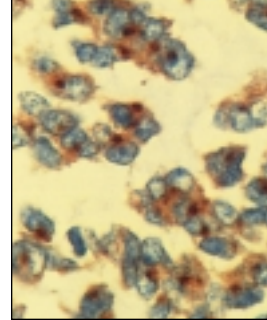


PROSTATE ADENOCARCINOMA: CELLULAR AND MOLECULAR ABNORMALITIES

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Introduction

Prostate cancer is the most common malignancy detected in men in the United States. The worldwide incidence of this disease is also rising, mainly due to demographic factors, such as the increasingly elderly population and, more importantly, the increasing number of cases identified following prostate-specific antigen (PSA) testing.

Adenocarcinoma of the prostate gland presents with a wide variety of clinical findings, ranging from asymptomatic, relatively latent tumors to highly aggressive, metastasizing cancers. Tumors predominantly arise from epithelial cells in the peripheral zone of the gland. Tumors that progress, if untreated, will extend into the prostatic capsule and seminal vesicles, and will ultimately metastasize to regional and distant sites such as lymph nodes and bone. Metastatic hormone-refractory disease is the most important cause of morbidity, treatment failure, and subsequent mortality from prostate cancer. The two main issues for clinicians and pathologists involved in prostate cancer are early detection of the cancer and identification of the prognostic factors that predict outcome in individual patients.¹

Although an understanding of some of the cellular and molecular defects in prostate cancer has recently begun to emerge, several unanswered questions remain regarding issues such as predisposition, disease expression, and progression of this cancer. For exam-

ple, how can indolent tumors be differentiated from aggressive, lethal ones? What is the cellular mechanism involved in predisposition and eventual progression of prostate cancers?

Recognition of cellular features, which accurately predict the behavior of prostate cancer occurring within a specific patient, is a major challenge facing contemporary urologic pathologists and clinicians.^{2,3} Anatomically localized prostatic adenocarcinomas frequently behave in a malignant fashion. Although progression may be slow for the many patients who choose follow-up by “watchful waiting,” many will die of the disease until biologically appropriate therapeutic approaches are developed to curb the otherwise inevitable malignant behavior. Once the disease has spread beyond the surrounding fibrous capsular region of the gland, treatment is often unsuccessful. Adding to the diagnostic challenge is the fact that a relatively large subset of prostate tumors advance slowly and are rarely the cause of death. Early detection of prostate cancer, preferably in the preinvasive phase (in lesions such as high-grade prostatic intraepithelial neoplasia), is important if a treatment can be found that will arrest development of the cancer.

Much research effort has also focused on developing prognostic factors that can predict outcome in individual patients with prostate cancer.^{2,3} The goal is to adapt the therapeutic approach to the clinical, morphological, and molecular features of each patient. Many of

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the clinically important predictive factors in prostate cancer are still derived from a pathologist's examination of tissue specimens using light microscopy. However, in the future, molecular markers will provide supplemental information and the challenge will be to assemble the information into a useable form to aid in the diagnosis, staging, and treatment of prostate cancer.

In this review article, we summarize current literature on accepted pathological and promising molecular markers that have relevance to the development and progression of prostate cancer. Additionally, many of the pathways discussed provide a fertile area for the development of new cancer treatments.

Development of Prostate Cancer

Despite the introduction of widespread PSA testing, approximately 20% of patients with prostate cancer will have extracapsular disease that is incurable with current treatments.^{4,5} The identification of risk factors for the development of prostate cancer and precancerous change within the prostate allow for not only earlier detection of prostate cancer, but also the introduction of lifestyle and chemopreventive strategies.

Histopathologic Markers

Prostatic Intraepithelial Neoplasia

Prostatic intraepithelial neoplasia (PIN) refers to morphological

appearances within a spectrum extending between histologically "normal" and "frankly malignant" prostatic epithelium.^{5,6} PIN is thought to arise in pre-existing ducts rather than through the formation of new acinar epithelium. This premise is based on histopathologic observation in which cell groups consistently exhibit cytologic abnormalities that characterize PIN growing adjacent to benign epithelium. PIN is characterized by a series of well-defined architectural and cytological criteria including progressive loss of the epithelial bicellular layers, with eventual disappearance of basal cells. PIN is divided into two grades, low and high (which replaces the previous categories of PIN1 (low grade), PIN2, and PIN3 (high grade)).^{7,8} To assist in distinguishing high-grade PIN from in situ or invasive cancer, the presence of basal cells may be confirmed immunohistochemically using antibodies AE1/AE3 or 34b-E12, which recognize high-molecular-weight cytokeratins. The importance of diagnosing PIN is that high-grade PIN is associated with the presence of synchronous prostate cancer in greater than 35% of cases.⁷

Atypical Small Acinar Proliferation

In approximately 5% of prostate biopsies, small foci of atypical glands that are suspicious for but not diagnostic of carcinoma are identified. Most pathologists are reluctant to diagnose prostate cancer when fewer than three glands demonstrate frank cytological atypia. Atypical small acinar proliferation is a distinct

pathological condition from high-grade PIN and is associated with the diagnosis of prostate cancer on subsequent biopsy in approximately 45% of cases (even more frequently than high-grade PIN).^{9,10} Whether atypical small acinar proliferation is a true premalignant condition or just a consequence of prostate biopsy sampling and fixation techniques is uncertain. Nevertheless, the frequent association with frank prostate cancer warrants rebiopsy and careful follow-up.

