic level.

The genomic alterations in prostate cancer that are responsible for sporadic cancer are mostly somatic changes. A number of genes have been identified for their role in sporadic prostate cancer. A small portion of prostate cancers have an obvious hereditary factor, and some genes with germline mutations in hereditary cancer have also been identified. For hereditary cancer, the relevant mutations are mainly at the sequence level; but for sporadic cancer, the relevant mutations include alterations in both sequence and copy number. Some sequence alterations are small deletions and/or nonsense mutations that cause truncation or frameshifting in the encoded protein. Such changes have obvious impact on gene function, and thus easily establish a gene's role in prostate cancer. So far, these alterations are mostly somatic. PTEN and ATBF1 are two examples in prostate cancer [Li et al., 1997; Sun et al., 2005]. Most sequence changes, however, are missense changes whose effect on gene function has to be determined by genetic, functional, or biochemical studies. Most sequence alterations, including cancer-associated single nucleotide polymorphisms (SNPs), belong to this category. A typical example is the *AR* gene, which has numerous sequence changes in prostate cancer. Another major group of changes includes the genes that have copy number change, which can also sometimes have sequence change. Increasing numbers of such genes are being identified, and may involve more cancer genes than other groups. The expression of this kind of gene is gene-dosage-dependent. Those with copy number loss are haploinsufficient. Two typical examples, the *NKX3-1* gene from 8p21 and the cyclin-dependent kinase inhibitor, CDKN1B (p27), are haploinsufficient but do not have somatic sequence alterations in prostate cancer. The MYC oncogene is an example of a gene with copy number gain but without sequence alterations in prostate cancer.

There are multiple approaches for identifying genetic alterations and validating their role in carcinogenesis, including linkage and association studies for genetic markers and SNPs, deletion/amplification mapping for somatic copy number changes, mutation analysis for known genes, functional evaluation of genes in different models, and biochemical and biological studies of genes and their pathways. Below we present the genes for which there is convincing

evidence for a role in prostate cancer based on one or more of these approaches.

ANXA7 (ANX7)

The annexin A7 (*ANXA7*) gene, which codes for a Ca^{2+}-activated GTPase, is located on human chromosome 10q21, a site frequently affected by chromosomal loss that has been hypothesized to harbor tumor suppressor genes (TSG). In the initial evaluation of ANXA7 as a candidate for the *10q21* gene, frequent loss of ANXA7 expression was observed in prostate cancer, especially in metastasis and local recurrences of hormone refractory prostate cancer. LOH at ANXA7 is also frequent in prostate cancer. In functional studies, restoration of ANXA7 expression in LNCaP and PC-3 prostate cancer cell lines significantly reduced cell proliferation and colony formation. An ANXA7 knockout mouse model was generated to further evaluate the biological function of ANXA7 in vivo [Srivastava et al., 2003]. Whereas null ANXA7−/− mice die during embryogenesis, the ANXA7 heterozygous mice (ANXA7+/−) develop, mature, and age normally, and more interestingly, demonstrate a cancer-prone phenotype. A broad range of spontaneous tumors have been detected in ANXA7+/− mice, including prostate cancer. The tumors still have one copy of the ANXA7 genome and express reduced levels of ANXA7 protein, indicating that ANXA7 is haploinsufficient. Microarray-based analysis of cancer tissues from ANXA7+/− mice showed a profound reduction in the expression of several other tumor suppressor genes, DNA repair genes, and apoptosis-related genes [Srivastava et al., 2003]. These studies indicate that ANXA7 is a haploinsufficient tumor suppressor gene whose hemizygous deletion in human prostate cancer results in reduced expression and function. At present, no studies have been conducted to determine if ANXA7 undergoes small deletions or point mutations in human prostate cancer.

AR (ANDROGEN RECEPTOR)

The prostate is an androgen-regulated organ, which has led to long-standing interest in the role of androgens in prostate carcinogenesis. Androgens are essential for the development of prostate as well as its growth and maintenance. The association between androgen levels and prostate cancer has been well established. Androgen action is mediated by the intracellular androgen receptor (AR), which belongs to the superfamily of ligand-dependent transcription factors. AR binds testosterone to stimulate the transcription of androgen-responsive genes and regulate the growth of both normal prostate gland and prostate cancer. Targeting the androgen-signaling axis (androgen ablation therapy) remains the predominant treatment regime for patients with metastatic prostate cancer. However, most prostate cancers eventually become resistant to androgen withdrawal treatment. Extensive studies over more than a decade indicate that the failure of androgen withdrawal therapy may not result from a loss of androgen signaling but, rather, from the acquisition of genetic changes that lead to aberrant activation of the androgen-signaling axis. As summarized below, two major types of genetic alterations in AR occur in hormone refractory cancers: (1) somatic mutations that result in decreased specificity of ligand-binding and inappropriate receptor activation by estrogens, progestins, adrenal androgens, glucocorticoids, and/or AR antagonists; and (2) genomic amplification of the *AR* gene, which can maintain an active androgen signaling axis even with very low levels of androgen. Germline polymorphisms in the trinucleotide repeat of the *AR* gene, which probably affect AR activities, have also been linked to increased prostate cancer risk, whereas other germline mutations are rare.

