

Oncogenes and Tumor Suppressor Genes in Prostate Cancer

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INTRODUCTION: CANCER GENES IN 2000

Our understanding of the genetic basis of human carcinogenesis while far from complete has increased greatly over the past two decades. It is now clear that there exists multiple classes of cancer-associated genes which contribute to the carcinogenic process when their functions are perturbed by either genetic or epigenetic mechanisms. The more traditional classes of tumor suppressors (contributing to cancer formation when inactivated) and oncogenes (procarcinogenic when activated) are now joined by the mutator genes which, when altered, result in a decreased ability to maintain fidelity of the genetic code and function (e.g., genes involved in DNA repair). These classes of genes have been identified largely by virtue of function-altering mutations, occurring either somatically or in the germ line, which play a major readily discernable role in tumor development. For prostate cancer, genes in each one of these classes have been identified, although genes uniquely involved in prostate-specific carcinogenesis (i.e., so-called "gatekeepers", or genes which directly and specifically regulate growth of prostate tumors by inhibiting their growth or promoting their death (1)) have not been found, a situation that will undoubtedly change as more effort is focused on this question. Similarly, while the concept of oncogenes and tumor suppressor genes has been very helpful in providing a basic framework for the mechanistic understanding of carcinogenesis, these concepts and categories need to be expanded to include the large array of genes in which sequence variations result in more subtle contributions to the carcinogenic process. As discussed in numerous presentations in this issue of *Epidemiologic Reviews*, genetic variants that modify inherited risk for prostate cancer are being identified at a rapid pace, and the role that these genes play needs to be included when considering prostate cancer-associated genes. This review will focus primarily upon more traditional tumor suppressor genes and oncogenes and the somatic alterations in these genes that have been implicated in prostate carcinogenesis.

MULTISTEP CARCINOGENESIS AND PROSTATE CANCER PROGRESSION

Human carcinogenesis is a complex process, one requiring a number of steps. For prostate cancer, early evidence for this multistep requirement was elegantly demonstrated in the studies of experimental carcinogenesis in rodent models. The pioneering studies of Thompson et al. (2) found that expression of a single potent oncogene (i.e., *RAS*) in normal prostate cells of the mouse is insufficient for transformation; the overexpression of a second oncogene (*myc*) is necessary before transformation becomes a frequent event. Even when expressing two oncogenes, not every cell becomes transformed, suggesting that further steps are necessary, e.g., inactivation of tumor suppressor genes and other growth regulatory elements. Similarly, Rhim et al. (3) demonstrated stepwise immortalization and transformation of human prostate epithelial cells by a combination of HPV-18 and *v-K-ras*. Although in clinical specimens of prostate cancer the requirement for multiple steps is less easily demonstrated, the finding of multiple genetic alterations as a common characteristic of prostate cancer, and human tumors in general, supports this concept (4).

Application of the multistep concept to human prostate carcinogenesis would suggest that incidental or latent cancers (i.e., the clinically undetected prostate cancers found in most aged men dying from non prostate cancer causes at autopsy) as well as putative precursor lesions (i.e., prostatic intraepithelial neoplasia (5)), will have undergone only a subset of the steps, "hits", or mutations necessary for progression to the fully malignant phenotype. Furthermore, this hypothesis would suggest that specific and discrete genetic alterations may be associated with different stages and even grades of prostate cancer. An example would be the poor prognosis for men whose prostate cancers have undergone extensive gain of sequences on the long arm of chromosome 8 (6), described below.

What are the molecular events responsible for the progression of prostate cancer, or, in other words, why and how does prostate cancer evolve from an indolent to a life-threatening disease? Is this evolution inevitable or are some prostate cancers destined never to progress to advanced disease, let alone clinically detectable disease, regardless of the time frame provided? Conversely, are some prostate cancers capable of metastasis very early in their natural history? Extensive effort has been focused on these questions, as it is critical to understand the mechanisms of prostate cancer progression in molecular genetic terms if therapeutic approaches aimed at preventing or stopping this progression are to be other than empirically based.