Molecular Markers

Family history is a major risk factor for the development of prostate cancer. Several large epidemiological studies have demonstrated familial clustering, suggesting that approximately 9% of prostate cancer may be due to an autosomal dominant inherited gene.¹¹ Using linkage analysis, loss of an area on chromosome 1 (1q24-25) has been identified that greatly increases the risk of developing prostate cancer. The chromosomal changes have been confirmed on independent analysis, and the area has been designated *HPC1* (human prostate cancer 1). A second area on the X chromosome (Xq27-28) has also been identified and designates *HPCX* (human prostate cancer X). Two additional areas on chromosome 1 (1p36 and 1q42-43) have been identified as having potential links to prostate cancer development. Unfortunately, the causative genes in each of these areas have not yet been conclusively identified.¹²

From a clinical standpoint, familial prostate cancer occurs at a

Table 1. — Predictive Pathological Factors in Prostate Cancer

Gleason score
WHO nuclear grade
Pathological stage
Surgical margins
Perineural invasion
Vascular or lymphatic invasion
Tumor volume
Histological type
DNA ploidy and proliferation index
Neuroendocrine differentiation

younger patient age than sporadic cancers. There is some suggestion that cancers associated with the *HPC1* gene are more biologically aggressive. However, several studies have shown little survival difference between inherited and sporadic prostate cancer. The identification of the genes involved in the development of prostate cancer not only will allow the development of blood tests to establish risk, but also will provide a window on the molecular events involved in prostate carcinogenesis.

Mechanisms of Prostate Cancer Progression

Pathological Markers

Prostate cancer is unusual in that it can be broadly categorized into two clinical presentations: (1) a latent form that is found at autopsy in approximately 30% of men over 50 years of age and in 60%-70% of men over age 80 and (2) a clinically evident form that is diagnosed in approximately 9% of men and is lethal in about 3% of men. Clearly, this is an oversimplification as there is considerable hetero-

geneity in prostate cancer in terms of prognosis and response to therapy. The recognition of cellular features that accurately predict the behavior of prostate cancer occurring within a specific patient is a major challenge facing contemporary urologic pathology. Thus, the interest in pursuing molecular markers of prostate cancer progression has expanded. The hope is that by dissecting the molecular pathways of prostate cancer progression, prognostic markers and therapeutic opportunities will be identified. In this section, well-known pathological predictors are summarized and newer molecular markers of prostate cancer progression are discussed (Table 1).

Gleason Score

Gleason score is one of the most useful prostate cancer grading systems and is universally accepted as a pathological indicator of biological behavior, correlating with stage and metastatic potential. The Gleason system for histologic grading of prostate tumors is based solely on morphology (Fig 1). Low-power microscopic examination of

biopsy specimens usually reveals tumor patterns ranging from small, well-differentiated glands to poorly differentiated sheets or cords of malignant cells. Five distinct glandular patterns are graded progressively from most to least differentiated. The grades of the two predominant patterns present in a surgical specimen are added to yield the final Gleason score. Patients with well-differentiated lesions (Gleason scores 2-4) usually have early-stage disease and a good prognosis. Gleason scores 8-10, however, are associated with a poor prognosis. Gleason score correlates well with other known prognostic factors such as tumor size, presence of pelvic lymph-node metastasis, and PSA level. Reports in the literature on the prognostic importance of Gleason score are extensive and will not be discussed further here.¹³⁻¹⁶

Perineural Invasion

The growth of tumor cells along the nerve sheaths is a common finding in radical prostatectomy specimens and is termed "perineural invasion." Perineural invasion has been identified in up to

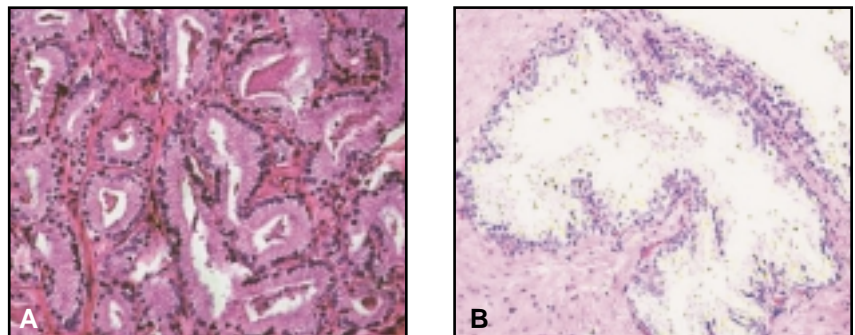


Fig 1. — Prostatic adenocarcinoma, well differentiated. (A) Simple glands formed by cells with mild nuclear anaplasia. Regular glandular growth with minimal stromal tissue between glands, Gleason score 3. (B) Normal prostatic tissue (hematoxylin-eosin, × 100).

38% of prostate biopsy specimens.¹⁷ Considerable controversy exists as to the significance of perineural invasion. Some studies suggest that this finding is associated with extracapsular disease in up to 95% of cases (of considerable importance when deciding whether to resect the neurovascular bundle controlling potency at the time of radical prostatectomy).¹⁸ However, other investigators found that perineural invasion was of no predictive value when other prognostic factors such as Gleason score and PSA level were accounted for by multivariate analysis.¹⁹ The finding is noted on needle biopsy pathology reports but is rarely used to make clinical or operative decisions.

Vascular or Lymphatic Invasion

Microvascular invasion is the finding of tumor cells within an endothelial lined space. Vascular invasion reflects the fact that the tumor has acquired the ability to invade blood vessels, which is one of the early steps in the metastatic process. The prognostic value of vascular invasion has been established in multiple tumor types, most notably in testicular cancer where its presence changes tumor stage from T1 to T2 and has significant clinical importance in deciding therapy.²⁰ Vascular invasion is found in approximately 38% of radical prostatectomy specimens and is associated with increasing tumor stage, grade, and nodal metastases. However, similar to perineural invasion, it falls out as a marker when other standard predictors of progression are included in multivariate analysis.²¹