Somatic Mutations of AR in Sporadic Prostate Cancer

After a somatic mutation was detected in exon E of AR's hormone binding domain in 1 of 26 specimens of untreated organ-confined stage B prostate cancer [Newmark et al., 1992], a large number of studies were published on the mutation status of AR in sporadic prostate cancer. The frequency of AR mutation varies greatly between different studies, from zero in some localized tumors in some studies to over 50% in some hormone refractory metastatic cancers in other studies. Overall, AR mutation is hardly detectable in localized primary prostate cancers, but is detected in about 10–30% of the hormone refractory cases and metastases of prostate cancer. Whereas androgen withdrawal therapy is an obvious factor that selects for cells with AR mutations to grow, AR mutations also occur in tumors that have not received androgen ablation therapy, and the presence of AR

mutation is related to rapid failure of subsequent hormonal therapies. It is clear that AR mutation is partially responsible for altered androgen responsiveness in some prostate cancers.

Whereas mutations of the AR can be in different functional domains as well as in 5′- and 3-translational regions (UTRs) of the gene, most of the detected mutations are base substitutions that affect the function of AR either directly or indirectly. For example, some mutations do not differ from wild-type AR in their ability to bind the synthetic androgen methyltrienolone, but they change AR's responses to other factors. In fact, a significant proportion of missense mutations in the *AR* gene collocate to specific regions of AR such as the ligand-binding domain, and such collocations in turn could help identify additional regions of critical function in the AR molecule. An online database, the androgen receptor gene mutations database (ARDB), has been established, which includes all the AR mutations detected in prostate cancer to date [Gottlieb et al., 2004].

AR Amplification in Hormone Refractory Prostate Cancer

In addressing the resistance of prostate cancer to endocrine therapy, frequent genomic amplification of AR has been found in recurrent prostate cancers from patients who had failed androgen deprivation therapy [Visakorpi et al., 1995]. This finding has been confirmed in other studies. Some tumors have both mutation and gene amplification of AR. AR amplification was most likely to occur in tumors that initially responded well to endocrine therapy and whose response duration was more than 12 months. Tumors that recurred earlier or those that showed no initial response to therapy did not contain AR amplification. It is possible that amplification of the *AR* gene is causative for the failure of androgen deprivation therapy in prostate cancer [Koivisto et al., 1997].

Microsatellite Mutation of AR in Germline DNA of Prostate Cancer Patients

The *AR* gene contains polymorphic trinucleotide microsatellite CAG and GGC repeats that code for a variable length of glutamine and glycine, respectively, in the AR protein. Different lengths of such repeats could affect protein function, as demonstrated in several other genes and diseases. Several studies have been published addressing microsatellite alteration in AR in prostate cancer. Whereas somatic alteration of the repeat length is very rare, a shorter CAG repeat has been repeatedly shown to be associated with increased risk of prostate cancer as well as more aggressive features of prostate cancer such as higher tumor stage and grade, metastasis, and mortality [Giovannucci et al., 1997]. The GGC repeat also appears to be associated with prostate cancer risk.

ATBF1

The q22 band of chromosome 16 (16q22) is one of the frequently deleted chromosomal loci in prostate cancer. Many studies have been conducted to map and clone the 16q22 tumor suppressor gene. At present, the ATBF1 transcription factor appears to be more interesting, as it is located in a smaller region of deletion [Sun et al., 2005], and undergoes frequent somatic mutations including frameshift/truncating mutations in sporadic prostate cancer [Sun et al., 2005]. A deletion of 21 or 24-nucleotides in the coding region has been detected not only in sporadic tumors but also in germline DNA from patients with prostate cancer [Sun et al., 2005]. ATBF1 also appears to undergo transcriptional downregulation. Functionally, ATBF1 expression is associated with reduced rate of cell proliferation, upregulation of the CDKN1A (p21) tumor suppressor, and downregulation of AFP oncoprotein. Therefore, ATBF1 is a reasonable candidate tumor suppressor gene in prostate cancer.

BRCA1 AND BRCA2

The tumor suppressor gene *BRCA1* on chromosome 17q21 has been linked to a subset of hereditary breast cancers. A linkage to chromosome 17q22 has also been detected in some hereditary prostate cancers. BRCA1 is thus a candidate for a susceptibility gene in prostate cancer. Multiple studies, however, have excluded a role for BRCA1 in prostate cancer, because truncating mutation of BRCA1 is rare in cases that showed linkage to 17q22, and the 17q22 susceptibility gene in prostate cancer remains to be identified. In some studies, a slightly increased risk of prostate cancer was detected in individuals carrying known BRCA1 truncating mutations, especially those with a younger age at diagnosis [Thompson and Easton, 2002]. In some studies, Ashkenazi

Jewish men were examined for the association between founder mutations in BRCA1 and prostate cancer risk, but no association has been detected between BRCA1 mutation and prostate cancer risk [Kirchhoff et al., 2004]. Even in some high-risk prostate cancer families (with at least three cases of prostate cancer), no BRCA1 truncating mutations have been detected. Therefore, the role of BRCA1 mutation in prostate cancer is quite limited.

The *BRCA2* gene, on the other hand, has been consistently shown to play a role in prostate cancer. The gene is located at 13q12, and its mutation accounts for 30–35% of familial breast cancers. An association between BRCA2 mutation and prostate cancer has been noticed in breast–ovarian cancer families with BRCA2 mutations. A common founder mutation (6174delT) has been identified in Ashkenazi Jewish people, and this allele is significantly associated with prostate cancer risk [Kirchhoff et al., 2004], although some studies did not detect a significant association when smaller numbers of cases were used. A founder mutation of BRCA2, 5-bp deletion, has been identified in the Icelandic population, and an association of this mutation with prostate cancer has also been detected. In familial prostate cancer, mutation of BRCA2 occurs in some families, and it has been estimated that germ-line mutations in BRCA2 may account for about 5% of prostate cancer in familial clusters. Mutation of BRCA2 is particularly significant in prostate cancers diagnosed at a younger age [Edwards et al., 2003]. In an extreme example, each of the men in a family (the father and four of his sons) was diagnosed with prostate cancer at exceptionally early ages (51, 52, 56, 58, and 63 years, respectively), and each one of them had BRCA2 truncating mutation (6051delA). The BRCA2 mutation does not occur in sporadic prostate cancer, though.

