Prognostic Factors in Prostate Cancer

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Abstract

The ability of traditional and newer molecular-based prognostic factors to predict the outcome of prostate cancer is of considerable interest to urologists, pathologists, and patients. In this review, a series of traditional and newer molecular-based prognostic factors are considered, including those that have achieved widespread use, newer tests that are beginning to be used in clinical practice, and emerging molecular markers that have yet to be widely validated in the published literature or clinical trials.

Prostate cancer is the most frequent new cancer diagnosis in American men, and the disease incidence increases with age more than any other cancer.1,2 During the past 15 years, in the era of serum prostate-specific antigen (PSA) screening, there has been a significant increase in the detection of prostate cancer.3-6 Prostate cancer is widely known to vary substantially in aggressiveness.7-13 Given the significant potential morbidity associated with aggressive treatment, there has been significant interest in the development of traditional morphologic feature–based and newer molecular-based prognostic factors that potentially could distinguish the indolent cases unlikely to progress during a man’s remaining lifetime from the invasive tumors capable of distant metastasis and producing androgen-independent, antiandrogen-resistant fatal disease.7-13

Morphologic Feature–Based Prognostic Factors

The pathologic staging and microscopic grading of prostate cancer are widely accepted as major morphology-driven prognostic factors for the disease.14 Additional morphologic markers include vascular space involvement and mitotic activity. In an attempt to stratify the potential clinical usefulness of prognostic and predictive factors, the College of American Pathologists has proposed classifying them into the following categories: category I, markers that are well supported by the literature and generally used in patient management; category II, markers that are extensively studied biologically and/or clinically but with few clinical outcome studies; and category III, markers that currently do not meet the criteria of category I or II.15 The College of American Pathologists classification of prognostic factors in prostate cancer is summarized in Table I.
Tumor Type

The morphologic variants of prostatic carcinoma, although relatively uncommon, can be associated with different disease progression patterns. Standard *acinar adenocarcinoma* arises in the peripheral zones of the prostate gland and accounts for more than 90% of all newly diagnosed prostate cancers. *Prostatic duct carcinomas* originate from larger dilated central ducts of the gland, immunostain positively for PSA and prostatic acid phosphatase (PSAP), and usually are of low to intermediate aggressiveness. *Endometrioid adenocarcinomas* are included in the prostatic duct carcinoma group. *Mucinous carcinomas* also stain for both PSAP and PSA, rarely respond to hormonal therapy, and often cause bone metastases. *Adenoid cystic carcinomas* are nonreactive for both PSA and PSAP and may be associated with distant metastasis. *Squamous and adenosquamous carcinomas* may develop in patients treated with radiation therapy or after conventional adenocarcinoma.