Neuroendocrine Differentiation

Prostate cancer may show divergent differentiation toward a neuroendocrine phenotype in the form of neuroendocrine small cell carcinoma or carcinoid-like tumors. More common, however, is focal neuroendocrine differentiation in prostate cancer, which may be pronounced in approximately 10% of carcinomas. The prognostic importance of focal neuroendocrine differentiation in prostate cancer is controversial. However, current evidence suggests that it has an influence on prognosis in hormone-resistant tumors or a role in the conversion to a hormone-resistant phenotype and has an influence on prognosis in this patient group.^{3,22} Chromogranin A appears to be the best overall tissue and serum marker of neuroendocrine differentiation, and thus serum chromogranin A concentrations may be useful in assessing the emergence or progression of hormone-resistant cancer.⁴

DNA Ploidy and Cell Proliferation

An assessment of the proportion of proliferating cells reflects tumor growth and may predict the likelihood of tumor progression. Studies using flow cytometry, mitotic index, and Ki-67 immunohistochemistry have suggested that proliferative indices may have prognostic importance in prostatic cancer. Flow cytometry, which measures the proportion of the cell population in different phases of the cell cycle, indicates that a high S-phase fraction and a high ratio of cells in S and G₂,

when compared with the M phase, correlate with a rapid tumor progression and short cancer-specific survival.²³ Immunochemical staining for Ki-67 reacts with an intranuclear antigen whose presence is detectable only in proliferating cells.²⁴ Expression of Ki-67 protein is required throughout the cell cycle, since abrogation of expression inhibits cell proliferation.²⁴ A highly significant correlation between Ki-67 expression and the degree of malignancy has been reported in many tumor types.²⁴ A correlation between the Ki-67 labeling index and prognosis in prostate cancer has been reported in several studies. Diaz et al²⁴ found no association between Ki-67 labeling index and histopathologic phenotype. However, a low levels of Ki-67 expression was independently associated with an improved prognosis. Proliferation index correlated with clinical stage and pathological grade, and all three discriminated between tumors confined to the prostate and those that had extended to seminal vesicles, lymph nodes, or bone.²³⁻²⁷

Molecular Markers

The molecular mechanisms underlying prostate cancer progression to metastatic disease and the hormone refractory state are poorly understood. No single event identified at the molecular level has been shown to correlate with the development and/or progression of human prostate cancer (Table 2). The biochemical and molecular mechanisms that control cellular interaction during prostate organogenesis, morphogenesis, and functional differentiation remain unde-

terminated. The inter- and intra-cellular signaling pathways that govern androgen receptor-mediated gene transcription and communicate with other signal transduction pathways remain largely uncharacterized. The importance of understanding stromal-epithelial interactions is underscored by the strong propensity of prostate cancer to metastasize to the bone and the subsequent unique osteoblastic response observed in patients with metastatic disease. This section focuses on some of the more common molecular abnormalities found in tumors and discuss their significance in the progression of prostate cancer.

Oncogenes

Oncogenes are altered normal genes that confer the malignant phenotype primarily by dysregulating the normal cellular mecha-

nisms of growth control. Oncogenes have important roles in malignant progression in many tumor types, most obviously chronic myelogenous leukemia, where the *bcr-abl* oncogene is a result of the chromosomal translocation t(9:22) (Philadelphia chromosome). In human prostate cancer, studies of oncogene activation and expression have been singularly unsatisfactory. Information from several sources indicates that presently no known oncogenes are specifically or consistently activated in either human or rodent prostate cancer.^{22,28-30} Additionally, no recognized oncogene markers provide insight into the pathogenesis or progression of prostate cancer.

Tumor Suppressor Genes

Tumor suppressor genes code for proteins that regulate negative cell growth. Loss of or diminished

expression of a tumor suppressor gene can lead to uncontrolled growth through a number of mechanisms including interference with the cell cycle, apoptosis, DNA repair, or cell adhesion.

The *retinoblastoma (Rb) tumor suppressor gene* was the first tumor suppressor gene to be identified. Loss of Rb function leads to uncontrolled growth due to the failure of the mutant proteins to bind and sequester the E2F transcription factors. Mutational inactivation of Rb is found in defined subsets of most human epithelial neoplasms, in addition to all retinoblastomas.^{31,32} One study has confirmed the presence of Rb mutations in a subpopulation of human prostate cancer and supports the probability that mutation of the Rb gene might be an important step in the genesis of at least some prostate cancers.^{33,34} Of interest, gene transfers of exogenous copies of wild-type Rb suppress tumorigenicity in several different tumor cell lines known to have pre-existing endogenous Rb mutations.

Loss of heterozygosity (LOH) for Rb has been shown in up to 60% of informative patients with prostate cancer. Loss of Rb expression occurred with similar frequencies in both early-stage and low-grade cancers, as well as advanced cancers.³⁵ However, a lack of correlation of LOH at 13q (location of Rb) with absent pRb expression suggests that the existence of another tumor suppressor gene in this region is important in prostate cancer.

Table 2. — Molecular Events Studied in Prostate Adenocarcinoma

Tumor Suppressor Genes:	Rb Wild p53 KAI1
Growth Factors and Cytokines:	Acidic fibroblast growth factor (aFGF) Basic fibroblast growth factor (bFGF) Platelet-derived growth factor (PDGF) Epidermal growth factor HER-2/neu (<i>c-erbB-2</i>) and <i>c-erbB-3</i> oncogenes Interleukin-6 Interleukin-11
Angiogenesis:	VEGF
Apoptosis:	Bcl-2 Bcl-X _L
Protein Kinase C	
Osteopontin	
Telomerase	
Genes for Adhesion Molecules:	CD44
STAT:	Stat3

The p53 tumor suppressor gene is the most frequently mutated gene in human tumors. The p53 gene plays an important role in regulating entry and progression through the cell cycle and/or inducing apoptosis in the presence of DNA damage. Loss of p53 function is relatively easy to identify with immunohistochemistry. Abnormal p53 expression has been found in many tumor types and in general predicts a poor prognosis.^{26,36-38} Approximately 16% of newly diagnosed primary prostatic carcinomas in the United States have abnormal p53 protein expression.^{34,39} Little correlation has been found with tumor stage and grade, a finding strongly suggesting that inactivation of this tumor suppressor gene is unlikely to be either a consistent or even an important event during the initiation phase of most human prostate cancers.^{8,26,36} However, as prostate cancer progresses to the hormone refractory phenotype, the incidence increases to approximately 50%, suggesting that p53 may have a role in prostate tumor progression. Altered p53 in these circumstances is associated with a poorer prognosis.