CDKN1B (p27/Kip)

Deletions of the p12–13 region of chromosome 12, including homozygous deletion, occur in prostate cancer, suggesting the existence of a tumor suppressor gene in this region [Dong, 2002]. Several candidates have been identified for the *12p12–13* gene. The *CDKN1B* (*p27/Kip1*) gene is more interesting, because it is able to inhibit cyclin-dependent kinases and block cell proliferation. Lower levels of CDKN1B expression predict recurrence and poor disease-free survival in prostate cancer and correlate with a number of prognostic morphologic features including higher tumor grade, positive surgical margins, seminal vesicle involvement, and lymph node metastasis. Although mutations of CDKN1B have not been detected in cancer specimens, hemizygous and homozygous deletions occur in prostate cancer. The *CDKN1B* gene is haploinsufficient, so hemizygous deletion reduces the expression of CDKN1B and affects its normal function through dosage reduction. More interestingly, both Cdkn1b nullizygous and heterozygous mice develop hyperplasia in prostates and are predisposed to tumors in multiple tissues when challenged with gamma-irradiation or a chemical carcinogen. Molecular analyses of tumors in Cdkn1b heterozygous mice show that the remaining wild-type allele is neither mutated nor silenced, indicating the haploinsufficiency of Cdkn1b in tumor suppression. In addition, concomitant inactivation of one Pten allele and one or both Cdkn1b alleles accelerates spontaneous neoplastic transformation and incidence of tumors of various histological origins in mice. In human hereditary prostate cancer, a series of epistatic PTEN and CDKN1B interaction analyses have presented evidence for an interaction between the PTEN locus and the CDKN1B locus in prostatic carcinogenesis. Cooperation has also been demonstrated between loss of Cdkn1b and loss of Nkx3-1 in mouse prostatic carcinogenesis [Gary et al., 2004].

A SNP variant in codon 109 of CDKN1B has been found to be associated with an increased risk of advanced prostate carcinoma [Kibel et al., 2003]. The association is particularly strong in patients with androgen-independent disease or those under the median age of diagnosis. In hereditary prostate cancer, additional sequence variants have been identified, and one of the SNPs, the C allele of −79C/T, appears to be overtransmitted from parents to their affected offspring [Chang et al., 2004], which is more evident in offspring whose age at diagnosis is <65 years.

CHEK2

As an important regulator of p53 in the DNA-damage-signaling pathway, the *CHEK2* gene has recently been evaluated for mutations in prostate cancer. A number of different

mutations, including some frameshift mutations, have been identified in both hereditary and sporadic prostate cancers [Dong et al., 2003]. Functional studies have demonstrated that the frameshift mutations cause abnormal splicing and/or reduced expression of CHEK2 [Dong et al., 2003]. A truncating mutation (1100delC), which abrogates CHEK2's kinase activity, was also associated with both hereditary and sporadic prostate cancer. Loss of the wild-type CHEK2 allele was not observed in any prostate cancers from five men who carried CHEK2-truncating mutations, suggesting that CHEK2 is haploinsufficient.

CYP17

The *CYP17* gene encodes the enzyme cytochrome P-450c17 alpha, which mediates both 17 alpha-hydroxylase and 17,20-lyase in the androgen biosynthesis pathway. A T > C transition in the 5′-promoter region of *CYP17* gene has been hypothesized to increase *CYP17* gene expression, but findings from several studies with smaller numbers of cases and controls have been inconsistent, with some showing an association between the C/C genotype and prostate cancer risk and some showing no differences. A genetic linkage analysis and family-based association analysis have also been conducted in familial prostate cancer. In 159 such families, each of which contains at least three first-degree relatives with prostate cancer, evidence for linkage at the *CYP17* gene region has been found. However, family-based association tests did not provide evidence for overtransmission of the polymorphism in the 5′-promoter region of CYP17 to affected individuals in the HPC families, suggesting that the *CYP17* gene or other genes in the region may increase the susceptibility to prostate cancer, but polymorphism in the 5′ promoter region has a minor role. A meta-analysis of 10 studies with CYP17 genotyping in 2,404 patients with prostate cancer and 2,755 controls suggests that the C/C genotype is a risk factor for sporadic prostate cancer only in men of African descent but not in men of European descent [Ntais et al., 2003].

CYP1B1

For another gene involved in the androgen metabolism, CYP1B1, at least four studies have been conducted to evaluate the association between genetic variants of this gene and prostate cancer risk. One or more variants have been shown to be associated with increased risk of prostate cancer in each of the studies [Chang et al., 2003], although biochemical studies are still lacking to characterize these variants in the expression and/or function of cytochrome P450 1B1.

