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Prostate Cancer
Old Problems and New Approaches*

Part II. Diagnostic and Prognostic Markers, Pathology and Biological Aspects

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Diagnostic and prognostic markers for prostatic cancer (PCa) include conventional protein markers (e.g., PAP, PSA, PSMA, PIP, OA-519, Ki-67, PCNA, TF, collagenase, and TIMP 1), angiogenesis indicator (e.g., factor VIII), neuroendocrine differentiation status, adhesion molecules (E-cadherin, integrin), bone matrix degrading products (e.g., ICPT), as well as molecular markers (e.g., PSA, PSMA, p53, 12-LOX, and MSI). Currently, only PSA is used clinically for early diagnosis and monitoring of PCa. The histological differential diagnosis of prostatic adenocarcinoma includes normal tissues such as Cowper's gland, paraganglion tissue and seminal vesicle or ejaculatory duct as well as pathological conditions such as atypical adenomatous hyperplasia, atrophy, basal cell hyperplasia and sclerosing adenosis. A common PCa is characterized by a remarkable heterogeneity in terms of its differentiation, microscopic growth patterns and biological aggressiveness. Most PCa are multifocal with significant variations in tumor grade between anatomically separated tumor foci. The Gleason grading system which recognizes five major grades defined by patterns of neoplastic growth has gained almost uniform acceptance. In predicting the biologic behavior of PCa clinical and pathological stages are used as the major prognostic indicators. Among the cell proliferation and death regulators androgens are critical survival factors for normal prostate epithelial cells as well as for the androgen-dependent human prostatic cancer cells. The androgen ablation has been shown to increase the apoptotic index in prostatic cancer patients and castration also promotes apoptotic death of human prostate carcinoma grown in mice. The progression of PCa, similarly to other malignancies, is a multistep process, accompanied by genetic and epigenetic changes, involving phenomenons as adhesion, invasion and angiogenesis (without prostate specific features). (Pathology Oncology Research Vol 2, No3, 191–211, 1996)

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4. Markers

4. 1. Diagnostic Markers

4.1.1. Prostatic Acid Phosphatase (PAP)

Human PAP is a non-specific phosphomonoesterase, synthesized and secreted into seminal plasma under androgen control.[1] This glycoprotein has a molecular weight of 100 kDa consisting of two identical subunits of approximately 50 kDa. The full length PAP cDNA encodes a predicted protein of 386 residues with a 32 amino acid signal peptide.[2] PAP is only a small portion (10-25%) of the total acid phosphatase in the serum of the normal adult man. To differentiate PAP from acid phosphatases from extraprostatic cell types, several methods were introduced, which include using substrates with higher specificity (sodium tymlphthalein, 1-naphthylphosphate), specific inhibitors [L(+)-tartarte] and the more reliable radioimmunoassays for PAP.[1] Despite the aforementioned alterations in PAP assays and substrates, difficulties remained with this marker. These disadvan-

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tages includes cross reactivity with serum acid phosphatases of non-prostatic origins, diurnal variations in serum PAP levels, transient elevations after prostatic examination and manipulation (e.g., digital rectal examination), and the instability of the enzyme which requires special specimen handling.[3] In addition, prostatic acid phosphatase is not prostate-specific. It is also synthesized by granulocytes, spleen, and pancreas.[1] These difficulties lead to high false negative and false positive rates, despite advancements in detection technology. For PCa detection, the sensitivity and specificity of the serum PAP determination has a reported range of 31-61% and 78-99%, respectively.[5] However, when the receiver operator characteristic (ROC) curves for PAP and a newer prostate marker, prostate specific antigen (PSA) in the diagnosis of PCa were compared, it was found that PSA is a superior test because it has higher sensitivity and specificity for all cutoff values.[6] Moreover, several studies have demonstrated that PAP is normal in 57-73% of patients with localized PCa. This limited ability to detect early PCa, together with that fact that many patients with elevated levels of PAP already have advanced disease, limits the applicability of screening and diagnosis of PCa using this tumor marker. Currently, PSA has already replaced PAP as the most useful prostate diagnostic marker in practice.

