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Molecular genetics of human prostate cancer

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Multiple factors contribute to the high incidence and prevalence of prostate cancer including race, ethnicity, diet, environment, widespread awareness through prostate-specific antigen screening and genetics. Linkage analysis has identified several candidate sites for hereditary prostate cancer gene loci. Molecular studies have also identified genes that are frequently altered in sporadic prostate cancer. It appears that due to the heterogeneity of prostate cancer, multiple genes may be involved in the neoplastic process.
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In developed countries, prostate cancer is the most commonly diagnosed nonskin malignancy in males. It is estimated that one in six males will be diagnosed with prostate cancer during their lifetime. Multiple factors contribute to the high incidence and prevalence of prostate cancer. Risk factors include age, family history, and race. Environmental exposures are clearly involved as well. Although the exact exposures that increase prostate cancer risk are unclear, diet (especially those high in animal fat such as red meat as well as those with low levels of antioxidants such as selenium and vitamin E), job/industrial chemicals, sexually transmitted infections, and chronic prostatitis have been implicated to varying degrees. The marked increase in incidence in prostate cancer that occurred in the mid-1980s, which subsequently leveled off in the mid- to late-1990s, indicates that widespread awareness and serum prostate-specific antigen screening can produce a transient marked increase in prostate cancer incidence. This review will examine recent developments in understanding the molecular basis of prostate cancer and some tools that should help us in future investigations.

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Hereditary prostate cancer

Currently, the evidence for a strong genetic component is compelling. Observations made in the 1950s by Morganti *et al*[1] suggested a strong familial predisposition for prostate cancer. Strengthening the genetic evidence is a high frequency for prostate cancer in monozygotic as compared to dizygotic twins in a study of twins from Sweden, Denmark, and Finland.[2] However, unlike the successful mapping and cloning of *BRCA1* and *BRCA2*, which explain a large proportion of hereditary breast cancers, genes conferring susceptibility to prostate cancer have been more elusive. Work over the past decade using genomewide scans in prostate cancer families has identified highrisk alleles, displaying either an autosomal dominant or X-linked mode of inheritance for a hereditary prostate cancer gene, from at least seven candidate genetic loci (Table 1). Of these loci, three candidate genes have been identified, *HPC2/ELAC2* on 17p[3] and *RNASEL* on 1q25,[4] and *MSR1* on 8p22–23.[5] In terms of *ELAC2*, while an initial attempt to confirm these findings was promising,[6] more recent reports find little evidence that *ELAC2* is linked to hereditary or sporadic prostate cancer.[7–10] RNASEL (encoding ribonuclease L) is a ubiquitously expressed latent endoribonuclease involved in the mediation of the antiviral and proapoptotic activities of the interferon-inducible 2-5A system.[11,12] Work now from several groups demonstrates that the reduction of RNASEL activity through mutation leads to decreased enzymatic activity.[4,13–15] Most recent work suggests that approximately 13% of prostate cancer cases in the population may be attributable to this

mutation,[4] although another recent study only found mutations in hereditary cases and not sporadic cases of prostate cancer.[16] This example of a novel mutation, which is not associated with complete loss of protein production but a decrease in its activity, demonstrates the complexity in understanding the development and progression of cancer. Furthermore, an *RNASEL* knockout mouse exists, which is devoid of any prostate-related phenotype. However, this mouse is susceptible to infections, an interesting observation given the increasing interest in proliferative inflammatory atrophy (PIA) as a putative precursor lesion in the development of prostate cancer. The other recently observed hereditary gene, *MSR1*, is a macrophage-specific receptor, which can bind polyanionic ligands, including Gram-negative and Gram-positive bacteria. *MSR1* knockout mice also have a reduced capacity to eradicate pathogens.[5]