### Table 1

**Prognostic Factors in Prostate Cancer**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Method of Detection</th>
<th>CAP Prognostic Marker Category</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PSA</td>
<td>ELISA</td>
<td>I</td>
<td>Standard practice</td>
</tr>
<tr>
<td>Pathologic stage</td>
<td>Routine pathologic examination</td>
<td>I</td>
<td>Standard practice</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>Routine pathologic examination</td>
<td>I</td>
<td>Standard practice</td>
</tr>
<tr>
<td>Surgical margins</td>
<td>Routine pathologic examination</td>
<td>I</td>
<td>Standard practice</td>
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<tr>
<td>Tumor volume</td>
<td>Needle biopsy summed percentages of involvement</td>
<td>Routine pathologic examination</td>
<td>II</td>
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<tr>
<td>Prostatectomy whole mounts</td>
<td>Special pathologic examination</td>
<td>II</td>
<td>In clinical use in some centers</td>
</tr>
<tr>
<td>Tumor type</td>
<td>Routine pathologic examination</td>
<td>II</td>
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</tr>
<tr>
<td>Tissue PSA</td>
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<td></td>
</tr>
<tr>
<td>Tissue PSMA</td>
<td>Immunohistochemical analysis</td>
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<td>Androgen receptor</td>
<td>Immunohistochemical analysis</td>
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<tr>
<td>Ki-67 (MIB-1) labeling index</td>
<td>Immunohistochemical analysis</td>
<td>Research only</td>
<td></td>
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<td>Other cell cycle (mitosis counts, PCNA, p27, p21, p34)</td>
<td>Immunohistochemical analysis</td>
<td>In clinical use in some centers</td>
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<tr>
<td>DNA ploidy</td>
<td>Flow or image cytometry</td>
<td>II</td>
<td>In clinical use in some centers</td>
</tr>
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<td>Cytogenetics</td>
<td>Karyotype and FISH</td>
<td>Research only</td>
<td></td>
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<tr>
<td>Morphometric features: roundness, texture</td>
<td>Image cytometry</td>
<td>III</td>
<td>Research only</td>
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<tr>
<td>Microvessel density</td>
<td>Immunohistochemical analysis; image cytometry</td>
<td>III</td>
<td>In clinical use in some centers</td>
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<td>Nuclear matrix proteins</td>
<td>Immunohistochemical analysis</td>
<td>Research only</td>
<td></td>
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<td>Cytokine expression</td>
<td>Immunohistochemical analysis</td>
<td>Research only</td>
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<td>Growth factors and receptors</td>
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<td>Cell adhesion molecules: E-cadherin, CD44, integrins</td>
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<td>Invasion proteases</td>
<td>Immunohistochemical analysis; bioassays</td>
<td>Research only</td>
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<td>Dominant oncogenes</td>
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</tr>
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<td>p53</td>
<td>Immunohistochemical analysis; sequencing</td>
<td>In clinical use in some centers</td>
<td></td>
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<td>PTEN/AKT</td>
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<td>Research only</td>
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<td>GST-π</td>
<td>Immunohistochemical analysis; gene methylation</td>
<td>Research only</td>
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<td>Immunohistochemical analysis; TUNEL assay</td>
<td>III</td>
<td>In clinical use in some centers</td>
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<td>Telomerase</td>
<td>TRAP assay; in situ hybridization</td>
<td>Research only</td>
<td></td>
</tr>
<tr>
<td>Microsatellite instability</td>
<td>PCR; electrophoresis</td>
<td>Research only</td>
<td></td>
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<tr>
<td>Human glandular kallikrein 2</td>
<td>Serum ELISA</td>
<td>III</td>
<td>Blood test in use in some clinical centers (prostate cancer detection)</td>
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<td>Hepsin</td>
<td>Microarray</td>
<td>Research only</td>
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<td>AMACR</td>
<td>Immunohistochemical analysis</td>
<td>Research only</td>
<td></td>
</tr>
<tr>
<td>Ubiquitin and proteolysis</td>
<td>Immunohistochemical analysis; bioassays</td>
<td>Research only</td>
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<tr>
<td>NFκB</td>
<td>Immunohistochemical analysis</td>
<td>Research only</td>
<td></td>
</tr>
<tr>
<td>Expression profiling</td>
<td>Microarray</td>
<td>Research only</td>
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</tbody>
</table>

AMACR, α-methylacyl-coenzyme A racemase; CAP, College of American Pathologists; ELISA, enzyme-linked immunosorbent assay; FISH, fluorescence in situ hybridization; GST, glutathione-S-transferase; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; PTEN, phosphatase and tensin homolog deleted from chromosome 10; RT-PCR, reverse transcription–polymerase chain reaction; TRAP, telomere amplification protocol; TUNEL, terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate–biotin nick-end labeling.

* From Bostwick et al. See the text for an explanation of the categories. Blank cells indicate that the marker has not been assigned to a category.
has been treated with estrogens.24-26 Signet-ring cell carcinomas are negative for neutral and acid mucins, immunoreactive for PSAP and PSA, and clinically aggressive.27,28 Small cell and neuroendocrine carcinomas are associated with a uniformly poor prognosis.29 Prostatic transitional cell carcinomas arise from the periurethral glandular epithelium or from metaplastic prostatic epithelium, are negative for PSA and PSAP, and often develop into aggressive tumors that do not respond to hormonal therapy.16,30 Lymphoepithelioma-like carcinomas are poorly differentiated carcinomas with a syncytial growth pattern, prominent lymphocytic stroma, and adverse clinical behavior.16 Carcinosarcomas and sarcomatoid carcinomas are rapidly progressive biphasic tumors featuring a sarcomatous component.16,31,32 Basal cell carcinomas and carcinoid tumors are additional rare prostatic neoplasms with adverse clinical outcomes.33