4.1.2. Prostate-Specific Antigen (PSA)

PSA is a single chain glycoprotein with 240 amino acids, having a molecular weight of approximately 34 kDa.[8] It is a member of the serine protease family and has trypsin-like and chymotrypsin-like activity.[9] PSA is synthesized by the epithelial cells of the prostatic acini and ducts and secreted as a normal constituent of seminal fluid. It has been shown to be present in the serum of men with both benign and malignant prostatic diseases. Since its discovery and the development of detection methods, PSA has been widely used as a tumor marker for screening, diagnosis and monitoring of PCa.[10]

It was originally thought that PSA is exclusively produced by the prostate. However, accumulating evidence suggest that other body tissues and cancer of non-prostatic origins may also synthesize PSA. PSA has been demonstrated in urine,[11] periurethral glands,[12] perianal gland,[13] saliva,[14] amniotic fluid,[15] milk of lactating women,[14] and serum of normal women.[15] Recently, PSA protein and/or mRNA has been demonstrated in tumors of the skin,[16] salivary glands,[17] kidney,[18] ovary,[19,20] lung,[19] and breast,[16,20] as well as myeloid leukemia[19] and non-Hodgkin lymphoma.[21] Therefore, PSA is no longer considered prostate-specific.

The quantitation of PSA levels in serum is achieved by using commercially available immunoassay kits that utilized monoclonal antibodies to identify epitopes on the PSA molecule.[22] The most common assays used in US are the Tandem-R PSA and Tandem-E PSA assays (Hybritech Inc., San Diego, CA) and the IMx PSA assay (Abbott Laboratories, Abbott Park, IL). For all assays mentioned, the age-specific reference ranges (cutoffs) are applicable.[3,23]

The role of PSA in the screening and early diagnosis of PCa has been well established.[3,24] Early experience has demonstrated that PSA testing detects pathologically organ-confined PCa in a large proportion of cases and that radical prostatectomy nearly always completely eradicates pathologically localized disease.[24] The overall detection rate of PCa in screening populations has a reported range of 2.2-5.7%.[24] As predicted, the combination of serum PSA and digital rectal examination was found to be superior to either modality alone in the diagnosis of PCa. However, as other tumor marker, PSA has some common shortcomings. First, PSA is not disease-specific. Elevated PSA levels could be found in a small proportion of normal males and approximately 25-86% of patients with benign prostatic hyperplasia (BPH) and prostatitis.[25] Recent data suggest that PSA is not prostate-specific (see above for details). Despite the fact that PSA is by no means a perfect marker, when the ROC curves for PSA, PAP, and a number of other tumor markers and diagnostic procedures were constructed and compared, PSA has been proven to have higher sensitivity and specificity than any other marker at any cutoffs.[6] Currently, PSA testing has replaced PAP as a tumor marker for screening and early diagnosis of PCa.

Recently, PSA density,[26] PSA slope,[27] PSA doubling time,[28] and free PSA[29] have been measured and calculated trying to improve the sensitivity and specificity of PSA testing. They, to a certain extent, provide an improvement.[3]

4.1.3. Prostate Specific Membrane Antigen (PSMA)

PSMA, a relatively new diagnostic marker, is a transmembrane glycoprotein with a molecular weight of approximately 100 kDa. Antibody studies have demonstrated that PSMA expression is highly restricted to prostate tissues and it is highly expressed in most of the normal intraepithelial neoplasia, and the primary and metastatic PCa specimens.[30,31] Recently, excellent work characterizing PMSA and its role as a PCa marker have been carried out.[31,32,33,34,35] The open reading frame (ORF) of the PSMA cDNA is 2.25 kb in length encoding for a protein of 750 amino acids with a predicted protein molecular weight of 84 kDa, excluding carbohydrate.[34] The fact that PSMA is an integral membrane protein suggests that this molecule may serve as a target for diagnostic imaging and therapeutic targeting modalities.[36]

The restriction of PSMA expression to the prostate is not absolute. Israeli et al demonstrated that the PSMA gene is highly expressed in prostate tissue,[34] however, it is also expressed at a detectable level in brain, salivary gland

and small intestine tissue. They further demonstrated that a patient with renal cell carcinoma tested positive for circulating PSMA producing cells.[32]

