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Commentary

Molecular Markers for Prostate Cancer Metastasis

Developing Diagnostic Methods for Predicting the Aggressiveness of Prostate Cancer

John T. Isaacs

From the Johns Hopkins Oncology Center and Brady Urological Institute, Department of Urology, Johns Hopkins University School of Medicine, Baltimore, Maryland

Magnitude of the Problem

One of every three cancers diagnosed in American males is of prostatic origin, making prostate cancer the most commonly diagnosed malignancy in males in the United States.[1] Annually, there will be 317,000 newly diagnosed cases and 41,000 deaths from prostate cancer within the U.S.[1] The incidence of prostate cancer in the U.S. has not been decreased by changes in lifestyle; in fact, the incidence rate of clinical prostatic cancer has increased steadily since the 1930s.[2] These high annual incidence rates translate into the human reality that one of every five American males will eventually develop clinical prostate cancer during their lifetime.[1] Prostatic cancer incidence increases with age more rapidly than any other type of cancer; less than 1% of prostate cancers are diagnosed in men under 50 years of age.[3] Thus, as the life expectancy of the male population increases over time, the incidence of clinical prostate cancer will also increase.[3] This is one explanation for why the annual incidence rate of clinical prostate cancer has increased steadily since 1935 to the present time.[2] Furthermore, during this same time frame, the death rate from the disease has doubled.[1] Because the incidence of prostate cancer increases as a function of age, there is a misconception that it is a disease of the very elderly. Approximately, 20% (ie, 65,400 cases) of prostate cancers diagnosed annually occur in men under the age of 65 years.[1] To place this in perspective, the 65,400 prostate cancers diagnosed per year in men under 65 years of age is equal to the total number of colorectal cancers diagnosed in men of all ages and exceeds the combined total of all renal and bladder cancers or the combined total of all leukemias and brain and central nervous system tumors.[1] Although the majority of prostate cancer cases are in men over the age of 65 years, the impact of the disease is still significant. The average life span of a man who dies of metastatic prostate cancer is reduced by 9.2 years.[4]

Substaging Prostate Cancer

The problem presented by prostate cancer is that currently it is difficult to predict the clinical course of the disease for individuals. Approximately 50% of men with prostate cancer have clinically advanced (ie, non-organ-confined) disease at the time of initial diagnosis.[5] One-third of the remaining 50% of men with organ-confined disease (initially determined by clinical staging) actually have micrometastatic disease at the time of surgery (as determined by subsequent pathological staging).[5] In some men, prostate cancer kills the patient within a year of diagnosis. In contrast, other men survive untreated

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Address reprint requests to Dr. John T. Isaacs, Johns Hopkins Oncology Center, 422 North Bond Street, Baltimore, MD 21231-1001.

for many years with localized disease without clinically detectable metastases.[6] If completely localized (ie, within the prostatic capsule), prostate cancer can be cured by surgery alone (ie, radical prostatectomy).[7–9]

Unfortunately, if diagnosed when non-organ-confined, prostate cancer is a fatal disease for which presently there is no curative treatment.[10] Because of the poor prognosis for men with metastatic prostate cancer, aggressive screening programs have been suggested for men starting at age 50 to permit early detection of prostate cancer while localized and potentially curable.

Screening for early-stage prostate cancer is logical, although there are complications with the proposed screening methods. Their basic limitation is that they are unable to predict accurately the prognosis of localized prostate cancer based upon histological grading alone.[7,8] The histological grade of the primary prostate cancer is evaluated using the Gleason grading system.[11] In this system, grading is based upon the degree of glandular differentiation and growth pattern of the tumor as it relates to the prostatic stroma.[11] The pattern may vary from well differentiated (grade 1) to poorly differentiated (grade 5). This system takes into account tumor heterogeneity by scoring both the primary and secondary tumor growth patterns. For example, if the majority of the tumor is well differentiated (grade 1) and the secondary growth pattern is poorly differentiated (grade 5), the combined Gleason sum would be a 6. Low (ie, <5) Gleason sum prostate cancers predictably have minimal aggressive behavior whereas very high (8–10) Gleason sum tumors are usually highly aggressive.[12] Unfortunately, the intermediate (5–7) Gleason sum tumors are highly unpredictable in their clinical aggressiveness.[12] This limitation is of particular importance as the majority of tumors (76%) fall into this intermediate Gleason sum category.[12] Thus, predicting the biological potential of the majority of prostate cancer in asymptomatic patients based upon histology alone is problematic.