Tumor Grade

The microscopic grade of a prostate cancer correlates significantly with the local extent of the disease, incidence of lymph node and bone metastasis, response to various therapies, and overall disease outcome.6-13 The 2-grade summation scoring system developed by Gleason has correlated with cell proliferation rate, aneuploid DNA content, oncogene activation, and tumor suppressor gene mutation6-13 and is predictive of rapid PSA progression.35 However, although the Gleason score of prostatic adenocarcinoma is clearly one of the strongest predictors of biologic behavior and metastatic potential, in most studies, it does not seem to be capable of predicting disease outcome when used alone.6-13 The correlation of Gleason score for the needle biopsy specimen with the final score at radical prostatectomy is best for moderately and poorly differentiated adenocarcinomas.36 Discrepancies between the Gleason score for biopsy specimens and corresponding radical prostatectomy specimens are greatest when Gleason scores are low and the quantity of tumor in the biopsy specimens is limited.37 In addition, it has been documented that grading accuracy for needle biopsy specimens might be higher when the grading pathologists are experienced subspecialists in urologic pathology.38 This may be manifest in the higher incidence of grade changes, upward or downward, at radical prostatectomy encountered when community hospital pathologists performed the original biopsy grading.38 Foci of Gleason patterns 4 and 5 seem to be predictive of adverse outcome even when present in only a minute or tertiary focus.39,40

Tumor Volume

Tumor volume is a significant predictor of pathologic stage, lymph node and distant metastasis, and overall disease outcome.34,41-43 The number and length of involvement of multiple (sextant or octant) needle biopsy cores has been successful at predicting overall tumor volume, pathologic stage, and disease outcome.44

Tumor Stage

Extraprostatic extension is reportedly very common in prostatic adenocarcinoma, with an incidence as high as 90% in 1 series.45,46 Patients with focal intracapsular penetration by the tumor are reported to have an intermediate prognostic risk between those with organ-confined disease and those with diffuse extraprostatic extension.47 The presence of perineural invasion in the needle biopsy specimen has been reported to be a specific marker for capsular penetration of the tumor in a prostatectomy specimen,48 although the overall prognostic significance of perineural invasion remains controversial.49

Seminal vesicle involvement by prostate cancer is associated with high tumor grade, large tumor volume, extraprostatic extension, lymph node metastasis, and poor prognosis.50 Positive margins of resection significantly affect disease outcome and correlate with high preoperative serum PSA levels, high tumor grade, and aneuploid DNA content.47,51-53 Vascular space invasion also has been associated with disease progression.54 The presence of nodal metastases is highly associated with significant tumor progression, with an overall incidence averaging 40%.55 Recent studies of micrometastasis detection using molecular methods have differed in their conclusions.56,57 Bone metastases might signify the progression to androgen-independent tumor growth and, with liver and other parenchymal metastases, often herald progression to an ultimate fatal disease outcome.

Molecular-Based Prognostic and Predictive Factors

A variety of ancillary procedures and molecular-based assays of genes and proteins (Table 1) have been studied for their ability to predict outcome and target therapy in prostate cancer.6-13,58,59

Tumor-Specific Proteins

Immunohistochemical staining and other methods designed to detect the cellular expression levels of PSA and PSAP have not been successful generally for predicting outcome in prostate cancer.5-13 However, in a recent study using fine-needle aspiration biopsy specimens and immunocytochemical analysis, loss of cellular PSA staining successfully predicted disease progression independent of clinical stage, cytologic grade, and DNA ploidy status in patients after endocrine therapy.60 Although individual cell anti-PSA
immunostaining decreases with tumor dedifferentiation, prostate-specific membrane antigen (PSMA) staining is maintained in both low- and high-grade tumors, including primary and metastatic lesions. In a recent study using prostatectomy specimens, increased PSMA expression was associated with biochemical disease relapse. Anti-PSMA staining also has been described in the endothelium of tumor blood vessels in carcinomas of other primary sites, including breast, colon, and lung. Prostate stem cell antigen seems to maintain or increase staining intensity in high-grade tumors and has been correlated with advanced stage and bone metastasis.