4.1.4. Prostatic Inhibin Peptide (PIP)

PIP is an abundant 94 amino acid protein secreted by prostatic epithelial cells, it is detectable in serum, urine as well as cultured PCa cells.[33] In contrast to PSA and PAP, the expression of PIP has been shown to be independent of androgen. A growing body of evidence indicate that detection of the PIP levels in tissue specimens, serum, and urine may help distinguishing BPH from PCa.[34-38] PIP has been demonstrated to be able to suppress DNA synthesis and growth of the prostate cells in vitro and in vivo.[39,40] PIP also can induce apoptosis of PCa cells in animal model system.[40] All above mentioned evidence suggest that PIP may have therapeutic potential. However, the prostatic specificity of PIP has been recently questioned. A recently identified alternatively spliced form of PIP, PSP57, was detectable not only in the prostate but also in the kidney and bladder.[41]

4.2. Prognostic Markers

4.2.1. PAP

Foti et al first demonstrated a correlation between serum PAP elevation and extent of PCa.[42] Elevated serum PAP levels also have been found to be associated with PCa showing poorly differentiated histological patterns and advanced stage, but not the metastatic state and the tumor volume.[43] A correlation between PAP distribution and differentiation status also was observed.[44] However, these results await to be confirmed. The value of PAP as a means to identify metastasis remains a point of controversy. Sixty to eighty-five percent and 22-28% of patients with pelvic lymph node involvement showed elevated and normal PAP levels, respectively, depending on different methods of assays. Investigation conducted by Oesterling et al demonstrated that all patients with an elevated serum PAP level had either extracapsular disease or lymph node metastasis.[45] However, in another series, no statistically significant correlation was observed between PAP and the presence of positive regional lymph nodes.[46] Since PAP testing has been found to add very little unique information to the diagnosis and prognosis of PCa in addition to other markers (e.g., PSA) and conventional procedures (e.g., pathologic staging, bone scan, and digital rectal examination),[47] together with drawbacks of the PAP testing per se (see above), many investigators believe serum PAP testing is not routinely necessary and many institutions no longer recommend this test in the staging of PCa.

4.2.2. PSA

A direct relationship has been demonstrated between serum PSA and tumor burden.[48] The fact that serum PSA levels overlap between stages has resulted in PSA's inability to predict exact pathological stage. However, a number of studies have shown that local clinical stage and tumor grade significantly enhance the predictive power of PSA to determine pathological stage.[49] A role for PSA as a predictor of outcome in PCa has been suggested by several investigators.[50] As a general rule, as PCa progresses it produces more PSA. However, some undifferentiated PCa cells become androgen-unresponsive and unable to produce PSA.[51] It was suggested that undifferentiated cells may acquire the ability to express a PSA-suppressing activity. Such a factor may be secreted into the stroma and influence the PSA expression of other cells.[37] This may partially explain that the lack of increase in serum PSA levels observed in a proportion of patients with hormone-resistant PCa.

Recently, RT-PCR assay has been developed to detect circulating PSA mRNA producing cancer cells for molecular staging.[32,51,52] Since blood cell PSA RT-PCR assay is detecting mRNA produced by the circulating malignant cells, a positive RT-PCR assay implicates hematogenous micrometastasis of PCa.[19,51,52] RT-PCR assay for detecting PSA mRNA producing PCa cells was first described by Gomella et al.[52] It was found that 50% (4/8) of D1-3 patients and none of the D0 PCa patients, BPH patients or normal female controls had positive PSA/RT-PCR results.[52] Such a PSA/RT-PCR assay has also been used to detect PSA mRNA producing cells in lymph nodes.[53] A more sensitive enhanced PSA/RT-PCR assay employing digoxigenin-modified nucleotides has been developed to detect one PSA mRNA producing cell in 100,000 non-PSA producing lymphocytes.[51] They found that no specimens from control females and males without cancer was positive for enhanced PSA/RT-PCR assay, however, 77.8% (14) of 18 metastatic PCa patients (as judged by positive bone scan) were positive.[51] In addition, 38.5% (25) of 65 patients with T1-2b (clinically localized) disease were found to be positive for this assay and the PSA/RT-PCR positivity in surgical-candidate patients correlated significantly with capsular penetration and surgical margin positivity.[51] However, due to the fact that the PSA gene is not exclusively expressed in prostate cells, interpretation of a positive result should be very cautious.[19] Moreover, the clinical significance of the presence of a single tumor cell in the circulation is unknown.