Although the molecular details of prostatic carcinogenesis have not been fully unraveled, it is clear that the development of a malignant prostate cancer cell from a normal prostatic glandular cell requires multiple transformation events.[13] Due to the multistep nature of prostatic carcinogenesis, cells that have undergone some but not all of the transformation steps are present within the prostates of aging men, and the clonal growth of these partially transformed cells produces morphologically detectable premalignant lesions in the gland.[14–16] A variety of such premalignant lesions have been demonstrated within the prostate and have been termed atypical primary hyperplasia, dysplastic hyperplasia, atypical hyperplasia, adenosis, intraductal dysplasia, adenomatous hyperplasia, and prostatic interepithelial neoplastic (PIN) by various authors dependent upon a variety of histological criteria.[14] For the sake of simplicity, these lesions will be subsequently referred to collectively as PIN lesions.

In the human,[15] as in animals,[17,18] not all premalignant PIN lesions progress to produce histologically detectable prostate cancer during the lifetime of their host, demonstrating that additional transformation steps must occur before these lesions can progress. In addition, it is well known that once PINs progress to the histologically detectable cancers, most of these never produce clinical symptoms during the lifetime of the host. Based on autopsy studies, 20% of men in the age range of 50 to 60 years and 50% in the 70- to 80-year range have histological deposits of cancer within their prostates but never produced clinical symptoms during their lifetime.[13] There are thus approximately 11 million men older than 50 years in the United States with such histologically detectable asymptomatic prostate cancer.[3,5] Despite the remarkable number of these asymptomatic cancers in the United States male population, the majority remain clinically silent (ie, neither life threatening nor life altering), and only a portion become clinically manifest during the lifetime of the host.[3,5]

These asymptomatic histological cancers have been referred to as latent, microscopic, incidental, dormant, and so forth, and there are problems with all of these various labels. For example, latent implies that the biological potential of these initially asymptomatic histologically detectable cancers is known. However, presently it is not possible to predict with accuracy in an individual patient which of these cancers will eventually progress to produce clinical disease based upon histological grading alone. The term microscopic is misleading because these asymptomatic lesions are by no means always microscopic. For example, data from the German Prostate Cancer Registry reveal that one-third of these prostatic cancers are greater than 1 cm in diameter when detected at autopsy.[19] In addition, these asymptomatic tumors are not always well differentiated histologically, and in one study only 58% of the prostate cancers found at autopsy were well differentiated.[20] Therefore, the term histological prostate cancer will be used to describe the prostate cancers that exist in most older men as this implies nothing about the biological potential of these tumors.

A fundamental issue with regard to prostatic carcinogenesis is whether histological prostate cancers already have completed all the malignant steps necessary to become clinically manifest. If histological prostate cancer already has undergone all of the malignant events necessary to produce an invasive clinical tumor, then the only additional requirement for histological cancer to produce clinical symptoms would be the time required for the growth of the tumor to a clinically detectable size. Alternatively, if a histological prostate cancer has not undergone all of the essential events necessary to produce a life-threatening or life-altering cancer, then not only would additional time be necessary but the occurrence of additional malignant events would be necessary also for these histological tumors to require therapeutic intervention.

The resolution of whether the majority of histological prostate cancers require, in addition to time, additional malignant events to produce clinically aggressive tumors is possible based upon available clinical data. If additional time (ie, tumor growth) is the only requirement for a histological prostate cancer to produce clinical disease, then the age-specific prevalence of clinically manifest prostate cancers should be similar in various male populations throughout the world if two conditions are met. These two conditions are 1) that the age-specific prevalence of histological prostate cancers must be similar between the male populations being compared and 2) that the life expectancy of the populations being compared must be the same. Both of these conditions are met for the male population in the U.S. *versus* Japan, thus allowing valid comparison.[13] Even though the age-specific prevalence of histological prostate cancer and the life expectancy is similar between Japanese and American males, there is more than a 10-fold difference in the age-specific prevalence of clinical prostate cancer between these two populations.[13] These data are thus inconsistent with the majority of histological prostate cancer having undergone all of the steps necessary to produce a clinically aggressive disease.