CYP3A4

The *CYP3A4* gene encodes another member of the cytochrome P450 supergene family, which is involved in the oxidation of testosterone for the deactivation of the hormone. A case-control study has demonstrated that an A > G variant in the 5′-promoter region of the gene, which may change a regulatory element of the gene but has not been confirmed in biochemical assays, was associated with higher tumor stage, tumor grade, and lymph node-metastasis [Rebbeck et al., 1998]. The association with tumor stage is most pronounced in men diagnosed at relatively old ages who reported no family history of prostate cancer [Rebbeck et al., 1998]. The CYP3A4 A > G genotype frequency in different ethnic groups broadly follows trends in prostate cancer incidence, presentation, and mortality in the United States. The variant's correlation with clinically more advanced prostate cancer has been confirmed in additional studies. Using two additional sequence variants of CYP3A4, an association with prostate cancer risk has also been confirmed, although the association is stronger in African Americans and weaker in European Americans [Zeigler-Johnson et al., 2004]. It should be noted that little is known about whether the sequence variants affect CYP3A4's function in vivo.

ELAC2/HPC2

The *ELAC2*/*HPC2* gene at 17p11 is the first candidate gene identified for human prostate cancer based on linkage analysis and positional cloning [Tavtigian et al., 2001]. It encodes a tRNA 3′ processing endoribonuclease. In different studies, at least two truncating or nonsense mutations have been found in some pedigrees of hereditary prostate cancer, which are expected to abolish the enzyme activity. In addition, two common missense variants in HPC2/ELAC2, Ser217Leu and Ala541Thr, which have not been shown to alter the enzymatic activities of ELAC2, have also been shown to be associated with prostate cancer in a sample of men drawn

from families with hereditary prostate cancer. However, in an analysis of unselected cases and controls for family history of prostate cancer, the majority of which are sporadic prostate cancer, only the carriers of both Leu217 and Thr541 alleles had an increased risk of prostate cancer, and the risk did not differ significantly by family history or race. There have been many studies addressing the role of the two variants of ELAC2 in susceptibility to prostate cancer, but findings among different studies have not been consistent, with some showing an association between one or both of the variations and prostate cancer risk, and some showing no association at all. A meta-analysis of several published studies suggest that the Thr541 allele, either alone or in combination with the Leu217 allele, is associated with prostate cancer risk [Camp and Tavtigian, 2002]. The results are most significant in the more extreme case/control comparison group, that is, men with familial prostate cancer versus low-risk control individuals [Camp and Tavtigian, 2002]. There is convincing evidence for the role of ELAC2 in prostate cancer, and it has been estimated that risk genotypes in ELAC2 may cause 2% of prostate cancers in the general population [Camp and Tavtigian, 2002].

EPHB2

The *EPHB2* gene encodes a receptor tyrosine kinase. It was identified as a tumor suppressor gene in prostate cancer by nonsense-mediated decay microarray analysis, and frameshift mutations of EPHB2 have been detected in about 10% of sporadic prostate cancer specimens [Huusko et al., 2004]. Restoration of EPHB2 function in DU 145 prostate cancer cells, which lack wildtype EphB2, suppresses clonogenic growth. In 72 probands from the African American Hereditary Prostate Cancer Study (AAHPC), a nonsense mutation, K1019X (3055A > T), has been identified. This germline mutation is more frequent in African Americans than in Caucasian men, and is significantly associated with an increased risk of prostate cancer in African Americans. It is possible that inactivation of EPHB2 affects cell migration and maintenance of normal tissue architecture.

GSTs

The genes for glutathione S-transferases, which are involved in the metabolism of carcinogens and the defense against reactive oxygen species, may link exposure to genome-damaging stress to increased genomic instability during prostatic carcinogenesis. Many studies have been conducted evaluating whether different GSTs such as GSTP1, GSTM1, and GSTT1 have genetic variants that may be associated with prostate cancer risk. *GSTP1* gene encodes the p-class GST. Loss of expression for GSTP1 is common in prostate cancer, and promoter methylation is a primary mechanism responsible for its loss of expression. The 313A > G germline genetic variant of the gene, which results in an amino acid substitution that alters the function of the enzyme, has been linked to an increased risk of prostate cancer [Harries et al., 1997]. Additional SNPs have been identified in not only GSTP1 but also two other members of the GST family, GSTM1 and GSTT1. Some of these SNPs have also been linked to prostate cancer risk. In a number of follow-up studies, however, the link between GST polymorphisms and prostate cancer risk has not always been validated [Ntais et al., 2005]. It appears that germline polymorphisms in GSTP1, GSTM1, and GSTT1 contribute to prostatic carcinogenesis only when specific environmental carcinogenic factors are present.

KLF5

The q21 band of chromosome 13 (13q21) is the second most frequently deleted locus in human prostate cancer. Deletion of 13q21 is also frequent in many other types of human malignancy. A number of deletion mapping studies have been conducted, and the minimal region of deletion has been narrowed to 200 kb, in which the transcription factor KLF5 is the only complete gene [Chen et al., 2003a]. KLF5 is another member of the Kruppel-like transcription factors that are involved in cell proliferation, differentiation, and carcinogenesis. The major form of deletion for KLF5 in human cancer is hemizygous deletion, and homozygous deletion is rare. In mice, knockout of both KLF5 alleles is lethal to embryogenesis, and knockout of one allele can still have an impact, indicating that KLF5 is haploinsufficient. Hemizygous deletion thus reduces KLF5 expression and impairs the function of KLF5 through haploinsufficiency, representing a common genetic mechanism for the loss of KLF5 function in prostate cancer. Although few mutations have been identified in KLF5 in cancer cells, there are

two other mechanisms that lead to inactivation of KLF5 in cancer cells. One is transcriptional silencing of KLF5, and the other is excessive protein degradation of KLF5 by the ubiquitin proteasome pathway. In in vitro functional studies, transfection of KLF5 into cancer cell lines suppresses cell proliferation in most of them, although KLF5 is stimulatory for cell growth in the TSU-Pr1 bladder cancer cell line. It is possible that loss of KLF5 cooperates with other genetic alterations in the development and progression of prostate cancer.