**Nuclear Hormone Receptors**

Although androgen receptor (AR) loss and clinical lack of benefit from antiandrogen therapy have been associated with high-grade and high-stage prostate cancer, AR activity has not independently predicted disease-related death. AR expression can be heterogeneous in prostate cancer, which might reflect AR genetic instability and the future development of androgen-independent tumor growth. Assays of AR activity have not been used to select patients for androgen ablation therapy before prostatectomy. Research interest in AR activity has focused on the relationship between expression of various growth factors and matrix metalloproteinases associated with prostate cancer progression and AR status. Although the development of androgen-independent tumor growth has been associated with various specific point mutations in the AR gene, disease outcome has not correlated with AR expression. Further characterization of AR activity in prostate cancer seems warranted to better understand the events that produce the capability of androgen-independent growth for some aggressive tumors and the interaction of AR with other prognostic markers.

**Cell Proliferation Markers**

Cell proliferation has been measured in prostate cancer by immunohistochemical staining using cell proliferation markers and by the calculation of the S phase from flow cytometry or image analysis–derived quantitative DNA histograms. Immunohistochemical analysis using the MIB-1/Ki-67 antibody is the technique of choice for measuring cell cycle progression in processed human tissues. A proliferative index of more than 16% MIB-1 staining has been associated with a highly adverse prognosis. MIB-1 overexpression also has been associated with primary therapy failure and has predicted prognosis even in patients with existing lymph node involvement. In contrast with other malignancies, S-phase calculations by flow cytometric analysis or image analysis have been less clinically useful in prostate cancer.
Cell Cycle Regulators

Cyclins

Cell cycle regulatory proteins have been linked to adverse outcome in prostate cancer. Cyclin D1 overexpression has been detected in approximately 10% of prostate cancers and has been linked to the development of bone metastases.

Cyclin-Dependent Kinase Inhibitors

In the G1 to S phase transition of the cell cycle, 2 families of cyclin-dependent kinase inhibitors have been described: the Cip/Kip and INK4 groups. Both p21 and p27 proteins, members of the Cip/Kip family, have been studied as prognostic factors in prostate cancer. Maintenance of p21 immunoreactivity is associated with prolonged disease-free survival. Loss of p27 expression has been associated with adverse disease outcome in a number of studies. In addition, the homeobox protein skp-2 has been shown to be expressed inversely to p27 in prostate cancer and might represent a drug target candidate for the disease. The p16INK4 tumor suppressor gene is rarely mutated in prostate cancer, but decreased protein expression has been associated with gene deletions and promoter hypermethylation. Interestingly, increased p16 immunostaining has been associated with the presence of prostate cancer, but this marker has not become a useful prognostic factor to date. Overexpression of p34cdc2 cyclin-dependent kinase, involved in the S to G2M transition of the cell cycle, has been associated with aggressive high-grade disease featuring an increased incidence of biochemical failure after primary therapy.

DNA Ploidy Determination

The majority of retrospective studies have shown that aneuploid DNA content in prostate cancer independently predicts a poor prognosis for the disease. DNA ploidy measurements have been performed on needle biopsy specimens by using the tissue section image analysis technique. An aneuploid DNA ploidy status determined on needle biopsy specimens has correlated successfully with the ploidy status of corresponding radical prostatectomy specimens and independently predicted disease outcome. DNA ploidy determination on needle biopsy specimens has been used to confirm biopsy grading, although this role is reduced substantially when grading of the biopsy specimen is performed by experts.

Molecular Genetics and Cytogenetics

A variety of molecular and cytogenetic abnormalities have been associated with prostate cancer. Although certain loci have been associated with the incidence of familial prostate cancer, disease heterogeneity has impeded the discovery of predisposition genes. The most frequent sites of losses of genetic material in prostate cancer, in decreasing order, are on chromosomes 13q, 8p, 6q, 5q, 16q, 18q, 2q, 4q, 10q, and Y. The most frequent gains are seen on chromosomes 8q, 17q, Xq, 7q, 3q, 9q, 1q, and Xp. Chromosomal loss at 8p22 with concurrent gain of 8c and 13q21 have been associated with adverse disease outcome. Also, loss at 8p21, the site of a prostate-specific homeobox gene NKX3.1, also has correlated with tumor progression. Comparative genomic hybridization studies of prostate cancer have found DNA copy number changes in as many as 65% of the analyzed tumors, with the most common chromosomal losses found at regions 13q21q33 (29%), 6q11q23 (24%), 16q, and 18q (each 18%) and the most common gains at 19 (18%).