These results demonstrate that 1) multiple malignant events are required for the development of histological prostate cancer, and that the probability of undergoing these multiple transformation events is similar in Japanese and American men, and 2) the progression from histological to clinical prostate cancer requires additional malignant steps, and the probability of undergoing these additional events is lower in the Japanese than the American men.[13] The probability of undergoing the additional malignant changes needed to produce a life-threatening clinically aggressive prostate cancer involves both genetic and environmental factors. In approximately 10% of American males, development of clinically aggressive prostate cancer involves germ-like inheritance of prostate cancer susceptibility genes.[21] Genetic linkage analysis has demonstrated that ~40% of patients with such familial prostate cancer is due to the inheritance of a prostate cancer susceptibility gene located on the long arm of human chromosome 1.[22] For the remaining 90% of patients with no family history of prostate cancer, environmental factors are critical. For example, when Japanese men migrate to California or Hawaii, the age-adjusted incidence for prostate cancer dramatically increases in the first and second generations and becomes more similar to the high rates of American men than to the low rates of native Japanese.[23,24] This increase in the incidence of clinical prostate cancer among Japanese men migrating to a high prevalence area for prostate cancer does not support an inherent resistance to the development of clinical prostate cancer in the Japanese man. In contrast to the increase in clinical prostate cancer cases, the prevalence of histological prostate cancer remains unchanged when Japanese men migrate to the United States (ie, Japanese men in Japan or in the United States or American men in the United States all have a similarly high age-specific prevalence of histological prostatic cancer).[25]

Clinical Importance of Distinguishing between Various Types of Histological Prostate Cancer

If all histological prostate cancers have undergone the malignant events necessary to produce a clinically aggressive cancer, it would seem prudent to detect and treat all of these histological prostate cancers, as the malignant potential of these tumors is similar and clinical outcome dependent only upon time (ie, the natural history is predictable). In contrast, as described above, histological prostate cancers are heterogeneous with only a proportion having completed the process required to produce a clinically aggressive cancer. The remaining proportion of these histological prostate cancers never undergo the additional events required to produce life-altering, let alone life-threatening effects despite host longevity and ample time for tumor growth.[5,6] Thus, this latter proportion of histological prostate cancers remain subclinical and do not require treatment. Presently, it is not possible to predict which histological cancers have undergone all of the steps needed

for progression to clinically aggressive cancer and which have not (ie, the natural history is unpredictable). Thus, the ability to predict which tumors have the capacity to manifest aggressive behavior requiring therapy becomes a critical issue as greater emphasis is placed upon screening for earlier detection of prostatic cancer.

This issue is critical because, if such a patient with histologically detectable prostate cancer is left untreated until definitive clinical evidence of metastatic disease outside of the prostate, the ability to cure such a metastatic patient with presently available therapy is lost.[10] If truly localized, prostatic cancer can be cured by radical prostatectomy.[7–9] However, if the cancer only appears by standard staging criteria to be localized but in reality has metastasized, then systemic therapy is required. Unfortunately, there is no prognostic method to identify cells possessing the metastatic phenotype within a primary prostate cancer population. This is significant because presently one-third of men at the time of treatment for presumed localized prostatic cancer already have clinically undetectable micrometastatic disease requiring additional systemic therapy.[26] At present, more than 150,000 men per year are candidates for therapy for localized disease.[1] What is needed are markers of metastatic ability that can be used to discriminate the ~50,000 patients per year undergoing local treatment who have micrometastatic disease requiring systemic therapy from the ~100,000 men per year without micrometastatic disease who require only local treatment or watchful waiting.

What is urgently needed is some type of diagnostic method that can be combined with histological grading to identify which histological prostate cancers have completed the progression to a stage that will produce a life-altering or life-threatening disease, thus requiring immediate therapeutic intervention at the time of initial diagnosis. Acquisition of metastatic ability by prostate cancer cells is the most lethal aspect of prostatic cancer progression. Once this has occurred, definitive therapy is required before the initially localized metastatic cells escape from the prostate. Thus, detection of specific molecular changes definitely associated with acquisition of metastatic ability by prostate cancer cells could be used, in combination with histological grading, to individually substage patients with histological prostatic cancer to those requiring no therapy *versus* those requiring local therapy *versus* those requiring local therapy plus systemic therapy.