It is clear, however, that these three genes do not account for the majority of hereditary prostate cancer cases. In addition, more than 10 other loci have been implicated by at least some groups. The discovery of highly penetrant prostate cancer genes has been particularly difficult for at least two main reasons. First, due to the advanced age of onset (median 60 years), identification of more than two generations to perform molecular studies on is difficult. Second, given the high frequency of prostate cancer, it is likely that cases considered to be hereditary during segregation studies actually represent phenocopies; currently, it is not possible to distinguish sporadic (phenocopies) from hereditary cases in families with high rates of prostate cancer. In addition, hereditary prostate cancer does not occur in any of the known cancer syndromes and does not have any clinical (other than a somewhat early age of onset at times) or pathologic characteristics to allow researchers to distinguish it from sporadic cases.[17] It is hoped that the formation of large international consortia that are collaborating and pooling families will provide some relief to these problems. Perhaps even more important in terms of inherited susceptibility for prostate cancer are common polymorphisms in a number of low penetrance alleles of other genes—the so-called genetic modifier alleles. The list of these variants is long, but the major pathways currently under examination include those involved in androgen action, DNA repair, carcinogen metabolism, and inflammation pathways.[18,19] It is widely assumed that the specific combinations of these variants, in the proper environmental setting, can profoundly affect the risk of developing prostate cancer.

Early molecular alterations in prostate cancer progression

Pathologists have long recognized focal areas of epithelial atrophy in the prostate.[20–22] These focal areas of epithelial atrophy, distinct from the diffuse atrophy seen after androgen deprivation, most often appear in the periphery of the prostate, where prostate cancers typically arise.[20,23–27] Epithelial atrophy may be associated with acute or chronic inflammation, contain proliferative epithelial cells, and may show morphological transitions in continuity with high-grade prostatic intraepithelial neoplasia (PIN) lesions, a putative prostate cancer precursor.[27,28] A transition from these atrophic lesion to carcinoma, with little or know recognizable PIN component can be observed.[22,29,30] Focal atrophy of the prostate exists as a spectrum of morphologies and areas containing it in the prostate can be quite extensive. Since these lesions have also been shown to have a higher proliferation index,[25–27,31] they have been termed PIA lesions.[27] In support of PIA as a prostate cancer precursor, chromosome 8 gain, detected by fluorescence *in situ* hybridization (FISH) with a chromosome 8 centromere probe, was found in human PIA, PIN, and prostate cancer.[31,32] Others have recently documented rare p53 mutations in one variant of PIA,[33] and work from one author group (ADM) shows that approximately 6% of PIA lesions show evidence of somatic methylation of the GSTP1 gene promoter.[34] Focal atrophy lesions may arise either as a consequence of epithelial damage from infection, ischemia, or toxin exposure or as a direct consequence of inflammatory oxidant damage to the epithelium.[27] Regardless of the etiology of PIA, the epithelial cells in these lesions exhibit molecular signs of stress, expressing high levels of GSTP1, GSTA1, and cyclooxygenase 2 (COX-2).[27,28,35,36] There is also mounting evidence that the atrophic

Table 1 Prostate cancer susceptibility loci identified by linkage analysis

Susceptibility loci	*Locus*	*Mode*	*Putative gene*	*Reference*
HPC1	1q24–25	AD	*RNASEL*[4]	Smith *et al.*[106]
PCAP	1q42.2–43	AD	?	Berthon *et al.*[107]
CAPB	1p36	AD	?	Gibbs *et al.*[108]
HPCX	Xq27–28	X-linked/AR	?	Xu *et al.*[109]
HPC20	20q13	AD	?	Berry *et al.*[110]
HPC2	17p	AD	*HPC2/ELAC2*[3]	Tavtigian *et al.*[3]
	8p22–23	AD	*MSR1*	Xu *et al.*[5]

Mode = suggested mode of inheritance; AD = autosomal dominant; AR = autosomal recessive.

luminal cells in PIA represent a form of intermediate epithelial cell[37]—similar to cells postulated to be the targets of neoplastic transformation in the prostate.[38–41] Therefore, both PIA and high-grade PIN may represent steps along a pathway in the progression to invasive prostate cancer (Figure 1). However, it is not clear if they represent separate pathways or steps along the same pathway.