Morphometrics

A variety of morphometric techniques have been applied on prostate cancer specimens with the nuclear roundness factor measurement achieving the most significant potential clinical usefulness. Prostate cancers featuring almost perfectly round nuclei typically are well-differentiated and slow-growing cancers. Tumors with irregular nuclear contours and correspondingly low nuclear roundness have been associated with high tumor grade and a propensity for the development of distant metastasis and shortened survival. A recent study of morphometric features in prostate cancer found that suboptimal circle fit and Feret diameter ratio measurements could predict disease relapse after radiation therapy.
Tumor Vascularity and Microvessel Density

Tumor angiogenesis also has correlated with adverse outcome in prostate cancer as measured by microvessel counting studies. Significantly higher microvessel counts have been obtained in areas of adenocarcinoma than in the benign tissues of radical prostatectomy specimens. Prostate cancers seem to have the greatest concentration of microvessels in the centers of the tumoral areas, which might account for the infrequency of necrosis in prostate cancer. Increased microvascularity has been found to correlate with the pathologic stage of the disease. Microvessel density has been associated with the presence of metastasis and with a significant risk for disease progression after radical prostatectomy in some studies but has failed to achieve significance as an outcome predictor in others. These conflicting results might reflect methodological differences in microvessel counting techniques. The application of microvessel counts to prostate cancer needle biopsy specimens, in which the counts could be used prospectively to plan therapy, has not received sufficient study.

Color Doppler flow has been shown to be an important aid to gray-scale sonography in the detection of prostatic carcinoma and has been correlated with tumor grade and stage. However, little evidence has been presented to date linking sonographic findings to microvessel counts in the respective tissue samples. In fact, in 1 study, microvessel density and tumor size were no different in specimens with normal or with increased color Doppler flow.

Nuclear Matrix Proteins

Nuclear matrix proteins function to maintain the structure, shape, and higher order of DNA organization within a cell. Nuclear matrix proteins have been characterized in prostate cancer and may define subsets of the disease with differing biology and clinical behavior. The expression of 1 nuclear matrix protein, YL-1, seems to be related to an aggressive type of prostate cancer and correlates with pathologic stage of disease.

Cytokines

In hormone-refractory prostate cancer, up-regulation of inflammation-associated interleukin (IL)-4, IL-6, and IL-10 has been described. Recently, serum IL-6 levels have been linked to adverse outcome. Tissue-based cytokine measurements have not been associated with prostate cancer prognosis.

Growth Factors

A variety of growth factors have been studied in prostate cancer, including the epidermal growth factor (EGF) and its receptor (EGFR). The results have been conflicting, ie, although EGF assays of prostate cancer specimens show higher levels than seen in the normal prostate, high-grade
tumors seem to have lower EGF content than do well-differentiated lesions. Increased EGFR expression has been linked to progression to androgen-independent disease in a study, and preclinical studies have suggested that anti-EGFR therapies, such as with small molecule tyrosine kinase inhibitor ZD1839 (Iressa, Astra Zeneca, Manchester, England), have possible clinical usefulness in treatment. Increased expression of basic fibroblast growth factor has been linked to adverse outcome. Overexpression of transforming growth factor β has been implicated in the growth of prostate cancer cell lines and a significant reduction in disease-free survival in clinical trials. Up-regulation of vascular endothelial growth factor in prostate cancer has been associated with adverse outcome in patients with clinically localized disease. Growth factors seem to operate in networks in the prostate, and further studies are necessary to elucidate the various interactions of these trophic proteins on disease outcome.

**Cell Adhesion Molecules**

Loss of expression of E-cadherin, a cell adhesion molecule, has been associated with adverse disease outcome in prostate cancer. Decreased expression of E-cadherin has been shown to associate with high tumor grade and aneuploidy. It has been further suggested that this deletion might involve the E-cadherin gene and that E-cadherin protein, in addition to its cell adhesion role, might be functioning as a tumor suppressor protein. Alternatively, the loss of E-cadherin expression in prostate cancer might be related to gene methylation. Further immunohistochemical and molecular genetic studies seem warranted to pursue this potential important association.