KLF6

KLF6 is a zinc finger transcription factor likely to have a role in cell proliferation and differentiation. It was reported as a tumor suppressor gene in prostate cancer because of its frequent loss of heterozygosity (LOH) and mutation as well as functional suppression of cell proliferation [Narla et al., 2001]. Somatic mutations of KLF6 in prostate cancer have been confirmed in additional studies, although the mutation frequency is not as high as originally reported [Chen et al., 2003b]. In addition, loss of KLF6 expression, probably by regulatory mechanisms, also occurs in prostate cancer [Chen et al., 2003b]. Although germline mutation of KLF6 is rare and KLF6 does not appear to play a role in hereditary prostate cancer, a germline SNP in KLF6 has been confirmed in a tri-institutional study of 3,411 men for a significant association with an increased relative risk of prostate cancer regardless of family history of disease [Narla et al., 2005]. This SNP generates a functional SRp40 DNA binding site and increases transcription of three alternatively spliced KLF6 isoforms, which produce variant KLF6 proteins that are mislocalized to the cytoplasm and antagonize wildtype KLF6 function.

MSR1

The p22 band of chromosome 8 is one of the chromosomal loci that are frequently deleted in prostate cancer and also linked to hereditary prostate cancer. Macrophage scavenger receptors (MSRs) are trimeric membrane glycoproteins that mediate the binding, internalization, and processing of a wide range of negatively charged macromolecules. The *MSR1* gene located at 8p22 has been reported as a strong candidate for the 8p22 prostate cancer susceptibility gene, because mutations in MSR1, including truncating mutations, have been shown to be associated with prostate cancer risk in both hereditary cases and sporadic cancers. Men of both African and European descent are affected [Xu et al., 2002]. The MSR1 protein has six predicted protein domains, and the truncating mutation Arg293X results in a dominant negative mutant of the gene. In case control studies, an association of sequence variants of MSR1 with prostate cancer risk has also been confirmed. In some studies, the risk factor of MSR1 mutation could not be confirmed. Haplotype analyses have showed a significant difference in the haplotype frequencies between prostate cancer cases and normal controls [Xu et al., 2003], and it appears that the observed association of MSR1 common variants and prostate cancer risk is independent of the effect of the known rare mutations.

MITOCHONDRIA DNA (mtDNA)

The mitochondrial genome is a circular strand of 16.5-Kb DNA that encodes 13 proteins essential for cellular energy production. The haploid DNA is semiautonomously maintained in mitochondria, and exists in multiple copies in each cell. Since the mtDNA lacks protective histones and has limited DNA repair ability, mutations can accumulate over time in various tissues throughout the body including the prostate. Extensive somatic mutations of the mtDNA in its coding regions, including deletion mutations, occur in prostate cancer. Mutations of mtDNA have also been detected in prostatic intraepithelial neoplasia (PIN) lesions, which have been considered a precursor of prostate cancer. Germline mutations of mtDNA also contribute to prostate cancer [Petros et al., 2005]. Tumors often produce increased reactive oxygen species (ROS), and mtDNA mutations that inhibit oxidative phosphorylation can increase ROS production and thus contribute to tumorigenicity. Functional analysis of a mutation of mtDNA validated this hypothesis in an experimental model of prostatic carcinogenesis [Petros et al., 2005].

MYC

The cellular proto-oncogene MYC has been well implicated in cell transformation. Although earlier studies did not detect obvious genomic amplification of MYC, which could be due to a

simple copy gain of 8q and more contamination of nontumor cells in the specimens, CGH studies have showed that gain of 8q, including 8q24 involving MYC and 8q21, is one of the most frequent alterations in prostate cancer [Knuutila et al., 1998]. In the LNCaP prostate cancer cell line, MYC has significant amplification and overexpression. The PC-3 line also has 8q gain. In a comprehensive study using the more definitive fluorescence in situ hybridization (FISH) method with probes for MYC (8q24), 8q centromere, and other chromosomes, about 40% of primary tumors and over 90% of metastases showed varying levels of MYC copy number increases [Jenkins et al., 1997]. Whereas primary tumors mainly have simple gain of MYC due to an extra copy of 8q, metastases have more frequent regional MYC amplification, suggesting that MYC is more commonly involved in prostate cancer progression. Substantial amplification of MYC correlated with increased MYC protein expression in the nucleus. MYC copy number gain becomes more frequent after patients receive androgen deprivation therapy (ADT). After ADT treatment, MYC copy number gain correlates with the proliferation rate indicated by the Ki-67/MIB1 index. Simple copy number gain of MYC in prostate cancer has been verified in additional studies using different methods, and in some studies gain of chromosome 8q including MYC is more frequent and is associated with higher tumor grade. In a transgenic mouse model, overexpression of Myc in the ventral prostate epithelial cells leads to the development of abnormalities similar to prostatic intraepithelial neoplasia (PIN) in humans, although no adenocarcinoma was observed in that study. In another transgenic mouse model overexpressing Myc in the prostate, not only PIN lesions but also prostatic carcinomas were induced [Ellwood-Yen et al., 2003]. Overexpression of MYC can also immortalize human prostatic epithelial cells. Therefore, gain of function of MYC is clearly an oncogenic factor in human prostate cancer.