Chromosomal Instability and Telomeres

Chromosomal instability is an important molecular mechanism during the pathogenesis of malignant transformation in human epithelial tissues,[42] yet the molecular mechanisms responsible for chromosome destabilization during carcinogenesis are largely unknown. One route to chromosomal instability is through defective telomeres.[43,44] Telomeres, which consist of multiple repeats of a 6-base-pair unit (TTAGGG), complexed with several different binding proteins, protect chromosome ends from fusing with other chromosome ends or other chromosomes containing double-strand breaks.[45] However, in the absence of compensatory mechanisms, telomeric DNA is subject to loss due to cell division and possibly oxidative damage. Telomere shortening leads to chromosomal instability that, in mouse models, causes an increased cancer incidence that is likely a result of chromosome fusions, subsequent breakage, and rearrangement.[46,47] Telomeres within human carcinomas are often found to be abnormally reduced in length. In recent work, the telomeres from prostate cancer were consistently shorter than those from cells in either of the adjacent normal prostate tissue.[48,49]

Most carcinomas arise from preinvasive intra epithelial precursor lesions.[50] These lesions show morphological features and molecular alterations characteristic of malignant neoplasia, including genetic instability[51] but occur within pre-existing epithelia and are confined within the basement membrane. If genetic instability helps to drive cancer formation, and telomeres shortening is a major mechanism leading to genetic instability, then telomere shortening should be present at the intraepithelial phase of carcinoma. Recently, an *in situ* telomere FISH technique was employed to demonstrate telomere shortening in the majority of high-grade PIN lesions, which are thought to be cancer precursor lesions of the prostate.[52] Interestingly, the telomere shortening found in high-grade PIN was restricted to the luminal cells and was not present in the underlying basal cells. This finding suggests that basal cells are not the direct precursor cells to high-grade PIN, but supports the concept that that cells with an intermediate luminal cell phenotype are the likely direct target cell of transformation in the prostate. Thus, telomere shortening is a prevalent biomarker in human prostate neoplasia occurring early in the process of prostate carcinogenesis.

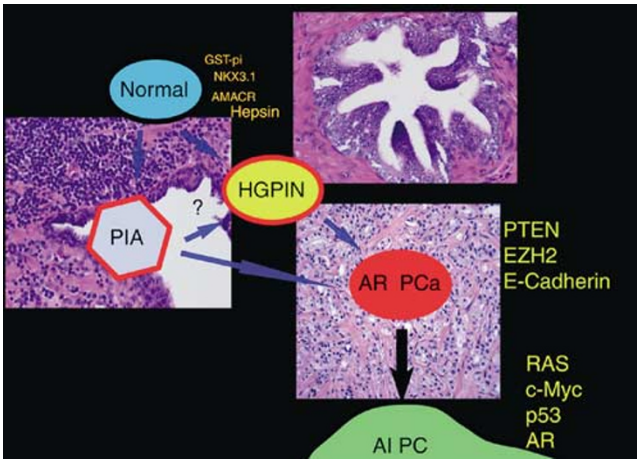

Figure 1 Prostate Cancer Progression. This schematic illustrates the putative steps in the development of prostate cancer. Normal appearing prostate epithelium may develop into clinically localized androgen responsive prostate cancer (AR PCa) through one or two pathways. Normal epithelium may undergo molecular alterations in caretaker genes such as GST-pi or NKX3.1 and develops into the intraepithelial neoplastic lesion, high-grade prostatic intraepithelial neoplasia (PIN). This process may also proceed through proliferative inflammatory atophy (PIA). Alternatively, AR PCA may develop through more than one pathway arising from PIA and high-grade PIN. Multiple genes have been found to be altered in advanced hormone refractory prostate cancer including PTEN, EZH2, and p53. Multiple mutations in the androgen receptor have also been identified.