Received for publication November 20, 2000, and accepted for publication April 17, 2001.

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CHROMOSOMAL ABNORMALITIES IN PROSTATE CANCER CELLS

The study of somatic changes arising during prostate tumorigenesis has progressed rapidly during the past years, being greatly aided by the development of several novel molecular cytogenetic technologies, including fluorescence in situ hybridization and comparative genomic hybridization. Together with loss of heterozygosity studies and karyotyping, these approaches have resulted in a comprehensive identification of the chromosomal regions involved in prostate tumorigenesis.

The most common chromosomal abnormalities in prostate cancer cells include losses of 8p, 10q, 13q, and 16q as well as gains of 7p, 7q, 8q, and Xq, as detected by comparative genomic hybridization (reviewed in Nupponen and Visakorpi (7)). Additionally, allelic loss is seen at 6q, 7q, 17p, 17q, and 18q (reviewed in Isaacs and Bova (8)). In many cases the aberrations seen in chromosomal arms consist of several distinct regions of loss or gain indicating multiple target genes in these regions. For example, allelic loss is seen at three separate regions of chromosome 13, 13q14, 13q22, and 13q31 (9), and gain of 8q results in additional copies of sequences at 8q21 and 8q23-24 (10). The complexities of these rearrangements have made it difficult to identify the genes targeted by these gains and losses. However, alterations in some specific genes have been characterized, and these studies are described below. Furthermore, a few chromosomal aberrations have been associated with clinical outcome. Such aberrations include deletions at 7q31 (11) and 13q (12) as well as losses of 8p and gains of 8q, which are more prevalent in recurrent cancers than in primary tumors (13, 14).

SPECIFIC ONCOGENE AND TUMOR SUPPRESSOR GENE ALTERATIONS IN PROSTATE CANCER

A number of genes have been found to be mutated in prostate cancer including *TP53*, *PTEN*, *RB*, *ras*, *CDKN2*, *AR* (androgen receptor), and *CTNGB1*. *ras* mutations are uncommon (<5 percent of cases) (15–18) as are point mutations of *RB* (19), although loss of one copy of *RB* readily occurs (20). To date the most consistently observed site of point mutations is in *TP53*, and these mutations are common only in advanced disease. Microsatellite instability is uncommon but detectable in prostate cancer (21), and the *MSH2* and *PMS2* genes have been found to be mutated in prostate cancer cell lines which exhibit this phenotype (22, 23).

Oncogenes

c-myc. Gain of 8q in prostate cancers was first described by Bova et al. (24). Gain of 8q is more prevalent in recurrent tumors (13) as well as in metastatic lesions (25) than in primary tumors. Accordingly, 8q gains are associated with a short progression-free interval (6, 14, 26) and the presence of lymph-node metastasis (27). The *c-myc* oncogene is located at 8q24, the other of the minimally amplified regions at 8q (10, 13, 25). This well-known oncogene plays

an important role in the regulation of cellular proliferation, differentiation, and apoptosis (reviewed in Grandori et al. (28)). Both over-expression and amplification of *c-myc* have been detected in prostate tumors (10, 29, 30). However, relatively few prostate tumors show high-level amplification of *c-myc* (10), indicating that there may exist other target genes for the 8q23-24 amplification in addition to those at the other minimally amplified region 8q21. In this respect, two other 8q genes, *PSCA* and the p40 subunit of translation initiation factor 3 are found to be frequently included in the gained regions of chromosome 8, and show increased expression in a subset of prostate cancers (31, 32).

ERBB2. In view of the promising therapeutic potential of the commercially available anti-*ERBB2* antibody, the role of this 17q oncogene in prostate cancer is of great interest. Using fluorescence in situ hybridization analysis, several groups have, however, failed to show high level amplification of *ERBB2* (33, 34), even though over-expression of the gene is a frequent event in prostate cancer, as well as an independent prognostic factor for the disease (35–37). An intriguing mechanism for the role of *ERBB2* in hormone-independent prostate cancer was recently presented by Craft et al. (38). In androgen-independent cancer cells, over-expression of *ERBB2* was able to “superactivate” the androgen receptor pathway, providing a clue to how prostate cancers can circumvent androgen deprivation therapy. Indeed, the commercial *ERBB2* antibody inhibits growth of prostate cancer cells in a xenograft model (39).