The E-cadherin interaction with β-catenin has received substantial study resulting from the linkage of β-catenin degradation and association with the APC gene. Expression of β-catenin on the cytoplasmic membrane indicates E-cadherin interaction, whereas transfer of β-catenin staining to the cytoplasm and nucleus indicates that β-catenin has initiated signal transduction mediated by the lymphoid enhancer factor complex, which has been associated with the up-regulation of various genes associated with adverse prognosis, such as cyclin D1. Anomalies of β-catenin expression have been described in prostate cancer and have been associated with disease progression.

The CD44 cell adhesion molecule also has been linked to outcome in prostate cancer. Loss of expression of the CD44 protein standard form has been associated with other adverse prognostic factors such as high tumor grade and aneuploid DNA content. Recent evidence points to promoter gene hypermethylation as the cause of the loss of CD44 standard form expression in prostate cancer. CD44 also is associated with production of a series of splice variant proteins that have been linked to adverse outcome in a variety of malignant neoplasms, including prostate cancer. The expression of CD44v6 might be a predictor of poor prognosis in organ-confined prostate cancer and useful for planning adjuvant therapy.

Integrins have been studied widely in prostate cancer and implicated as potential indicators of aggressive disease. Decreased expression of the α5 integrin subunit and the laminin 5 subunit have been linked to adverse disease outcome.

**Tumor Invasion–Associated Proteases**

Cathepsin D, a lysosomal protease and autocrine mitogen, has been associated with prognosis in breast cancer. In prostate cancer, increased tumor cathepsin D immunoreactivity has been correlated with pathologic stage and with tumor grade and DNA content. Increased serum levels of soluble urokinase plasminogen activator receptor have been linked to progressive prostate cancer. Finally, localization of tumor collagenases and matrix metalloproteinases has been linked to the development and progression of prostate cancer.

**Dominant Oncogenes**

In comparison with their significance in adenocarcinomas of the respiratory and gastrointestinal tracts, the roles of dominant oncogenes in the development and progression of prostate cancer seem limited. The ras genes,
commonly mutated in epithelial adenocarcinomas in the gastrointestinal, hepatobiliary, and respiratory tracts, are not altered frequently in human prostate cancers, cell lines, or experimental models. Amplification of the myc gene has been studied in prostate cancer but could not be linked to disease progression. Mutations of myc might be associated with the development of hormone-refractory disease.

The HER-2/neu (c-erb-b2) Gene

The presence of HER-2/neu (c-erb-b2) gene amplification or overexpression of the protein has been associated with adverse outcome in breast cancer, and testing for HER-2 has achieved standard-of-practice status for selecting therapy for breast cancer patients. For prostate cancer, the results of immunohistochemical analysis–based studies have conflicted but generally favor that overexpression of the HER-2/neu protein is associated with an adverse outcome. The results of fluorescence in situ hybridization–based studies also have varied, with some finding extra copies of the gene in hormone-naive and hormone-treated cases and others not detecting amplification. HER-2/neu messenger RNA (mRNA) levels have been correlated with metastatic disease and androgen-independent, hormone-refractory, progressive disease. Early clinical trials using the humanized anti–HER-2/neu monoclonal antibody trastuzumab (Herceptin) have not shown significant responses. Other anti–HER-2/neu–targeted therapies for prostate cancer are in early clinical development.

Tumor Suppressor Genes

p53

The assessment of p53 status in prostate cancer has included both molecular techniques (single-strand conformation polymorphism and direct sequencing) and immunohistochemical analysis (multiple methods and reagents). Positive immunostaining for p53 has been associated with the detection of the more stable mutant protein and predicts the presence of p53 gene mutation with about 80% to 90% accuracy. By using a variety of antibodies and immunohistochemical techniques, p53 protein expression has been reported frequently in prostate cancer, with average immunoreactivity ranging from 13% to 23%. A positive association between nuclear p53 immunoreactivity and aggressive biologic behavior of prostate cancer has been confirmed in multiple studies. Mutations of p53 seem to be frequent in metastatic prostate cancer. Although immunohistochemical analysis can be an inaccurate predictor of p53 gene status, when molecular biologic techniques are used, it has been reported that 42% of prostate cancers can harbor mutant p53 sequences. Mutations of the p53 locus in benign prostate tissue have been reported, suggesting that p53 mutations might occur early in the pathogenesis of prostate cancer. New studies of p53 status, including functional assays, must be performed on needle biopsy specimens to achieve prognostic value for prospective treatment planning in prostate cancer.