NKX3-1 (NKX3.1, NKX3A)

According to numerous genetic analyses of prostate cancer, deletion of the short arm of chromosome 8 (8p), especially the region at 8p21 involving NKX3-1, is the most frequent chromosomal locus deletion in human prostate cancer. Although several genes have been considered as the target gene of deletion at 8p21, the *NKX3-1* gene appears to be the most convincing. *NKX3-1* is a prostate-specific gene in humans. It is expressed at a higher level in adult prostate but its expression is reduced in prostate cancer cells. The gene is haploinsufficient, so the hemizygous deletion detected by CGH is a major mechanism reducing the expression and function of NKX3-1. Several studies have evaluated the mutations of NKX3-1 in prostate cancer, but have not detected significant mutations, so genomic deletion is the major alteration that weakens the function of NKX3-1 by haploinsufficiency.

Several studies have examined the effect of Nkx3-1 knockout on the prostate of mice. Homozygous mutant mice for Nkx3-1 are viable and fertile, but exhibit defective branching morphogenesis of the prostate and palatine glands. When the mice age, epithelial cells of the mutant prostate develop significant hyperplasia that are similar to PIN lesions in human prostate. Heterozygous mice also develop PIN-like hyperplasia in prostates, indicating the haploinsufficiency of Nkx3-1. Prostate-restricted targeting of a conditional Nkx3-1 allele results in adult-specific deletion of Nkx3-1 and also causes the formation of PIN [Abdulkadir et al., 2002]. In cell culture and xenograft assays, Nkx3-1 displays moderate suppression of growth rate and tumorigenicity. Crossing Nkx3-1 knockout mice with Pten knockout mice combined with phenotypic analysis has demonstrated that simultaneous loss-of-function of Nkx3-1 and Pten in mice results in striking cooperativity by 6 months of age, as shown by the increased incidence of high-grade PIN and carcinoma in situ lesions. Cooperation has also been shown between Nkx3-1 and Cdkn1b (p27), the latter is at another frequently deleted locus (12p12) in prostate cancer [Dong, 2002] and suppresses the proliferation of prostatic epithelial cells [Gary et al., 2004]. *NKX3-1* has become one of the well-characterized prostate cancer genes.

P53

P53 is a tumor suppressor gene whose mutations are implicated in the molecular genetics of many human malignancies. In fact, p53 is probably the most frequently mutated gene in human cancer. Nucleotide alterations, most commonly single point mutations, have been shown to not only abrogate the p53 suppressor

function but also contribute to the transformed phenotype. Whereas wildtype p53 protein degrades quickly in normal cells, mutated p53 alleles typically encode abnormally stable p53 proteins that accumulate to high levels in tumor cell nuclei. Immunohistochemistry (IHC) staining with anti-p53 antibodies has been used to evaluate p53 mutation. Using the IHC method in combination with mutation verification, abnormal nuclear p53 accumulation and p53 mutation have been observed in prostate cancers, although the mutation rates vary among different studies and among tumors of different aggressiveness (ranging from 3% to 42%). It is clear now that mutations of p53 are rare in primary prostate cancer, but are more common in prostate cancers of higher tumor stage, higher tumor grade, metastases, or androgen-independent tumors. In locally advanced prostate cancer, p53 mutation is associated with increased incidence of distant metastases, decreased progression-free survival, and decreased overall survival [Grignon et al., 1997]. Although in some studies, the association between p53 mutation and tumor grade and stage is not detectable, the association with metastasis has been repeatedly detected. Prostate cancers metastasizing to bone, in particular, appear to have the most frequent p53 mutations [Meyers et al., 1998]. Some primary carcinomas of the prostate contain more than one p53 mutation, suggesting the possibility of intratumoral heterogeneity of mutation of this gene. Mutations of p53 also occur frequently in prostate cancers treated with chemicals or radiation. It seems that exon 7 of p53 is more susceptible to mutation in prostate cancer. For patients with p53 mutations, combined radiation and hormone therapy results in reduced time to the development of distant metastases [Grignon et al., 1997]. It is likely that mutation of p53 impairs genomic stability, leading to genomic amplification of the *AR* gene during hormone therapy, since hormone-refractory prostate cancers with *AR* gene amplification showed more frequent p53 mutations than those without *AR* gene amplification. Mutation of p53 has also been detected in benign prostatic hyperplasia (BPH), and the mutation in BPH may be a tumor risk factor. Overall, *p53* gene mutation is more likely a late event in the progression of prostate cancer and is associated with advanced stage, metastasis, and the transition from androgen-dependent to androgen-independent growth.

PON1

Human serum paraoxonase eliminates carcinogenic lipid-soluble radicals. The expression of the main human paraoxonase gene *PON1* varies widely in humans, and certain PON1 polymorphisms are associated with different serum levels of PON1 protein. One SNP in the coding region of PON1, I102V, has been shown to be associated with decreased serum paraoxonase activity. Genotyping of 1,595 men for this PON1 mutation showed an increased risk for developing prostate cancer among 102V allele carriers [Marchesani et al., 2003]. In addition, patients with familial prostate cancer are more likely to be carriers of the PON1 I102V mutation than control subjects. Association of the PON1 102V allele with an increased risk for prostate cancer has been confirmed in another population.