Molecular alterations in sporadic prostate cancer

While mutations in any of the classic oncogenes and tumor suppressor genes are not found in high frequency in primary prostate cancers, a large number of studies have identified nonrandom somatic genome alterations. Using comparative genomic hybridization (CGH) to screen the DNA of prostate cancer, the most common chromosomal alterations in prostate cancer are losses at 1p, 6q, 8p, 10q, 13q, 16q, and 18q and gains at 1q, 2p, 7, 8q, 18q, and Xq.[53–56] Numerous genes have now been implicated in prostate cancer progression. Several genes have been implicated in the earliest development of prostate cancer (Table 2). The pi-class of glutathione *S*-transferase (GST), which plays a caretaker role by normally preventing stress-related damage, demonstrates hypermethylation in a high percentage of prostate cancers, thus preventing the expression of this protective gene.[57–59] *NKX3.1*, a homeobox gene located at 8p21, has also been implicated in prostate cancer.[60–63] Although no mutations have been identified in this gene,[61] recent work suggests that decreased expression is associated with prostate cancer progression.[62] *PTEN*, a tumor suppressor gene located at 10q23, was originally found to be mutated in primary brain tumors and breast and prostate cancer cell lines.[64,65]

Table 2 Selected genes associated with prostate cancer progression

Abbreviation	*Gene name(s)*	*Locus*	*Functional role*	*Molecular alteration*
GST-pi	Glutathione *S*-transferase pi	11q13	Caretaker gene	Hypermethlyation
NKX3.1	NK3 transcription factor homolog A	8p21	Homeobox gene	No mutations
PTEN	Phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	10q23.3	Tumor supressor gene	Mutations and haplotype insufficiency
AMACR	Alpha-methylacyl-CoA racemase	5p13.2–q11.1	β-Oxidation of branched-chain fatty acids	Overexpressed in PIN/Pca
Hepsin	Hepsin	19q11–q13.2	Transmembrane protease, serine 1	Overexpressed in PIN/Pca
KLF-6	Kruppel-like factor 6/COPEB	10p15	Zinc-finger transcription factor	Mutations and haplotype insufficiency
EZH2	Enhancer of zeste homolog 2	7q35	Transcriptional memory	Overexpressed in aggressive Pca
p27	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	12p13	Cyclin-dependent kinases 2 and 4 inhibitor	Downregulated with Pca progression
E-cadherin	E-cadherin	16q22.1	Cell adhesion molecule	Downregulated with Pca progression

Pca = prostate cancer; PIN = prostatic intraepithelial neoplasia.

PTEN encodes a phosphatase active against both proteins and lipids, and is also commonly altered in prostate cancer progression. PTEN is believed to regulate the phosphatidylinositol 3′-kinase/protein kinase B (PI3/Akt) signaling pathway, and therefore mutations or alterations lead to tumor progression.[66] As with many putative prostate cancer genes, PTEN is also associated with a number of other tumors. Mutations are less common than initially thought in prostate cancer; however, tumor suppressor activity may occur from the loss of one allele, leading to decreased expression of PTEN (i.e. haploinsufficiency).[67] A number of other genes have also been associated with prostate cancer, including *p27*[68–70] and *E-cadherin*.[71,72] p53 mutations are late events in prostate cancer and tend to occur in advanced and metastatic prostate tumors.[73]