KAI1 tumor suppressor gene is located at human chromosome 11p11.³⁵ Evolutionarily conserved and distributed widely in human normal tissues, KAI1 encodes one member of a structurally distinct family of leukocyte surface glycoproteins. All members of this family characteristically contain four transmembrane domains and a large extracellular N-glycosylation domain. Although the precise functions of these proteins are

unconfirmed, their membrane localization and extensive glycosylation suggest that they function in cell-cell interactions and cell-extracellular matrix interactions⁴⁰ and that they are important in invasion and metastasis. N-glycosylation of these molecules is consistent with their presumed role in metastasis suppression because the association between processing of N-linked oligosaccharides and metastatic phenotype is well documented.⁴⁰ The protein product of the gene is reported to be reduced in human cell lines derived from metastatic prostate cancers.

PTEN encodes a lipid phosphatase that leads to a decreased sensitivity to cell death. PTEN is located on 10q23, a region that is commonly lost in prostate cancer.⁴¹ PTEN knock-out mice appear to develop prostatic hypertrophy and dysplasia. Although there is some disagreement about the frequency of PTEN mutations, decreased expression of the protein is commonly found in prostate cancer. For these reasons PTEN may be important in the initiation of prostate cancer.^{41,42}

P27 is a cyclin-dependent kinase inhibitor that prevents progression of the cell cycle at the G₁-S phase transition. Decreased p27 expression, unlike loss of function of p53, is rarely due to gene mutation but rather to decreased protein production or, more commonly, to an increased rate of destruction by mechanisms such as protein phosphorylation and ubiquitination. Decreased p27 expression is an important step in expression

of the malignant phenotype and has been found to be an independent prognostic factor in breast and colon cancer. Several studies have demonstrated that decreased p27 expression is relatively common in prostate cancer and correlates with increasing tumor grade.⁴³ Additionally, target disruption of the p27 gene in mice leads to hypertrophy of several tissue types including the prostate.⁴⁴

Growth Factors and Growth Factor Receptors

Growth factors regulate mitogenesis and differentiation of cells by binding to specific cell surface receptors. Over the last few years, many growth factors and their receptors have been identified and grouped into several families based on their structural and functional properties. Although the exact mechanism of action of each of these is not fully understood, it follows the following general pathway: AA ligand (growth factor) binds to its cognate receptor (growth factor receptor), activating a tyrosine kinase (which is part of the receptor's intracellular domain), and this in turn initiates a cascade of events controlling a number of processes including cell proliferation, differentiation, and apoptosis. While these processes are common to a diverse array of tissues, there is also evidence that growth factors promote the tumor cell motility and invasion that are associated with the metastatic phenotype. Progression of prostate cancer, in common with other solid tumors, is accompanied by modifications in the expression of a number of these

growth factors and their receptors as well as changes from paracrine to autocrine growth. In the case of prostate cancer, a significant body of work has examined the role of growth factors in epithelial-stromal interactions. Evidence from tissue recombination experiments suggests that aberrant growth factor signaling from the stromal component has an important role in cancer progression.^{45,46} Particular attention has been paid to the interaction between bone-derived mesenchymal cells and prostatic epithelial cells because of the propensity of prostate cancer to metastasize to the bone and cause osteoblastic type metastasis.

At least four families of growth factors appear to influence prostate cancer progression through a number of autocrine and paracrine loops: (1) transforming growth factor-beta, (2) fibroblast growth factor, (3) epidermal growth factor, and (4) insulin-like growth factor. Unfortunately, elevated growth factor production, increased growth factor receptor expression, and/or abnormalities of growth factor signaling have not been found to consistently correlate with stage, grade, or progression of prostate cancer to a sufficient extent to allow for their use as clinical predictors. The role of each of these families in the development and progression of prostate cancer is discussed in previous reviews.^{45,47}

Androgen Receptor Signaling

Androgens are required for normal prostate development and growth. Androgen withdrawal in

prostate cancer leads to significant initial apoptosis; however, there is inevitable outgrowth of a hormone-refractory clone that will eventually lead to death. For these reasons, tremendous scientific effort has focused on the role of the androgen receptor and the pathways it regulates. Androgens (primarily dihydrotestosterone) bind to the androgen receptor in the cytoplasm. The complex is then translocated to the nucleus where it binds to specific areas of DNA known as androgen response elements, causing activation of androgen-regulated gene expression.⁴⁸ In hormone-refractory prostate cancer, direct androgen receptor signaling is not necessary since, in the majority of cases, downstream signaling takes over for the lack of androgen-mediated signals. In more advanced prostate cancer, receptor gene amplification, mutation, and/or polymorphism may play a role in tumor progression, but in general, other pathways are more important.⁴⁹

Angiogenesis

Angiogenesis is the growth of new blood vessels from existing blood vessels. It is required for many physiological processes such as wound healing, and it involves several pathways including the secretion of angiogenic substances, activation of endothelial cells, degradation of capillary membranes, and endothelial cell migration. Oxygen and nutrient diffusion alone are insufficient to support tumor growth greater than 2-3 mm³ without the development of new blood vessels. Tumors that

have the capacity to readily induce angiogenesis tend to be of higher grade and have a worse prognosis. Evaluation of angiogenesis in the prostate by looking at microvessel density has shown an association between an increasing number and level of disorganization of blood vessels as the progression from normal prostate to benign prostatic hyperplasia to malignancy occurs. Microvessel density varies from area to area within a tumor, thus potentially limiting the utility of vessel counts in biopsies to consistently predict prognosis in either organ-confined or more advanced prostatic cancer.⁵⁰ However, other studies have suggested that the overall density of microvessels is relatively uniform throughout the tumor.^{51,52}