PTEN

The long arm of chromosome 10 (10q23) is one of the most frequently deleted chromosomal regions in human prostate cancer, as demonstrated by numerous molecular and cytogenetic studies. In 1997, different groups simultaneously identified PTEN as a strong candidate for the 10q23 tumor suppressor gene [Li et al., 1997]. The *PTEN* gene has nine exons that encode a 403-amino acid protein of a dual-specific phosphatase with putative actin-binding and tyrosine phosphatase domains. Among the several genes from chromosomal regions that are frequently deleted in prostate cancer, PTEN is one of the few that are implicated in prostate cancer by frequent somatic mutations in aggressive prostate cancer. The mutations include many frameshift deletions. PTEN appears to be more frequently mutated in metastases of prostate cancer, indicating a role for PTEN in the progression of prostate cancer. Mutations of PTEN in localized prostate cancers have been found at lower frequencies; and the frequencies differ among studies largely due to differences in tumor grade and stage in the study populations, ranging from zero in some studies to 16% in some other studies [Dong et al., 2001]. Loss of PTEN expression has also been shown to correlate with higher grade primary

prostate cancer. Germline mutations of PTEN are associated with Cowden disease, in which patients are at increased risk for certain cancers. In hereditary prostate cancer, however, PTEN does not play a significant role, although nonparametric linkage regression analysis and ordered subset analysis have suggested an interaction between PTEN and CDKN1B.

Pten is also one of the few genes whose deletion clearly induces prostate cancer in mice, as demonstrated not only in conventional knockout mice, but also in conditional knockout mice [Wang et al., 2003]. It has also been demonstrated that loss of Pten cooperates with loss of other genes such as Nkx3-1 and Cdkn1b (p27) in the development and progression of prostate cancer. In addition, introduction of Pten into cancer cells that lack Pten function negatively regulates cell migration and survival and induces cell cycle arrest and apoptosis via negative regulation of the phosphatidylinositol 3′-kinase/protein kinase B/Akt signaling pathway. In fact, the PTEN molecular pathway is among the best studied in prostate cancer so far. Genetic, functional, and molecular studies have firmly established a role for PTEN in human prostate cancer.

RAS

The three RAS oncogenes, HRAS at 11p15, KRAS at 12p12, and NRAS at 1p13, encode the 21-kDa RAS oncoprotein. Point mutations in these genes convert the normal cellular genes into abnormally activated oncogenes, which can lead to uncontrolled cell proliferation and tumorigenesis. In earlier studies, few RAS mutations could be detected using the NIH3T3 transformation assay or oligodeoxynucleotide hybridization assay. Several studies have been published by hybridizing PCR amplified tumor DNA into allele-specific oligonucleotide probes that cover common mutations of RAS such as codons 12 and 61 of HRAS and KRAS and codons 12, 13, and 61 of NRAS, but overall the mutation frequency has been low (0 of 24 samples in one study and 1 of 19 primary prostate cancers in another study). One of four prostate cancer cell lines has a mutation in codon 12 of KRAS. SSCP combined with DNA sequencing has not detected more frequent mutations of RAS in American patients either.

In prostate cancers from Japanese patients, however, PCR and DNA hybridization with sequence-specific oligonucleotides demonstrated significantly more frequent somatic RAS mutations (24% or 16/68 cases including 11 in codon 61 of HRAS, 4 in codon 12 of NRAS, and 2 in codon 61 of KRAS) [Anwar et al., 1992]. Mutations of RAS are associated with higher tumor stage and higher tumor grade. Even in clinically silenced prostatic carcinomas discovered in Japanese men at autopsy, the same methods showed significant mutations of RAS (6 of 22 samples in codon 12 of KRAS). Using the more sensitive PCR–SSCP analysis and Mutant-Allele-Specific Amplification (MASA) method, an analysis of 81 Japanese prostate cancers for RAS mutations showed RAS mutations in 20 of 81 (24%) samples (13 in codon 12 of KRAS, 5 in codon 61 of HRAS, and 2 in codon 13 of HRAS); and again, mutations of RAS were significantly more frequent in higher-stage tumors. Therefore, RAS mutations appear to be more frequent in Japanese men than in American men in the three commonly analyzed codons. This hypothesis has been further confirmed in a study in which latent prostatic carcinomas discovered at autopsy in men from different ethnic backgrounds, including Japanese, Caucasian, African American, and Colombian, were analyzed by PCR and hybridization, and frequent RAS mutations were found only in Japanese men [Watanabe et al., 1994]. It is unknown if genetic factors or environmental factors cause the difference in RAS mutation rates among different ethnic groups, although the latter seems more likely.

RNASEL/HPC1

The *RNASEL* gene at 1q25 encodes the 2′-5′-oligoadenylate(2–5 A)-dependent RNase L (RNASEL). By using positional cloning and candidate gene methods, a nonsense mutation and a mutation in an initiation codon of RNASEL have been shown to segregate independently in two hereditary prostate cancer 1 (HPC1)-linked families [Carpten et al., 2002]. The Arg462Gln variant of RNASEL, which has three times less enzymatic activity than the wildtype, has been found to be significantly associated with prostate cancer risk. Both heterozygous and homozygous genotypes of this SNP cause an increased risk of prostate cancer. A founder frameshift mutation in RNASEL, 471delAAAG, was identified in Ashkenazi Jews, and this mutation is also associated with

prostate cancer risk. Another truncating mutation, E265X, found in Finnish men, is also associated with increased risk of prostate cancer. These mutations are often associated with early onset of disease. For the Arg462Gln variant, significant differences have been detected between prostate cancer patients and controls in familial but not in sporadic prostate cancer. This SNP is also associated with a younger age of disease onset. Variants of RNASEL have also been shown to be associated with familial prostate cancer in Japanese men. These variants showed no significant association with prostate cancer in Swedish and German populations. While mutations of RNASEL have been detected in prostate cancer cell lines, they rarely occur in sporadic prostate cancer. On the other hand, the 541E variant showed a positive association with metastatic sporadic prostate cancer in European Americans. In one study, the RNASEL Arg462Gln variant appeared to be associated with low-grade and early-stage disease in family history-negative European Americans. In family history-positive individuals, the Arg462Gln variant was inversely associated with low-grade and low-stage disease. In African Americans, Arg462Gln was associated with positive family history high-stage disease. In functional studies, RNASEL regulates cell proliferation and apoptosis through the interferon-regulated 2–5 A pathway and has been suggested as a candidate tumor suppressor gene.