Proposed Markers of Metastatic Prostate Cancer

Metastasis is highly selective and consists of a series of sequential, interrelated steps that include growth, vascularization, invasion, survival in the circulation, adhesion, extravasation, and proliferation at the distinct site.[27] Based upon this realization of the multistep nature of metastasis, in this issue of The American Journal of Pathology, the report of Greene et al[28] describes a multivariate *in situ* hybridization (ISH) analysis to test the correlation between the expression of a series of specific genes and metastatic potential of human prostate cancer sublines xenografted into nude mice. These genes include epidermal growth factor receptor, basic fibroblast growth factor (bFGF), interleukin-8 (IL-8), 72-kd and 92-kd type IV collagenase, E-cadherin, and multidrug resistance (*mdr*-1) gene. The genes analyzed were selected to reflect growth (ie, epidermal growth factor receptor and bFGF), angiogenesis (ie, IL-8), invasion (72-kd and 92-kd collagenases), adhesion (E-cadherin), and drug resistance (*mdr*-1).

For these studies, the expression of these genes was determined by ISH in the low metastatic parental PC-3M human prostate cancer cells and a low (ie, PC-3M-Pro4) and a high (ie, PC-3M-LN4) metastatic variant when grown *in vitro* in cell culture. These results demonstrated that the highly metastatic PC-3M-LN4 cells express higher levels of bFGF, IL-8, *mdr*-1, and type IV collagenase than the low metastatic PC-3M-Pro4 and PC-3M.[28] In addition, when these cells were orthotopically implanted into the prostates of nude mice, the PC-3M-LN4 cells continued to express higher levels of bFGF, IL-8, and type IV collagenase than the lower metastatic PC-3M cells.[28] These results demonstrate that such an ISH approach has the ability to define inter- and intratumor heterogeneity and the potential to identify individual primary prostate cancers with metastatic ability. As ISH can be performed on formalin-fixed, paraffin-embedded tissues, archival tissue can be tested to determine whether such an approach accurately predicts which cancers are aggressive based upon such criteria as disease recurrence and/or time of patient survival. A series of additional independent investigators likewise have focused upon identifying prognostic factors for identifying aggressive prostate cancer that can be assayed retrospectively in such archival tissue to test their predictive values. Most of these factors are specific proteins the expression of which can be measured either at the mRNA level via ISH or at the protein level via immunocytochemical staining (ICS). The logic

behind analyzing expression of several of these proteins will be discussed.

An explanation for the lack of metastatic ability of certain prostate cancers has been proposed based upon the role of the development of new blood vessels (ie, angiogenic) in prostatic carcinogenesis. There are two distinct phases during prostatic carcinogenesis with regard to tumor blood vessel development. During the first, or prevascular, phase, which may persist for years, cells that have undergone several but not all of the transformation steps undergo a limited amount of net growth producing premalignant prostatic intra-epithelial neoplastic (PIN) lesions.[14] Most of these PIN lesions do not continue net growth and do not progress to produce histologically detectable cancer.[14] Even the histological cancers that do progress to cancer remain of limited virulence unless they undergo conversion to the second, or angiogenic, phase. Folkman et al[29] have demonstrated that induction of angiogenesis is a critical step in carcinogenesis involving the conversion of hyperplastic lesions with low tumorigenic ability into cancerous lesions that can produce continuously growing tumors. These studies demonstrated that angiogenic ability appears first in a subset of hyperplastic lesions before onset of aggressive cancer, demonstrating that hyperplasia *per se* does not require angiogenesis.

Furusato et al[30,31] demonstrated that the majority of histological prostate cancers detectable in autopsy material in men dying with no clinical indication of prostate cancer had very low blood capillary density ratios compared with prostate cancers that produced clinical symptoms and metastasized. It has been demonstrated that the intensity of angiogenesis within a variety of human cancers, including prostate, can predict the metastatic ability of the cancer.[32,33] Coupling these observations with the demonstration that the growth of primary tumors beyond the size of 2 to 3 mm^3 is critically dependent upon the induction of tumor angiogenesis[34] suggests that quantitation of angiogenesis should be a highly effective method for predicting the aggressiveness of prostate cancer. Such quantitation is possible using ICS with anti-factor-8-related antigen antibodies to identify endothelial cells.[32] Recently, it has been demonstrated that certain cancers, including prostate cancer, can enzymatically produce anti-angiogenic factors from precursor proteins.[35–37] For example, plasminogen activator can be enzymatically processed to produce a peptide fragment, termed angiostatin, that has potent ability to inhibit angiogenesis.[35–37] Thus, decreased production of endogenous inhibitors of angiogenesis such as angiostatin may be predictive of aggressive prostate cancers.[37]