The most studied angiogenic factors in prostate cancer are basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF).⁵³ Conflicting results exist for both of these growth factors, with higher urinary levels being reported in benign prostatic hyperplasia compared to prostate cancer, and higher serum levels in patients with prostatic tumors compared to patients with benign prostatic conditions. Significant tissue levels of VEGF are present in prostate cancer and in PIN lesions, the expression being highest in association with neuroendocrine cells and correlated with an altered pattern of vascularization.⁵⁴ The VEGF expression is down-regulated by hormonal manipulation, except in the population of neuroendocrine cells. All this indicates that VEGF may contribute to the

establishment, progression, and regression of prostate neoplasia.⁵³ Clearly, tumor-induced angiogenesis is an essential step in the progression of malignant neoplasms and the development of metastases.⁵⁵ The therapeutic potential of angiogenesis inhibitors is under active investigation.⁵⁴

Apoptosis

Much attention is currently being focused on molecular control of programmed cell death (apoptosis) and methods to alter the balance toward enhancing tumor cell death in cancer therapy. Rates of apoptosis become altered during initiation, progression, and metastasis of human prostate cancers. Androgen deprivation first produces a reduction in tumor bulk, with androgen-dependent cells undergoing apoptotic cell death. However, there is continued proliferation of androgen-independent cells resulting in a cancer that is no longer responsive to antiandrogen therapy.³⁵

The oncogenic protein Bcl-2 and its family of related gene products occupy a central role in the regulation of apoptosis.^{56,58} Bcl-2, Bcl-X_L, Bcl-w, and Mcl-1 appear to be death antagonists, while Bax, Bak, Bcl-X_S, Bad, and Bik are death agonists.^{1,22,48,56,58} We have shown by immunohistochemical analysis of normal prostate that Bcl-2 expression is found primarily in the androgen-independent basal cells adjacent to the basement membrane and not in the differentiated luminal secretory cells. The luminal cells are androgen dependent and

undergo apoptosis after withdrawal of testosterone. An elevated level of Bcl-2 is a poor prognostic marker in early prostate cancer⁵⁷ and is associated with poor histological differentiation, a high proliferation rate, and an attenuated lymphocytic infiltrate.^{30,36,37} Strong expression of Bcl-2 protein is frequently associated with metastatic disease and is often seen in advanced hormone-refractory tumors.^{30,57}

Telomerase

The life span of a cell is determined by two controls: (1) regulations of the cell cycle and (2) the so-called mitotic clock, which is determined by the length of the telomere. The progressive shortening of the telomere at the time of each cell division eventually results in exposure of the tips of the chromosome to a variety of degrading enzymes, leading ultimately to activation of apoptotic pathways. Telomerase is a ribonucleoprotein enzyme that stabilizes telomere length by adding hexameric (TTAGGG) repeats to the telomeric ends of the chromosomes, thus compensating for the continued erosion of telomerase that occurs in its absence. The enzyme is expressed in embryonic cells and adult male germline cells, but it is undetectable in normal somatic cells except for proliferative cells of renewal tissues. In contrast to normal cells, tumor cells show no net loss of average telomere length with cell division, suggesting that telomere stability may be required for cells to escape from replicative senescence and proliferate indefinitely. The incidence of telomerase activation in prostate

cancer is between 47%-92% in various studies, although most show telomerase activity in approximately 90% of prostate adenocarcinoma cases.⁵⁹ The results of various techniques for sample collection (surgery or needle biopsy) provide similar data in terms of the incidence of telomerase positive samples, whereas major heterogeneity was reported in tissue samples collected in tissues adjacent to cancers, isolated prostatic intraepithelial neoplasia, or benign prostatic hyperplasia samples. Further follow-up of patients in whom increased telomerase activity is found in prostatic tissues but who demonstrate no evidence of cancer would clarify whether this marker may predict the development of prostate cancer. Because of the significant stromal infiltrate in prostate cancers, estimates of telomerase activity may be low as the surrounding normal cells will have little or no activity.⁵⁹

Cell Adhesion Molecules

Many studies have shown altered expression, particularly loss, of adhesion molecules during progression from normal prostatic epithelium to invasive prostate cancer. CD44 is a ubiquitous family of cell-surface glycoproteins that exist in multiple variant isoforms and serve as extracellular receptors for hyaluronic acid, collagen, and fibronectin, thus mediating cell-cell and cell-matrix interaction.²⁸ CD44v is variably expressed by epithelial cells and has attracted considerable attention because it is involved in tumor progression and appears to be specifically associated with the

development of the metastatic phenotype. In most studies examining tumor cell aggressiveness, CD44s or CD44v expression is increased. Transfection of the CD44v6 gene and its expression in cell lines is able to confer metastatic potential.^{28,29,60} Normal human prostatic epithelium expresses immunohistochemically detectable levels of CD44H, and neoplastic prostatic epithelium has been shown to contain CD44s protein as well as CD44v6 mRNA.^{28,60} In contrast to nonmalignant epithelium, most intense staining of prostatic neoplasia occurs in luminal epithelium. Abnormal expression of CD44v6 occurs in more than 80% of locally invasive and metastasizing human prostate cancers. However, of particular diagnostic importance is the finding of high levels of CD44v6 protein expression in significant numbers of morphologically benign prostatic biopsies containing atypical basal cell hyperplasia or of both low- and high-grade PIN.²² Thus CD44v has the potential to act as a marker for the progression of PIN to cancer.