4.2.3. PSMA

Data accumulated to date does not conclusively define a role for PSMA as a prognostic marker for PCa. In a series where 165 primary PCa, 79 lymph node metastases, 7

bone metastases. 27 BPH, 21 prostatic intraepithelial neoplasia (PIN), and 12 normal prostate tissue specimens were examined, expression of PSMA was found to correlate positively with pathologic grade, but not with clinical stage. The PSMA expression level is high in poorly differentiated and metastatic PCa, however, expression level in primary PCa does not correlate with nodal status, extracapsular penetration, or seminal vesicle positivity.[31] An alternatively spliced variant (PSMA') of PSMA mRNA has been recently identified and the ratio of expression of PSMA/PSMA' has been found to be a potential index for measuring PCa progression.[35] Nevertheless, a large series of PCa patients with defined pathological stages will be needed to further evaluate the potential of PSMA/PSMA' ratio as a useful prognostic indication.

A blood RT-PCR assay of PSMA, like that of PSA, has been recently developed for molecular staging of PCa.[32,33] Cama et al[32] used RT-PCR assay to detect circulating PSMA and/or PSA mRNA producing cells. They found that the sensitivity of the PSA test is higher than that of the PSMA test. Sixteen (80%) and 10 (50%) of 20 metastatic PCa patients had positive PSA and PSMA assay in blood samples, respectively. Twenty-seven and 19 of 80 patients with clinically localized PCa had positive PSA and PSMA test, respectively. The positivity of the PSA RT-PCR assay, but not that of the PSMA RT-PCR assay, correlates with pathological stage of PCa. However, when a nested RT-PCR assay was developed to detect PSMA and/or PSA producing cells, the sensitivity of the PSMA assay was found to be much higher that of the PSA assay.[33] In this study, the authors detected micrometastases in 2 BPH patients by the PSMA assay, suggesting the false positive rate for the PSMA test may be higher than that of the PSA test. Nevertheless, PSMA RT-PCR assay requires further study.

4.2.4. 12-Lipoxygenase (12-LOX)

12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE], a metabolite of arachidonic acid by 12-LOX, has been shown to play a pivotal role in invasion and metastasis. Recently, 12(S)-HETE has been shown to enhance PCa cell invasion[54] and the ability of prostate tumor cells to generate endogenous 12(S)-HETE has been correlated positively with their metastatic potential.[55] All these results suggest that the enzyme responsible for 12(S)-HETE production (i.e., 12-LOX protein or mRNA levels) may be prognostic markers for the aggressiveness of this cancer. The 12-LOX mRNA expression levels in 122 matched prostate normal and cancer tissues were measured by quantitative RT-PCR and in situ hybridization (ISH). ISH demonstrated weak expression of 12-LOX mRNA in basal cells of normal secretory glands. 12-LOX mRNA levels were elevated in PCa cells and the expression correlated with the differentiation status and invasiveness. In RT-PCR, overall, 46 (38%) of 122 evaluable patients showed elevated level of 12-LOX mRNA in PCa tissues compared to the matched normal tissues. A statistically significantly greater number of cases were found to have an elevated level of 12-LOX among T3, high grade, and surgical margin positive than T2, intermediate grade, and surgical margin negative prostatic adenocarcinomas. This data suggest that 12-LOX may serve as a correlative marker for a more aggressive phenotype of human PCa and hence for poor prognosis. This enzyme also may be a novel target for the development of anti-invasive and anti-metastatic agents.[56]

4.2.5. Tumor Suppressor Gene – p53

Wild-type p53 has been shown to be a suppressor of cell growth and transformation, causing a G_1 block in cell cycle progression and in certain cell types precipitating apoptosis. Mutations in the p53 gene have been demonstrated to be the most common genetic alterations in human cancers. Functional inactivation may result from genetic aberrations within the p53 gene, most frequently missense mutations, or inactivation by interacting with viral and cellular oncoproteins. Loss of wild-type p53 function leads to deregulation of the cell cycle checkpoint and DNA replication, defective or inefficient DNA repair, selective growth advantage and, as a result, tumor formation and progression.[57]

The role of p53 in human PCa is still unclear and remains controversial. While a number of groups demonstrated a high p53 mutation and/or protein accumulation rate in PCa,[58,59] others reported rare mutations.[60,61,62] Such frequency differences of the p53 mutation in PCa among various groups could partially be due to the geographic or demographic factors as well as methods used for detecting p53 abnormalities (see above).[63]