PTEN/AKT-1

The PTEN (phosphatase and tensin homolog deleted from chromosome 10) also known as MMAC1 (mutated in multiple advanced cancers 1) tumor suppressor gene is deleted or mutated in a wide variety of malignant neoplasms and in prostate cancer cell lines, xenografts, and clinical samples. Loss of expression of PTEN has been associated with down-regulation of the cyclin-dependent kinase inhibitor p27 and adverse outcome with increasing tumor grade and stage in prostate cancer.

p16

Abnormal expression of the G1-S cell cycle regulator p16 has been detected in prostate cancer clinical samples. Inactivation of p16 in prostate cancer has been associated with methylation of the promoter region of the p16 gene with silencing of mRNA and protein production. Recently, the loss of expression of p16 has been associated with prostate cancer progression, metastasis, and disease relapse.

KAI-1

The KAI-1 gene, mapped to chromosome 11p, has been implicated as a tumor suppressor gene in prostate cancer, but altered expression of this gene has not been associated with prognosis.

pRB

The retinoblastoma gene on chromosome 13 also might function as a tumor suppressor in prostate cancer, but probably is altered in only a small subset of cases. The immunohistochemical staining score independently predicted disease outcome in 1 study. Other known tumor suppressor genes that might have a role in prostatic carcinogenesis but have not been linked to disease outcome are the DCC (deleted in colorectal cancer) gene on chromosome 18q and the APC (adenomatous polyposis coli) gene on chromosome 5q. The APC gene might be important, however, in its association with degradation of the β-catenin protein and blockade of β-catenin–mediated nuclear transcription of cell cycle promoters such as cyclin D1.

Drug-Resistance Genes

Multidrug Resistance

The multidrug resistance factor has been implicated as having a role in the development of resistance of metastatic
prostate cancer to conventional cytotoxic chemotherapy.\textsuperscript{188} The expression of the multidrug resistance biomarker P-glycoprotein was correlated with tumor grade, stage, and PSA levels in one study\textsuperscript{186} and with tumor stage in another.\textsuperscript{189}

\textit{Glutathione-S-Transferase-\textpi}

The glutathione-S-transferase (GST)-\textpi gene is involved in the intracellular detoxification of drugs and toxins. GST-\textpi is deactivated in the vast majority of prostatic carcinomas by the hypermethylation of CpG island promoter sequences, resulting in loss of expression of the GST-\textpi protein.\textsuperscript{190,191} Although it has little value as a prognostic factor, GST-\textpi methylation detection shows substantial promise as a urine-, semen-, or blood-based assay for the detection of prostate cancer.\textsuperscript{192-194} The high frequency of GST-\textpi gene silencing by methylation also has raised the possibility that this biomarker could be the target of a chemoprevention program for prostate cancer.\textsuperscript{195}

\textbf{Apoptosis and bcl-2}

Expression of bcl-2 has been studied in prostate cancer, initially by immunohistochemical techniques, and found to react with primary and metastatic prostate cancer specimens obtained from patients with tumors refractory to hormonal therapy.\textsuperscript{196} Immunoreactivity for bcl-2 is most intense in basal cells rather than secretory cells\textsuperscript{197} and may be limited to normal prostatic and seminal vesicle epithelium and to rare cases of poorly differentiated but not well-differentiated prostatic carcinomas.\textsuperscript{198} In prostate cancer, overexpression of bcl-2 protein is not associated with rearrangements in the 2.8-kilobase major breakpoint region or with accumulation of p53 protein.\textsuperscript{199} Several studies have linked the overexpression of the bcl-2 antiapoptosis protein with decreased expression of the proapoptotic protein bax and adverse outcome in prostate cancer associated with resistance to cytotoxic chemotherapy in patients with hormone-refractory disease.\textsuperscript{13,200,201} Other studies, however, have not found prognostic significance for bcl-2 expression.\textsuperscript{202,203}