Signal Transducers and Activators of Transcription (STAT)

Constitutive activation of certain members of the signal transducers and activators of the transcription (STAT) family, particularly the Stat3 protein, has been associated with malignant cellular transformation in various leukemias, multiple myeloma, and solid tumors such as breast cancer, head and neck cancer, and prostate cancer.⁶¹⁻⁶⁵ Growth factor receptors with tyrosine kinase activity, cytokine receptors (such as IL-6), and nonreceptor

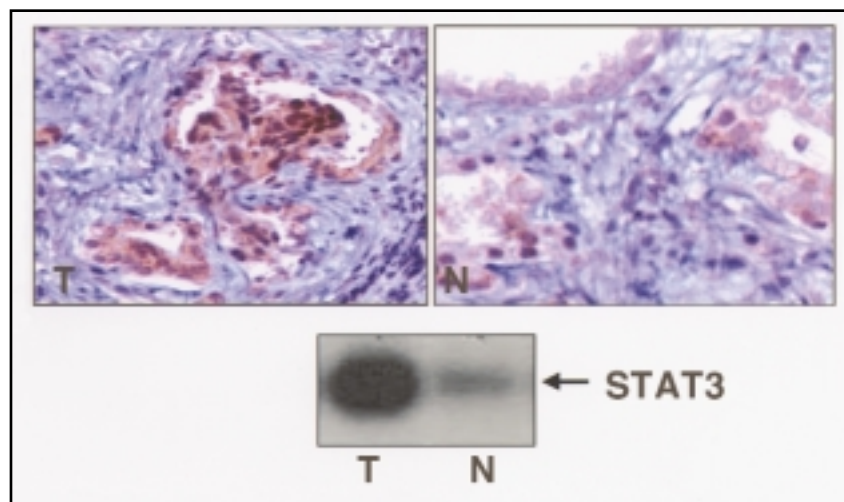


Fig 2. — Immunohistochemical staining of Stat3 in prostate cancer. A representative sample of matched prostate adenocarcinoma (T) and prostate normal tissue (N) at high magnification (600 \times). Detection of phospho-Stat3 by immunohistochemistry (red stain) correlates well with increased Stat3 DNA-binding activity as detected by the molecular assay electrophoretic mobility shift assay (below).

tyrosine kinases have critical roles in the activation of Stat3 signaling. We have previously demonstrated that constitutive activation of Stat3 occurs with high frequency in human prostate cancer cell lines and primary tumor specimens. Furthermore, we have shown that selective tyrosine kinase inhibitors block the growth of prostate cancer cell lines in culture.⁶⁵ Blocking Stat3 signaling directly with dominant-negative Stat3 protein or indirectly with tyrosine kinase inhibitors blocks growth of human multiple myeloma, breast carcinoma, and head and neck cancer cells.^{61,62} Thus, mounting evidence indicates that constitutive activation of Stat3 by tyrosine kinase signaling pathways contributes to malignant progression. We demonstrated in recent studies that blocking Stat3 signaling in human prostate carcinoma cells with tyrosine kinase inhibitors or transfection with antisense Stat3 oligonu-

cleotides induces apoptosis. Moreover, we showed that increased Stat3 DNA-binding activity by biochemical assay in primary prostate tumors, compared to adjacent non-tumor tissues, correlated well with immunohistochemical detection of activated phospho-Stat3 in tumor cells (Fig 2).⁶⁵ Collectively, these findings provide evidence that constitutive activation of Stat3 occurs frequently in human prostate carcinoma and is important for growth and survival of prostate cancer cells. Current studies are directed at evaluating Stat3 as a potential diagnostic/ prognostic factor in prostate cancer as well as a novel molecular target for new therapeutic approaches.

Conclusions

The molecular mechanisms of prostate development and progression are complicated and likely

involve the interaction of tumor suppressor genes, oncogenes, growth factors, adhesion molecules, signal transduction proteins, and angiogenesis. Moreover, the biological potential of prostate cancer is highly variable and cannot be satisfactorily predicted by histopathologic criteria alone. Further research is needed to better understand these mechanisms and pathways and thus prevent or potentially alter the diagnosis and treatment of patients with prostate cancer. The areas of cellular signaling, cell surface receptor activity, and the interactions of prostate cancer cells with soluble and matrix-associated molecules deserve significant attention.