Recent advances in genomic and proteomic technologies suggest that molecular signatures of disease can be used for diagnosis,[74,75] to predict survival,[76,77] and to define novel molecular subtypes of disease.[78] Several studies have used cDNA microarrays to characterize the gene expression profiles of prostate cancer in comparison with benign prostate disease and normal prostate tissue.[79–84] Several interesting candidates include *AMACR*, *Hepsin*, *KLF6*, and *EZH2*. Alpha-methylacyl-CoA racemase (*AMACR*), an enzyme that plays an important role in bile acid biosynthesis and β-oxidation of branched-chain fatty acids,[85,86] was determined to be upregulated in prostate cancer after examination of several independent gene expression data sets.[79,80,82,87–89] These findings were supported by different groups on the protein level even when using different types of antibodies for immunoblot analysis and high-density tissue microarrays (TMA).[79,87–89] Hepsin, a cell-surface serine protease, was determined to be overexpressed in localized and metastatic prostate cancer when compared to benign prostate or benign prostatic hyperplasia in several expression array experiments.[79–81,90] By immunohistochemistry, hepsin was found to be highly expressed in PIN, suggesting that dysregulation of hepsin is an early event in the development of prostate cancer.[79] Kruppel-like factor 6 (*KLF6*) is a zinc-finger transcription factor of unknown function, which is mutated in a subset of human prostate cancer.[91] Loss-of-heterozygosity analysis revealed that one *KLF6* allele is deleted in over 70% of primary prostate tumors. The retained allele is mutated in over 70% of these tumors. Functional studies suggest that wild-type KLF6 upregulates p21 (WAF1/CIP1) in a p53-independent manner and reduces cell proliferation, suggesting that KLF6 is a tumor suppressor gene. *EZH2* (enhancer of zeste homolog 2), a member of the polycomb gene family, is a transcriptional repressor known to be active early in embryogenesis,[92,93] showing decreased expression as cells differentiate. Recent work has demonstrated that *EZH2* is highly overexpressed in metastatic hormone refractory prostate cancer as determined by cDNA and TMA analysis.[94] *EZH2* was also seen to be overexpressed in localized prostate cancers that have a higher risk of developing biochemical recurrence following radical prostatectomy, suggesting a possible diagnostic utility as a biomarker. These studies suggest a potential clinical application in the diagnosis of prostate cancer. However, like many other genes that have been mentioned, these alterations are not specific to prostate cancer and may be observed in other neoplasms.

Androgen receptor and prostate cancer development

The androgen receptor (AR) plays a critical role in prostate development.[95] It has been known for many years that withdrawal of androgens leads to a rapid

decline in prostate cancer growth with significant clinical response. This response is short-lived and tumor cells re-emerge, which are independent of androgen stimulation (androgen independent). Numerous mutations have been identified in the androgen receptor gene (reviewed by Gelmann[96]). It has been hypothesized that through mutation, prostate cancers can grow with significantly lower circulating levels of androgens. In addition to common mutations, the amino-terminal domain encoded by exon one demonstrates a high percentage of polymorphic CAG repeats.[97] Shorter CAG repeat lengths have been associated with a greater risk of developing prostate cancer and prostate cancer progression.[98,99] Clinical trials such as the Prostate Cancer Prevention Trial (PCPT) are drawing to a close and should provide important clinical and molecular data on the role of decreasing the amount of available dehydroxytestosterone (DHT), the most active form of testosterone. Patients on this trial received long-term administration of the 5-hydroxyreductase inhibitor, fenasteride, which lowers levels of circulating DHT. One potential interesting result will be to observe the variability in response due to known polymorphisms in the 5-hydroxyreductase gene as over 50-fold differences have been detected in the effect of fenasteride on 5-hydroxyreductase activity.

Emerging molecular techniques: proteomics, laser capture microdissection, and bioinformatics

Proteomics is also being applied to serum samples to identify unique profiles.[100] This work promises to identify proteins that may be used for the prognosis and diagnosis of prostate cancer. Currently, several proteomic approaches are being used, including two-dimensional (2-D) electrophoresis and SEDI-TOF proteomics (recently reviewed by Adam *et al*[75]). The 2-D approach uses protein size and electrical charge to separate out proteins. Multiple gels from patients with and without prostate cancer are compared to help identify points that appear in one but not the other populations. Once unique proteins are identified, they can be isolated and further characterized using size and fragmentation patterns using proteases for protein digestion. This approach is laborious; however, new bioinformatics approaches may make virtual interpretation of proteins possible. The SEDI-TOF approach allows for the characterization of extremely small samples (eg, laser capture microdissection). The output is a protein profile that can be inferred. However, further characterization is not possible with this method. As in the recent example from Petricoin III *et al*,[100] they were able to identify discrete prostate cancer-related protein bands. However, the identity of these bands is unknown and therefore is of limited use. Alternatively, one can image the use of protein or expression array profiles to identify patients at highest risk for developing a disease state (eg, prostate cancer) or even which patients would benefit from a treatment protocol.