BCL2. Amplification of chromosome 18q is present in over a third of prostate tumors (7). The anti-apoptotic oncogene *BCL2* is located at 18q21.3. Over-expression of *BCL2* is seen frequently in recurrent tumors (40, 41), but seems not to be caused by amplification of the gene (7). The role *BCL2* is suggested to play in prostate cancer is interesting. Bcl-2 expression inhibits apoptosis of prostate cancer cells subjected to androgen deprivation (42). If this hypothesis holds true, *BCL2* would present a very attractive therapeutic target, potentially reducing the risk of recurrent cancer.

Androgen receptor. In addition to *BCL2*, the androgen receptor gene (*AR*) has been implicated in recurrence of prostate cancer. Visakorpi et al. (13) found frequent amplification of chromosome arm Xq in recurrent tumors, whereas Xq is very rarely amplified in primary tumors. The group went on to confirm that the *AR* gene was the target of this amplification (43). Amplification leading to over-expression of *AR* after androgen deprivation therapy is an understandable way of how prostate tumor cells overcome the decreased levels of circulating androgens. An additional means of enhancing androgen receptor signaling after androgen deprivation prostate cancer cells develop is activating mutations in *AR* (44, 45), although these tend to be rare.

Tumor suppressor genes

Chromosome 8. The genetic regions exhibiting allelic loss or chromosomal deletions most frequently in prostate cancer are two separate sites on chromosome 8p, 8p23, and 8p12-p22 (10, 24, 46). Loss of 8p appears to be an early event in prostate cancer development, as prostate intraepithelial neoplasias also show loss of heterozygosity at this

location (47). However, no clear candidates for the specific genes involved have appeared, although several genes, including *NKX3A*, *MSR1*, *N33*, and *PTK2B* have been actively investigated (48, 49).

TP53. *TP53* mutations are uncommon in localized disease but become quite frequent in deposits of metastatic prostate cancer, particularly those to bone (50–55). Observed heterogeneity of *TP53* mutations within different tumors in the same gland, and within different regions of the same gland, appears to be a somewhat unique feature of prostate cancer (56, 57). Furthermore, loss of heterozygosity and point mutation of *TP53* do not appear to be tightly coupled in this disease (58). A large number of studies have examined the prognostic significance of nuclear p53 protein immunostaining in both localized and advanced prostate cancer (59–73), and although the results are somewhat disparate, two conclusions can be drawn: 1) p53 staining tends to be very heterogeneous, resulting in problems for scoring and interpretation of staining results and inconsistencies due to sampling biases, and 2) in general, tumors with positive p53 staining are associated with a worse prognosis.

PTEN. A series of studies have examined prostate cancer specimens for alterations in the dual function phosphatase gene (*PTEN*) and found that this gene is inactivated by a combination of mechanisms including hemi- and homozygous deletion (74–79), point mutation (74, 75, 78), and promoter methylation (79). These changes are observed most commonly in advanced disease and may play a role in the acquisition of metastatic potential. However, McMenamin et al. (80) demonstrated that the majority of clinically localized prostate cancers had abnormal PTEN protein expression, with one in five cases being completely negative.

Wu et al. (81) demonstrated that in prostate cancer cells lines with inactivated *PTEN*, the AKT/phosphoinositide 3-kinase pathway is constitutively activated due to increased accumulation of the PTEN substrate PIP3. Activation of this pathway results in suppression of apoptosis and increased cell survival. These findings have stimulated extensive interest in these pathways as novel therapeutics targets in advanced prostate cancer.