The value of using p53 mutation as a prognostic marker for PCa is still in debate. The correlation between p53 abnormalities and PCa progression have been reported in a number of studies.[60,61,64,65,66] Bookstein et al[64] reported that 23% of stage III or IV tumors and 4% of stage 0-II tumors had abnormal nuclear p53 accumulation and that 20-25% of advanced cancers, but none of early PCa had mutations of the p53 gene. In another series of 92 patients, all tumors with p53 protein accumulation and/or mutations were metastatic (stage D), poorly differentiated, and androgen independent.[66] However, two studies suggested that p53 abnormalities may be an early event in PCa progression.[58] Such controversy could only be resolved by investigation of larger number of patients. Chen et al indicate that p53 abnormalities (allelic deletion, low expression, MDM2 overexpression and mutation) occur at a high rate during PCa development and that the frequency of p53 alterations appears to correlate with tumor grade/stage.[60] Most recently, Kubota et al[67] screened PCa specimens for p53 gene mutations in exons 1-11 and found that 9% of well and moderate-

ly differentiated and 30% of poorly differentiated PCa had p53 mutations. This result also supports that p53 mutation is a late event in the development of PCa.

4.2.6. Microsatellite Instability (MSI)

MSI (also known as replication errors or mutator phenotype), has been demonstrated in hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, and a number of sporadic cancers.[68] The human HNPCC genes (i.e., the hMSH2, hMLH1, hPMS1, and hPMS2 genes) have been cloned recently and found to be mutator genes.[69,70,71] Gao et al[68] screened 57 patients with prostatic adenocarcinoma for possible MSI at 18 microsatellite marker loci on 12 chromosomes. Thirty-seven of them showed positive MSI in at least one of the 18 microsatellite loci. A significant increase in frequency of MSI was found in invasive and high grade groups at loci on chromosome 6p, 8p, 10q, 11p, 13q, and 17p, but not on 3p, 5q, 7p, 16q, 18q, and Xq. Overall, more positive cases were found among invasive (70%) compared to noninvasive (58%), and among the high grade (87%) compared to low grade (57%) prostatic carcinoma. In six cases with positive lymph nodes (N_1 or N_2), five (83%) patients showed positive MSI. The correlation between MSI and high grade in PCa suggests that MSI may have the potential value as a prognostic marker for PCa. In another study by Uchida et al, 63% of poorly differentiated and 46% of stage D cancers were found to be positive for MSI. Statistically significant difference in well to moderately differentiated and poorly differentiated cancer was demonstrated.[72]

4.2.7. Oncoantigen 519 (A-519; Fatty Acid Synthase)

OA-519 is a 270 kDa protein found in the cytosol of breast and prostate carcinomas.[73] It has been recently shown to be a fatty acid synthase.[74] The potential prognostic value of OA-519 in PCa has recently been evaluated. Shurbaji et al[75] analyzed 42 PCa specimens for OA-519 expression. It was found that the proportion of positively stained cases increased with advancing clinical stage, with 25% of stage A cases expressing OA-519, and 46%, 67%, and 64% of stage B, C, and D, respectively, expressing OA-519. In another series, OA-519 has been demonstrated to be a predictor of pathologic stage independent of Gleason score in PCa.[75] In this study, OA-519 staining of the primary PCa was shown to be highly predictive in separating cases with organ-confined disease or capsular penetration vs. cases with seminal vesicle invasion or lymph node metastases.

4.2.8. Proliferation Markers

Ki-67 is a marker for cell proliferation. The Ki-67 staining index has been shown recently to correlate with the 5-bromodeoxyuridine (BrdUrd) labeling index.[76] In a series, Ki-67 staining score has been demonstrated to correlate in a statistically significantly manner with poor differentiation, lymph node metastases, and poor survival.[77] Mixed results were generated regarding the prognostic value of another proliferation marker, proliferating cell nuclear antigen (PCNA), in PCa. Carroll et al[78] showed that PCNA correlates with clinical stage and metastases, but not tumor grade. In another series, Harper et al[79] failed to demonstrate significant correlation between PCNA score and metastatic status. However, life table analysis indicated that the patients with the lower PCNA score survived significantly longer than those with the higher PCNA scores. Certainly, the potential prognostic value of PCNA in PCa remains to be examined further.