\textbf{Telomerase}

Telomerase activity, measured by the telomere amplification protocol assay, is present in virtually all prostate cancers, with increasing levels associated with high-grade disease.\textsuperscript{204-206} Telomerase detection has been advocated as a potential technique to confirm the presence of microfoci of prostate cancer in needle biopsy specimens.\textsuperscript{204,205} Telomerase measurements have been tested for their ability to assess the status of surgical resection margins after prostatectomy.\textsuperscript{207} Molecular methods also have featured telomerase testing as a means to detect prostate cancer in seminal fluid.\textsuperscript{192,208} However, given its wide expression range in most prostate cancers, the measurement of telomerase has not been useful as a prognostic factor, although it has been linked to high-grade disease.\textsuperscript{209}

\textbf{Microsatellite Instability}

Microsatellite instability (MSI) has not been studied widely in prostate cancer. Initial studies of clinical specimens failed to demonstrate widespread MSI.\textsuperscript{210} Additional studies suggested that MSI might be related to early prostate cancer development,\textsuperscript{211} and a recent study linked MSI and \textit{hMSH2} gene expression to biochemical disease relapse.\textsuperscript{212}

\textbf{Unclassified Biomarkers}

\textit{Human Glandular Kallikrein 2}

The human glandular kallikrein (hK) family of proteins includes PSA, which also is known as hK3.\textsuperscript{213} This secreted protein recently has shown promise for improving the specificity of serum-based prostate cancer screening compared with serum PSA levels.\textsuperscript{214-216} To date, hK2 expression in prostate cancer tissue has not been linked to prognosis.

\textit{Hepsin-like Protease}

By using transcriptional profiling, in addition to hK2, PSA (hK3), and PSMA, hepsin, a serine protease also known as hepsin-like protease, is a highly overexpressed mRNA that is particularly characteristic of high-grade tumors.\textsuperscript{217-219} Most recently, overexpression of hepsin mRNA detected by expression profiling on DNA microarrays has been associated with adverse disease outcome in prostate cancer.\textsuperscript{220,221}

\textit{\textalpha-Methylacyl-Coenzyme A Racemase}

Numerous recent transcriptional profiling studies of prostate cancer specimens have detected significant up-regulation of \textit{\textalpha-methylacyl-coenzyme A racemase} (AMACR), a peroxisomal and mitochondrial enzyme.\textsuperscript{221-223} Although AMACR expression has been linked to prostate cancer differentiation, it has not been identified as a prognostic marker for the disease.\textsuperscript{224} Most recently, AMACR immunostaining has been used to identify minute foci of prostate cancer in needle biopsy specimens, with variable results.\textsuperscript{225}

\textbf{Nuclear Factor \textkappaB and the Proteasome}

The nuclear transcription factor \textkappaB (NF\textkappaB) complex has a role in cancer development and progression through its influence on apoptosis.\textsuperscript{226} NF\textkappaB has been shown to be activated in human and androgen-independent prostate cancer cells.\textsuperscript{227} The ubiquitin-proteasome pathway has been studied in prostate cancer as a regulator of the NF\textkappaB signal transduction pathway, downstream proapoptotic (bax) and antiapoptotic (bcl-2) protein expression,\textsuperscript{226,228,229} and cell cycle regulatory proteins.\textsuperscript{230} The recently discovered proteasome inhibitor PS-341 (bortezomib [Velcade]) has been associated
with decreased production of bcl-2, inhibition of NFκB, and prevention of acquired resistance to chemotherapy in prostate cancer experimental systems.231 NFκB overexpression in prostate cancer recently has been linked to adverse disease outcome.232,233

Transcriptional Profiling

In the past several years, transcriptional profiling of clinical specimens has been introduced as a method for discovering new biomarkers of the disease and for the prediction of its outcome.217-223,234-237 Although the bioinformatic methods necessary to evaluate the large data sets associated with these procedures have not been standardized, significant common findings238 indicate that this technology holds substantial promise for the discovery of new prognostic factors that may be used in the future to predict the outcome of the disease and permit more individualized selection of primary therapy.

Conclusions

The major prognostic markers described in this review are summarized according to their current clinical use in Table 1. The classic morphologic feature–based factors of tumor type, grade, volume, and stage are used widely and generally considered to be the standard of practice. The Ki-67 cell proliferation index, p53 and bcl-2 immunostaining, and DNA ploidy analysis also are used in many laboratories as adjuncts for predicting outcome in the disease. The remaining prognostic and predictive markers discussed in this review continue to be under evaluation to assess their usefulness in determining prognosis and guiding the selection of therapy for the disease.

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