SRD5A2

The development and progression of prostate cancer is influenced by androgens. Steroid 5-alpha-reductase 2 (SRD5A2) converts testosterone to more bioactive dihydrotestosterone and is critical to the development of the prostate. Different alleles are associated with different activities of SRD5A2. A TA dinucleotide repeat polymorphism exists in the 3′-UTR of the gene, and its allele with longer TA repeats is more common in African American. In case-control studies, the role of this polymorphism in prostate cancer susceptibility is still inclusive. Another SNP, V89L, which reduces in vivo SRD5A2 activity, is most frequent in Asians, intermediate in Caucasians, and lowest in African Americans. Although many studies have failed to detect a significant association of this variant with prostate cancer or plasma androstanediol glucuronide levels, some studies have showed that men with the V allele of the *SRD5A2* gene are at greater risk of being diagnosed with prostate cancer and having disease progression than those with the L allele. In one study, the V89L variant is only associated with metastases at the time of diagnosis but not associated with altered prostate cancer risk. It appears that the association is more obvious in prostate cancer patients with younger ages at diagnosis [Cicek et al., 2004].

Another SNP variant, A49T, which leads to increased conversion of testosterone to dihydrotestosterone, significantly increased the risk of clinically significant disease in African-American men and Hispanic men [Makridakis et al., 1999]. Association of this variant has been confirmed in additional studies. The A49T variant is especially associated with more aggressive features of prostate cancer such as extracapsular disease, higher tumor stage, metastasis, worse biochemical disease-free survival, higher tumor grade, and younger age at diagnosis. Although there are also studies that have failed to detect an association of this variant with prostate cancer, evidence for prostate cancer linkage in the SRD5A2 locus has been observed in hereditary prostate cancer.

VDR

Prostatic cells express vitamin D receptor (VDR), which mediates the functions of 1,25-dihydroxy vitamin D. Low circulating levels of 1,25-dihydroxy vitamin D (1,25-D) have been implicated as a risk factor for prostate cancer. In addition, 1,25-D exhibits significant antineoplastic properties both in vitro and in vivo, and these antiproliferative effects appear to be mediated through the vitamin D receptor (VDR). A large number of studies have been conducted to examine if VDR polymorphisms, which often affect VDR activities, are associated with prostate cancer. Although some studies detected no association detected between VDR polymorphisms and prostate cancer risk, more studies have shown a positive association in different populations including Caucasian American, African American, Chinese, Japanese, European, and Indian [Medeiros et al., 2002]. Carriers of less-active VDR alleles usually have an increased risk of prostate cancer. In addition

to sporadic prostate cancer, association with VDR polymorphisms has also been detected in familial prostate cancers, especially those with a younger age of disease onset.

VDR polymorphisms appear to interact with other factors to contribute to the development and progression of prostate cancer. For example, high-activity alleles of VDR are inversely associated with prostate cancer risk in the presence of high sun exposure [John et al., 2005]. For polymorphism associated with VDR function as indicated by altered levels of 1,25-D, an association with prostate cancer has been detected in men with lower but not higher plasma 25-hydroxyvitamin D levels. VDR polymorphisms have also been associated with increased risk of more aggressive cancer with higher tumor grade, metastasis, and locally advanced tumors. In addition, VDR polymorphisms have been associated with prostate cancer risk in patients in the highest tertile of plasma IGFBP-3.

PERSPECTIVE

The genes discussed above are well implicated in human prostate cancer. Most of these genes still need to be examined by genetic, functional, and biochemical approaches to dissect their precise role in prostate cancer and understand the molecular pathways by which they affect prostatic carcinogenesis. Additionally, many more genes with a role in prostate cancer remain to be identified. For example, many chromosomal regions have been identified by molecular cytogenetic studies, especially CGH, for either copy number gain or loss in prostate cancer. Such regions are believed to harbor tumor suppressor genes or oncogenes [Knuutila et al., 1998; Dong, 2002], but many of the regions still do not have a convincing target gene. Additional studies are needed to identify and validate genes for more chromosomal regions that are either deleted or amplified in prostate cancer. For example, 7q31 has a small region that is frequently deleted in prostate cancer, but the target gene is still not well established. In many cases, multiple genes from a region may simultaneously play a role in prostatic carcinogenesis. In addition, there are many germline variants of genes that may affect gene function and thus modify prostatic carcinogenesis. Discovering these variants is another way by which more genes relevant to prostate cancer will be implicated. In familial prostate cancer, many loci have been mapped by different laboratories but only a few genes have been identified. More familial prostate cancer genes will be discovered in the future. Large scale sequencing of cancer genomes is another approach that will implicate more genes in prostate cancer by revealing tumor-specific gene mutations. As critical genetic alterations are identified and their pathways understood, the detection and treatment of prostate cancer will be vastly improved.

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