Laser capture microdissection (LCM) is a technique that should allow for a more precise understanding of the cells' activity and represents an important improvement on standard microdissection techniques, which are limiting in the study of prostate cancer due to its infiltrative nature.[101] LCM offers laser precision and can achieve transfer and isolation of single cells. LCM was developed by Emmert-Buck *et al*[102] at the National Cancer Institute (NCI) of the National Institutes of Health. LCM was born out of a need to isolate pure populations of tumor, normal, and dysplastic tissues as part of the Cancer Genome Anatomy Project (CGAP) project (http://cgap.nci.nih.gov).[103,104] LCM now allows the investigator to ask questions regarding individual cells and the surrounding stromal tissues.

A rapidly emerging field, bioinformatics, is starting to alter the way research is being conducted. Using information from large databases, *in silico* studies can be conducted to discover and validate new candidate genes and pathways significant in areas such as the development of prostate cancer. For example, Rhodes *et al*[105] recently identified lists of significant prostate cancer-related genes by performing a meta-analysis on publicly available cDNA expression array data sets. This study was also able to extrapolate prostate cancer-related pathways by piecing together data from multiple studies. This approach has now become available on an Internet-based website called ONCOMINE (www.oncomine.org) that allows users to perform a meta-analysis on genes of interest and contains links to other websites that provide information regarding their genes of interest. Pathologists will play an important role in this field due to our close relationship data information systems and a need for appropriate protection of patient-sensitive information available on our pathology data systems.

In summary, multiple factors contribute to the high incidence and prevalence of prostate cancer including race, ethnicity, diet, environment, widespread awareness through prostate-specific antigen screening and genetics. Linkage analysis has identified several candidate sites for hereditary prostate cancer gene loci. Molecular studies have also identified genes that are frequently altered in sporadic prostate cancer. It appears that due to the heterogeneity of prostate cancer, multiple genes may be involved in the neoplastic process.

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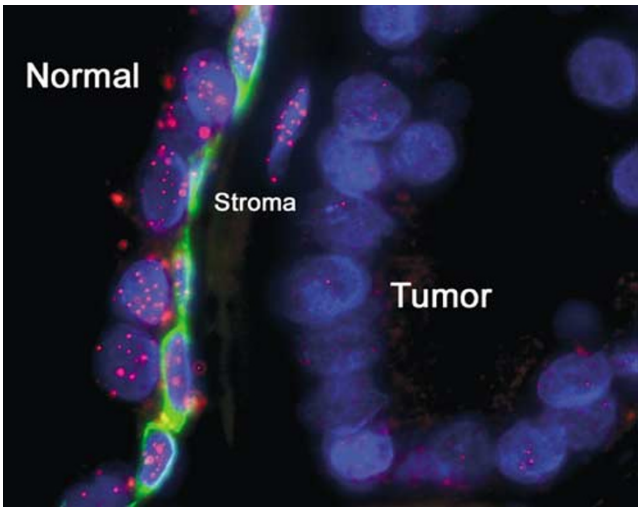

Figure 2 Direct Visualization of Telomere Shortening in Prostate Cancer. Telomere FISH signals (Red) within the cell nuclei (Blue) are diminished in prostate cancer compared to the adjacent normal gland. Green fluorescence indicates basal epithelial cells (basal cell-specific cytokeratin antibody).

(MAR) P50CA90381 (MAR) and P50CA58236 (AMDM), R01 CA84997(AMDM), KO8 CA78588 (AMDM), and R01AG21404 (MAR).

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