Acquisition of metastatic ability by prostate cancer cells involves not only increased protein expression but also decreased expression of metastasis suppressor proteins other than angiostatin. This conclusion is based upon observations that, when highly metastatic prostatic cancer cells are fused with non-metastatic prostatic cancer cells, the metastatic ability of the resultant somatic cell hybrid is suppressed without suppression of the tumorigenicity if the hybrid cells retain all of the chromosomes from both of the parental lines. This conclusion is further supported by the observation that, when such hybrids undergo nonrandom chromosomal loss, high metastatic ability is re-expressed.[38] This suggests that, for a prostate cancer cell to become highly metastatic, metastatic suppressor gene(s) must be inactivated by either mutation, allelic loss, or epigenetic inactivation (eg, hypermethylation).

To determine the chromosomal location of human prostate cancer metastasis suppressor gene(s), the technique of microcell-mediated chromosome transfer has been used to introduce specific human chromosomes into highly metastatic rat prostatic cancer cells. Such microcell-mediated chromosomal transfer has demonstrated that, located within human chromosomes 8, 10, 11, and 17 are metastasis suppressor genes for prostate cancer.[39–42] Both loss of heterozygosity[43–46] and comparative genomic hybridization[47,48] analysis have documented that loss of genetic material from human chromosome 8p, 10q, 16q, and 17p is common in both primary and metastatic sites of human prostate cancer. Although loss of heterozygosity or comparative genomic hybridization analysis has not previously identified chromosome 11 as a site of common loss of genetic material in human prostate cancer, positional cloning has identified genes located on human chromosome 11p13–12.1 that can suppress metastatic ability of prostate cancer cells.[49] A gene located on human chromosome 11p11.2 has been isolated and demonstrated to suppress metastasis when introduced into rat prostate cancer cells.[49] Expression of this gene, designated KAI1, is reduced in cell lines derived from human metastatic prostate tumors.[49] The KAI1 gene encodes a protein of 267 amino acids, with four hydrophobic transmembrane domains and one large extracellular hydrophilic domain with three potential *N*-glycosylation sites.[49] KAI1 is evolutionarily conserved, is expressed in many human tissues, and encodes a member of a structurally distinct family of leukocyte surface glycoproteins.[49] By ICS, high levels of KAI1 protein are detected in the epi-

thelial but not stromal compartment of normal prostatic and benign prostatic hyperplasia tissue.[50,51] In epithelial cells, KAI1 protein is expressed on the plasma membrane.[50,51] KAI1 protein expression is down-regulated in more than 70% of the primary prostatic cancers from untreated patients.[50,51] In untreated patients, down-regulation of KAI1 protein occurred in all of the lymph node metastases examined.[50,51] In patients with metastatic disease who had failed androgen ablation therapy, more than 90% of the primary prostatic cancers had down-regulation, with 60% having no KAI1 protein expression.[50,51] In additional studies, KAI1 expression was documented to be inversely correlated to both Gleason score and clinical state.[51] Primers derived from the sequences flanking each exon of KAI1 were used to analyze KAI1 mutation and allelic loss by the method of PCR single-strand conformational polymorphism. Using this method, no point mutation or allelic loss was detected in metastases.[50] No allelic loss was detected in primary and lymph node metastases via microsatellite analysis using the marker D11S1344, which is located in the region of KAI1.[50] These results demonstrate that KAI1 protein expression is consistently down-regulated during the progression of human prostatic cancer to a metastatic state and that this down-regulation does not commonly involve either mutation or allelic loss of the KAI1 gene.[50]