References

- Montironi R, Schulman CC. Precursors of prostatic cancer: progression, regression and chemoprevention. *Eur Urol*. 1996;30:133-137.
- Bostwick DG, Montironi R, Sesterhenn IA. Diagnosis of prostatic intraepithelial neoplasia: Prostate Working Group/consensus report. *Scan J Urol Nephrol Suppl*. 2000;205:3-10.
- Bostwick DG, Grignon DJ, Hammond ME, et al. Prognostic factors in prostate cancer. College of American Pathologists Consensus Statements 1999. *Arch Pathol Lab Med*. 2000;124:995-1000.
- Bostwick DG, Foster CS, Algaba F, et al. Prostate tissue factors. In: Murphy G, Denis L, Khoury S, et al, eds. *Prostate Cancer: Second International Consultation on Prostate Cancer*. Plymouth: Plymbridge Distributors; 2000:162-201.
- Partin AW, Murphy GP, Brawer MK. Report on Prostate Cancer Tumor Marker Workshop 1999. *Cancer*. 2000;88:955-963.
- Park S, Shinohara K, Grossfeld GD, et al. Prostate cancer detection in men with prior high grade prostatic intraepithelial neoplasia or atypical prostate biopsy. *J Urol*. 2001;165:1409-1414.
- Sakr WA, Partin AW. Histological markers of risk and the role of high-grade prostatic intraepithelial neoplasia. *Urology*. 2001;57:115-120.
- Sakr WA, Ward C, Grignon DJ, et al. Epidemiology and molecular biology of early prostatic neoplasia. *Mol Urol*. 2000;4:109-113;discussion 115.
- Bostwick DG, Amin MB, Dundone P, et al. Architectural patterns of high-grade prostatic intraepithelial neoplasia. *Hum Pathol*. 1993;24:298-310.
- Bostwick DG, Foster CS. Predictive factors in prostate cancer: current concepts from the 1999 College of American Pathologists Conference on Solid Tumor Prognostic Factors and the 1999 World Health Organization Second International Consultation on Prostate Cancer. *Semin Urol Oncol*. 1999;17:222-272.
- Carter BS, Bova GS, Beaty TH, et al. Hereditary prostate cancer: epidemiologic and clinical features. *J Urol*. 1993;150:797-802.
- Karayi MK, Neal DE, Markham AF. Current status of linkage studies in hereditary prostate cancer. *BJU Int*. 2000;86:659-669.
- McNeal JE, Gleason DE. Gleason's classification of prostatic adenocarcinomas. *Ann Pathol*. 1991;11:163-168.
- Gleason DE. Atypical hyperplasia, benign hyperplasia, and well-differentiated adenocarcinoma of the prostate. *Am J Surg Pathol*. 1985;9:53.
- Gleason DE. Histologic grading of prostate cancer: a perspective. *Hum Pathol*. 1992;23:273-279.
- Gleason DE. Undergrading of prostate cancer biopsies: a paradox inherent in all biologic bivariate distributions. *Urology*. 1996;47:289-291.
- Aihara M, Wheeler TM, Ohori M, et al. Heterogeneity of prostate cancer in radical prostatectomy specimens. *Urology*. 1994;43:60-66;discussion 66-67.
- Bastacky SI, Walsh PC, Epstein JI. Relationship between perineural tumor invasion on needle biopsy and radical prostatectomy capsular penetration in clinical stage B adenocarcinoma of the prostate. *Am J Surg Pathol*. 1993;17:336-341.
- Egan AJ, Bostwick DG. Prediction of extraprostatic extension of prostate cancer based on needle biopsy findings: perineural invasion lacks significance on multivariate analysis. *Am J Surg Pathol*. 1997;21:1496-1500.
- Ulbricht TM. Testis risk and prognostic factors. The pathologist's perspective. *Urol Clin North Am*. 1999;26:611-626.
- Bigler SA, Deering RE, Brawer MK. Comparison of microscopic vascularity in benign and malignant prostate tissue. *Hum Pathol*. 1993;24:220-226.
- Lijovic M, Fabiani ME, Bader J, et al. Prostate cancer: are new prognostic markers on the horizon? *Prostate*. 2000;3:62-65.
- Mora LB, Moscinski LC, Diaz JJ, et al. Stage B Prostate cancer: correlation of DNA ploidy analysis with histological and clinical parameters. *Cancer Control*. 1999;6:587-591.
- Diaz JJ, Mora LB, Austin PE, et al. Predictability of PSA failure in prostate cancer by computerized cytometric assessment of tumoral cell proliferation. *Urology*. 1999;53:931-938.
- Korkolopoulou P, Christodoulou P, Kapralos P, et al. The role of p53, MDM2 and c-erb B-2 oncoproteins, epidermal growth factor receptor and proliferation markers in the prognosis of urinary bladder cancer. *Pathol Res Pract*. 1997;193:767-775.
- Sasor A, Wagrowska-Danilewicz M, Danilewicz M. Ki-67 antigen and P53 protein expression in benign and malignant prostatic lesions. Immunohistochemical quantitative study. *Pol J Pathol JC*. 2000;51:31-36.
- Xie W, Wong YC, Tsao SW. Correlation of increased apoptosis and proliferation with development of prostatic intraepithelial neoplasia (PIN) in ventral prostate of the Noble rat. *Prostate*. 2000;15:44:31-39.
- Aaltomaa S, Lipponen P, Viitanen J, et al. Prognostic value of CD44 standard, variant isoforms 3 and 6 and -catenin expression in local prostate cancer treated by radical prostatectomy. *Eur Urol*. 2000;38:555-562.
- Aaltomaa S, Lipponen P, Ala-Opas M, et al. Expression and prognostic value of CD44 standard and variant v3 and v6 isoforms in prostate cancer. *Eur Urol*. 2001;39:138-144.
- DiPaola RS, Kumar P, Hait WN, et al. State-of-the-art prostate cancer treatment and research. A report from the Cancer Institute of New Jersey. *N J Med*. 2001;98:23-33.
- Gao M, Ossowski L, Ferrari AC. Activation of Rb and decline in androgen receptor protein precede retinoic acid-induced apoptosis in androgen-dependent LNCaP cells and their androgen-independent derivative. *J Cell Physiol*. 1999;179:336-346.
- Gao X, Honn KV. Recessive oncogenes: current status. *Pathol Oncol Res*. 1995;1:7-22.
- Singh A, Jones RF, Friedman H, et al. Expression of p53 and pRb in bladder and prostate cancers of patients having both cancers. *Anticancer Res*. 1999;19:5415-5417.
- Tamboli P, Amin MB, Xu HJ, et al. Immunohistochemical expression of retino-