p16 (*CDKN2A*). The finding of frequent homozygous deletions in a wide variety of cancer cell lines focused attention upon the *CDKN2A* gene, a negative regulator of cell cycle progression located at chromosome arm 9p21 (82). A relatively high frequency of homozygous (approximately 20 percent) (83) and hemizygous losses of *CDKN2A* have been observed in clinical specimens of prostate cancer (84), although point mutations appear to be uncommon (85). In the case of loss of heterozygosity, loss events in the vicinity of *CDKN2A* are more common in metastatic deposits of prostate cancer (43 percent versus 20 percent in primary tumors), and in a small but detectable fraction of tumors (approximately 15 percent) *CDKN2A* shows evidence of inactivation by promoter methylation (84). Whether all of the allelic loss events at 9p21 in prostate cancer are associated with *CDKN2A* inactivation, or whether they reflect inactivation of a neighboring gene (e.g., *p15*), has not been determined.

p27 (*CDKN1B*). A number of studies indicate that reduced levels of the cyclin kinase inhibitor (p27) are associated with a more aggressive prostate cancer phenotype (86–89), although the mechanism of this down regulation is not clear. Interestingly, Kibel et al. (90) described a homozygous deletion of *CDKN1B* in a lethal case of prostate cancer and a high frequency of loss of heterozygosity of *CDKN1B* in advanced prostate cancers in general. Thus, it is possible that, in addition to increased ubiquitin-mediated p27 protein degradation that has been demonstrated in colon and other cancers, in prostate cancer at least a subset of lesions may inactivate this gene via deletion. Thomas et al. (91) suggested the use of p27 protein expression analysis in biopsy specimens from patients with clinically localized cancer to preoperatively identify men with a high risk of recurrence.

GSTP1. *GSTP1*, which codes for the phase II detoxification enzyme glutathione S-transferase π , has been found to be extensively methylated in the promoter region in a completely cancer-specific fashion, with concomitant absence of expression (92). In fact, this epigenetic event, being found in over 90 percent of all prostate cancers as well as in prostatic intraepithelial neoplasia lesions, is the most common genomic alteration yet observed in prostate cancer. The mechanism by which this region becomes specifically methylated in prostate cancer, and the basis for its apparent selection in the carcinogenic pathway, is unclear at present. As this enzyme is a key part of an important cellular pathway to prevent damage from a wide range of carcinogens, the inactivation of this activity may result in increased susceptibility of prostate tissue to both tumor initiation and progression resulting from an increased rate of accumulated DNA damage. Indeed, reactivation of this or a similar cellular defense pathway, perhaps by dietary intervention, has been proposed as a treatment strategy aimed at blocking the progression of initiated prostate cancer foci.

Metastasis suppressor genes: *CDH1*, *KAI1*, *MAP2K4*. Aberrations in two genes have been associated with metastatic prostate cancer. The *CDH1* gene for the cell adhesion molecule E-cadherin on 16q has been extensively studied in prostate cancer progression. Reduced expression of E-cadherin or its accessory protein α -catenin are frequent events in advanced prostate cancer (93). Although, allelic loss at 16q is common in prostate cancers, reduced expression of E-cadherin seems not to be caused by this mechanism (94). The *KAI1* gene at 11p11.2 shows decreased expression in metastases and suppresses metastasis in an animal model (95). The down-regulation of the gene is not caused by mutation or allelic loss (96) but, rather, by post-transcriptional events. More recently, the gene coding for mitogen-activated protein kinase kinase 4 (*MAP2K4*) has been implicated as an important prostate cancer metastasis suppressor gene (97).

FUTURE DIRECTIONS

Many questions remain in this area. Genes responsible for prostate-specific carcinogenesis, if such genes exist, remain to be identified. Susceptibility genes identified through studies of prostate cancer families should be helpful in this regard. Little is known about the ethnic- and race-specific

patterns of gene mutation which may be important in explaining variation in prostate cancer incidence and mortality rates that exist in different populations, and how such patterns may be affected by environmental exposure. New technologies, such as cDNA microarrays, should provide a systematic description of the alterations in gene expression profiles that accompany prostate carcinogenesis, which would be of great help in prioritizing genes for further mutation or polymorphism studies. Additionally, high throughput, chip-based sequencing and genotyping technologies should provide unprecedented access to the variations in genomic DNA that are responsible for prostate cancer development.

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