In addition to KAI1, CD44 is another metastasis suppressor gene located on human chromosome 11.[52] The CD44 gene is located on human chromosome 11p13 and encodes an integral transmembrane glycoprotein that participates in specific cell-cell and cell-extracellular matrix interactions.[53] CD44 is encoded by 20 exons over a length of approximately 60 kb, at least 10 of which are variably expressed due to alternative splicing or the nuclear RNA.[54,55] CD44 is involved in cell adhesion, serving as a receptor for the extracellular matrix components hyaluronic acid and osteopontin.[56–58] Although CD44 appears to function in lymphocyte homing, lymphocyte activation, and extracellular matrix adhesion, the precise functions of each of the CD44 isoforms are less clear.[53] CD44 has been proposed to play a major role in tumorigenicity or metastasis in different types of tumor cells.[53] Individual isoforms differ in their ability to enhance[59] or decrease[60] tumorigenicity or metastatic potential when overexpressed on tumor cells. Down-regulation of the standard 85-kd form of CD44 expression both at the mRNA and protein level correlates with metastatic potential within the Dunning system of rat prostatic cancer sublines.[52] Transfection-induced enhanced expression of the 85-kd standard form of CD44 in the highly metastatic rat prostatic cells greatly suppresses their metastatic ability to the lungs without suppression of their *in vivo* growth rate or tumorigenicity.[52] CD44 is normally expressed on the plasma membrane of human prostatic glandular cells (ie, the cells of origin for prostate cancer).[53] CD44 expression is down-regulated in human prostate cancer progression with down-regulation being correlated with high tumor grade, aneuploidy, and distant metastasis.[61] These clinical observations are in agreement with the data that enhanced expression of the standard CD44 isoform in rat prostatic cancer cells inhibits their *in vivo* metastasis ability, whereas down-regulation of CD44 protein expression is associated with acquisition of metastatic ability. These results suggest that CD44 is a metastasis suppressor for prostatic cancer and that immunocytochemical detection of decreased expression of the standard form of CD44 may be useful in predicting the aggressiveness of prostate cancers.

The finding of frequent loss of heterozygosity on chromosome 16q[43–45] has led to an examination of putative candidate suppressor genes in this region. The E-cadherin gene maps to chromosome 16q22, and the product of this gene has been demonstrated to play a critical role in embryogenesis and organogenesis by mediating epithelial cell-cell recognition and adhesion processes.[62–65] E-cadherin protein levels are frequently reduced or absent in cancer cell lines, and such lines are often more fibroblastic in morphology and invasive in experimental assays.[66,67] In addition, experimental inactivation of E-cadherin with either antibodies or antisense RNA results in the acquisition of invasive potential and transfection of invasive adenocarcinoma cells with E-cadherin cDNA rendering the expressing cells noninvasive.[68]

The first studies to examine E-cadherin in prostate cancer were carried out in the Dunning rat model.[69] These studies found a strong correlation between the lack of E-cadherin and metastatic and/or invasive potential. This correlation was strengthened by the direct observation of the progression of a noninvasive, E-cadherin-positive tumor to an E-cadherin-negative, highly metastatic tumor.[69] These observations have been extended to human prostate cancer in a series of over 90 samples of prostate cancer for E-cadherin protein levels by immunohistochemistry.[70] Whereas all benign samples stained with uniform, strong intensity at cell-cell borders, approximately one-half of the tumors examined showed reduced or absent E-cadherin protein staining.[70] When compared with Gleason grade, there was a

strong association between high grade and aberrant E-cadherin staining.[70,71] In fact, no tumors with Gleason sum 9 and 10 had normal staining, whereas all tumors with a sum less than 6 had normal staining patterns.[70,71] Aberrant E-cadherin immunocytochemical staining is a powerful predictor of poor outcome, both in terms of disease progression and overall survival.[70,71]

Alterations of the tumor suppressor gene p53 have been thought to play a role in prostate carcinogenesis since the original observation of loss of genetic material from the distal portion of chromosome 17p in human prostate cancers[43] and the identification of p53 gene mutations in three of five prostate cancer cell lines.[72] Since these initial observations, a number of investigators have focused on defining the role of p53 alterations in clinical prostate cancer. Because mutations of the p53 gene commonly prolong the half-life of its protein product, immunocytochemical staining has been used as a measure of gene inactivation. In localized prostate cancers, immunoreactive p53 polypeptide has been reported in 0 to 79% of the cases, although in most series, less than 10% of the low-stage tumors stain positively.[73–84] Cancers that invade the prostatic capsule have staining rates of 10 to 20%, whereas 40 to 95% of the bone metastases stain positively.[78–82] Mutational analysis and sequencing of the p53 gene has confirmed that tumors that are positively staining for p53 harbor mutations and that mutations appear to be more common with increasing tumor stage.[79–88]