- blastoma and p53 tumor suppressor genes in prostatic intraepithelial neoplasia: comparison with prostatic adenocarcinoma and benign prostate. *Mod Pathol.* 1998;11:247-252.
35. Kwabi-Addo B, Giri D, Schmidt K, et al. Haploinsufficiency of the Pten tumor suppressor gene promotes prostate cancer progression. *Proc Natl Acad Sci USA.* 2001;98:11563-11568.
36. Stackhouse GB, Sesterhenn IA, Bauer JJ, et al. p53 and bcl-2 immunohistochemistry in pretreatment prostate needle biopsies to predict recurrence of prostate cancer after radical prostatectomy. *J Urol.* 1999;162:2046-2047.
37. Takayama H, Shin M, Nonomura N, et al. p53 mutations in prostatic intraepithelial neoplasia and concurrent carcinoma: analysis of laser capture microdissected specimens from non-transition and transition zones. *Jpn J Cancer Res.* 2000;91:941-947.
38. Yu D, Liu F, Liang Z. Detection and clinical pathological significance of the expression of P21, P185, p53 proteins and mutation of ras, p53 genes in transitional cell carcinoma of the bladder [Chinese]. *Zhonghua Bing Li Xue Za Zhi.* 1996;25:202-205.
39. Kubota Y, Fujinami K, Uemura H, et al. Retinoblastoma gene mutations in primary human prostate cancer. *Prostate.* 1995;27:314-320.
40. Hu WL, Li YQ, He HX, et al. KAI1/CD82 gene expression in benign prostatic hyperplasia and late-stage prostate cancer in Chinese. *Asian J Androl.* 2000;2:221-224.
41. Li P, Nicosia SV, Bai W. Antagonism between PTEN/MMAC1/TEP-1 and androgen receptor in growth and apoptosis of prostatic cancer cells. *J Biol Chem.* 2001;276:20444-20450.
42. Burton JL, Oakley N, Anderson JB. Recent advances in the histopathology and molecular biology of prostate cancer. *BJU Int.* 2000;85:87-94.
43. Guo Y, Sklar GN, Borkowski A, et al. Loss of the cyclin-dependent kinase inhibitor p27(Kip1) protein in human prostate cancer correlates with tumor grade. *Clin Cancer Res.* 1997;3(12 pt 1):2269-2274.
44. Fero ML, Rivkin M, Tasch M, et al. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. *Cell.* 1996;85:733-744.
45. Matrisian LM, Cunha GR, Mohla S. Epithelial-stromal interactions and tumor progression: meeting summary and future directions. *Cancer Res.* 2001;61:3844-3846.
46. Dunsmuir WD, Gillett CE, Meyer LC, et al. Molecular markers for predicting prostate cancer stage and survival. *BJU Int.* 2000;86:869-878.
47. Thompson TC, Truong LD, Timme TL, et al. Transforming growth factor beta 1 as a biomarker for prostate cancer. *J Cell Biochem Suppl.* 1992;16H:54-61.
48. Linja MJ, Savinainen KJ, Saramaki OR, et al. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. *Cancer Res.* 2001;61:3550-3555.
49. Matsuda T, Junicho A, Yamamoto T, et al. Cross-talk between signal transducer and activator of transcription 3 and androgen receptor signaling in prostate carcinoma cells. *Biochem Res Commun.* 2001;283:179-187.
50. Jones A, Fujiyama C, Turner K, et al. Angiogenesis and lymphangiogenesis in stage 1 germ cell tumours of the testis. *BJU Int.* 2000;86:80-86.
51. Brawer MK. Quantitative microvessel density. A staging and prognostic marker for human prostatic carcinoma. *Cancer.* 1996;78:345-349.
52. Kay PA, Robb RA, Bostwick DG. Prostate cancer microvessels: a novel method for three-dimensional reconstruction and analysis. *Prostate.* 1998;37:270-277.
53. Sugamoto T, Tanji N, Sato K, et al. The expression of basic fibroblast growth factor and vascular endothelial growth factor in prostatic adenocarcinoma: correlation with neovascularization. *Anticancer Res.* 2001;21:77-88.
54. Mazzucchelli R, Montironi R, Santinelli A, et al. Vascular endothelial growth factor expression and capillary architecture in high-grade PIN and prostate cancer in untreated and androgen-ablated patients. *Prostate.* 2000;45:72-79.
55. Ali IU, Senger DR, Smith LE. Angiogenesis as a potential biomarker in prostate cancer chemoprevention trials. *Urology.* 2001;57(4 Suppl 1):143-147.
56. Kucuk O, Sarkar FH, Sakr W, et al. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. *Cancer Epidemiol Biomarkers Prev.* 2001;10:861-868.
57. Diaz JJ, Pow-Sang JM, Mora LB, et al. Cytometric analysis of Fas and Bcl-2 expression in normal prostatic epithelium and prostate cancer. *Urol Oncol.* 2000;5:149-154.
58. Baltaci S, Orhan D, Ozer G, et al. Bcl-2 proto-oncogene expression in low- and high-grade prostatic intraepithelial neoplasia. *BJU Int.* 2000;85:155-159.
59. Orlando C, Gelmini S, Selli C, et al. Telomerase in urological malignancy. *J Urol.* 2001;166:666-673.
60. Brewster SF, Oxley JD, Trivella M, et al. Preoperative p53, bcl-2, CD44 and E-cadherin immunohistochemistry as predictors of biochemical relapse after radical prostatectomy. *J Urol.* 1999;161:1238-1243.
61. Garcia R, Bowman TL, Niu G, et al. Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. *Oncogene.* 2001;20:2499-2513.
62. Catlett-Falcone R, Landowski TH, Oshiro MM, et al. Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. *Immunity.* 1999;10:105-115.
63. Fernandes A, Hamburger AW, Gerwin BI. ErbB-2 kinase is required for constitutive stat 3 activation in malignant human lung epithelial cells. *Int J Cancer.* 1999;83:564-570.
64. Lou W, Ni Z, Dyer K, et al. Interleukin-6 induces prostate cancer cell growth accompanied by activation of stat3 signaling pathway. *Prostate.* 2000;42:239-242.
65. Mora LB, Buettner R, Seigne J, et al. Constitutive activation of Stat3 in human prostate tumors and cell lines: Inhibitors of Stat3 signaling block growth of prostate cancer cells. *Cancer Res.* 2001. In press.