Because p53 alterations are more common in high-stage tumors, it has been inferred that inactivation of this gene is important in the progression of human prostate cancer. *In vitro* and *in vivo* studies offer some support to this hypothesis. Expression of wild-type p53 in prostate cancer cell lines with mutant alleles suppress their growth.[72] However, several uncertainties remain regarding the role of p53 alterations in human prostate cancer. First, it has not been clearly demonstrated that p53 function is absent from clinical prostate cancers with p53 mutations. In a large variety of human tumors, p53 is inactivated by a common mechanism (ie, mutation of one copy of the gene and loss of the second).[89] Of the many studies relating p53 to prostatic carcinoma, only three have investigated both copies of the gene. Isaacs et al[72] found a missense mutation in one of two primary tumors with loss of one copy of chromosome 17p. Bookstein et al[79] found allelic loss of chromosome 17p in three of six prostatic tumors with documented p53 gene mutations. In a metastatic prostate cancer, Effert et al[85] found chromosome 17p allelic loss coupled with a p53 gene mutation; however, further analysis revealed that the deletion did not include the p53 gene. Thus, of the nine clinical prostatic tumors in which both copies of the p53 have been analyzed, only four show conclusive evidence of inactivation of both alleles. From these limited data, it remains uncertain whether alteration of one copy of the p53 gene, either by mutation or loss, necessarily implies that the gene is functionally inactivated. Equally uncertain is whether detection of p53 alterations offers prognostic information or aids in the selection of appropriate therapy in patients with prostate cancer.

To determine the role of p53 inactivation in the progression of clinical prostatic carcinomas, 67 tumors derived from patients with clinically localized disease were evaluated for chromosome 17p and p53 gene allelic loss, p53 gene mutations using single-strand conformational polymorphism and direct sequencing, and p53 protein expression using ICS.[90] Of 55 informative tumors, 10 demonstrated loss of 17q or the p53 gene; however, only a single tumor had a mutation in its remaining p53 allele.[90] Significant p53 overexpression was observed in 2 of 38 tumors, and 9 others had faint staining of a few nuclei (<1%). p53 overexpression occurred in no informative tumor with allelic loss or mutation. In a 1- to 7-year follow-up, positive ICS did not confer an increased risk of recurrence (risk of recurrence, 0.86; $P = 0.78$), whereas allelic loss of chromosome 17p appeared to be highly correlated with recurrence (risk of recurrence, 3.7; $P = 0.003$).[90] In an unrelated group of 42 patients with metastatic prostate cancer, p53 overexpression was found in 26 tumors (62%), and 15 (36%) had high-grade staining.[90] Neither the presence nor the degree of expression correlated with time to progression or time to death.[90] This series suggests that p53 gene inactivation is rare in primary prostatic tumors, not essential to the development of prostate cancer metastases, and of limited use as a prognostic marker in patients with primary or metastatic disease.[90] Another gene or genes on chromosome 17p may be involved in prostate cancer progression.

Conclusion

This commentary has reviewed a series of proteins the level of expression of which may be useful in predicting the clinical aggressiveness of newly diagnosed localized prostate cancer. These proteins, which are summarized in Table 1, can be either over- or underexpressed in the progression of localized to

Table 1. *Possible Prognostic Factors for Identifying Aggressive Prostate Cancers*

Parameters that in metastatic prostate cancers are associated with	
Increased expression	Decreased expression
bFGF[28]	KAI-1[49–51]
IL-8[28]	CD44 (standard form)[52]
mdr-1[28]	E-cadherin[70,71]
72-kd type IV collagenase[28]	Normal p53[73–88]
92-kd type IV collagenase[28]	Angiostatin[37]
Mutant p53[73–88]	β_4 integrin subunit[91]
Angiogenesis (factor-8-related antigen)[32,33]	γ-2 laminin 5 subunit[92]

metastatic prostate cancer. As correctly pointed out by Greene et al,[28] a multivariate analysis in which the level of expression of several of these parameters is combined appears critical if such analysis is to accurately predict the aggressive nature of individual prostate cancers. The use of both ISH to measure specific mRNA expression and ICS to measure specific protein expression are complementary approaches and can be performed on archival paraffin blocks to retrospectively test correlations between these parameters and a variety of clinical features (ie, response to therapy, disease recurrence, host survival etc).

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