4.2.9. Epithelial Cadherin (E-cadherin)

E-cadherin is a calcium-dependent cell adhesion molecule which has been shown to play an important role in maintaining the epithelial phenotype. Mutational inactivation of E-cadherin has been demonstrated in a number of carcinomas and downregulation of E-cadherin has been shown to have a close relationship with invasion and metastases.[80] E-cadherin has now been recognized as a new invasion/metastasis-suppressor gene.[80] The first line of evidence suggesting E-cadherin involvement in PCa came from studies of rat Dunning PCa model. It was demonstrated that while E-cadherin was expressed in normal rat prostate and the well- or moderately differentiated, noninvasive Dunning tumors, E-cadherin in invasive sublines was undetectable at either protein or mRNA levels.[81] Subsequently, Umbas et al[82] showed that approximately 50% of human PCa specimens had reduced or absent levels of E-cadherin protein and that E-cadherin expression inversely correlated with tumor grade, suggesting E-cadherin may have prognostic value. When a larger series was followed-up, a statistically significant inverse correlation was found between E-cadherin expression and grade, stage, and more importantly, overall survival.[83] Although not an independent predictor of prognosis, E-cadherin expression certainly warrants further study as a potential prognostic marker for PCa progression.

4.2.10. Factor VIII

It has been well established that angiogenesis is required for tumor growth and metastasis. Immunostaining endothelial cells in PCa tissue using antibodies against factor VIII has been employed recently to measure microvessel density. In a series of 74 PCa tissues, the mean microvessel count within carcinomas from patients without metastasis were significantly lower than that within carcinomas from patients with metastasis.[84] An increase in microvessel density in poorly differentiated tumors also was observed. This assay within invasive tumors may be valuable in choosing therapeutic options in early PCa.

4.2.11. Neuroendocrine Differentiation

Neuroendocrine cells are a recognized component of prostatic ducts and acini.[85] Plasma levels of chromogranin-A, a marker for neuroendocrine carcinoma, were found to be elevated in stage D2 PCa.[86] Neuroendocrine differentiation has been demonstrated to correlate with high Gleason score and poor overall survival.[87] There is some evidence to suggest that prostatic cancer cells with neuroendocrine differentiation are resistant to hormonal therapy and eutopic and ectopic hormone production may have potential screening and monitoring value for PCa.[85]

4.2.12. Tissue Factor (TF)

Tissue factor is a membrane-associated protein responsible for activating the extrinsic pathway of blood coagulation. It potentiates factor VII and initiates the most important pathway of blood coagulation *in vivo*.[88] TF expression has been demonstrated to be higher in a variety of cancers (e.g., gastric, colorectal, ovarian, and renal cancers) than in their benign counterparts.[89] In one series, urinary TF (UTF) levels were measured in 53 patients with PCa as well as 3 control groups. It was found that UTF levels were higher in patients with PCa when compared to healthy controls (not age-matched), those undergoing endoscopic surveillance for superficial transitional cell carcinoma of bladder, and men with histologically proven BPH.[89] In patients with PCa, bone scan positive patients had higher levels of UTF than bone scan negative patients. However, an overall correlation between UTF and PSA levels may indicate that UTF may relate to disease bulk, instead of the aggressiveness of the disease. Supportive evidence of association between tissue factor and tumor progression came from investigation of experimental PCa.[90] Using a rat PCa model, it was suggested that procoagulant activity reflects the malignant phenotype and may serve as a marker for human PCa.

4.2.13. Type I Collagen Degradation Product

Type I collagen is the major structural protein in bone accounting for approximately 90% of the organic matrix of bone.[91] Therefore, bone metastasis could be studied by following the metabolism of type I collagen. Kylmala et al[92] investigated 17 PCa patients with mixed sclerotic and lytic (S+L) metastases and 23 patients with predominantly sclerotic (S) metastases. It was found that the serum crosslinked carboxy-terminal telopeptide of type I collagen (ICTP) level in S+L group was significantly higher than that in S group. Serum ICPT level was found to inversely correlate with overall survival. Urinary levels of collagen cross-link metabolites, pyridinoline and deoxypyridinoline, measured by high pressure liquid chromatography (HPLC) had also been demonstrated to correlate with PCa progression.

4.2.14. Serum Metalloproteinases and Their Inhibitors

Tumor progression is in part the result of the activity of proteinases that facilitate invasion and metastasis by degrading the extracellular matrix.[93] A metalloproteinase, interstitial collagenase, and tissue inhibitor of metalloproteinases 1 and 2 (TIMPs 1 and 2) had been studied for their potential prognostic value in PCa.[94] It was found that patients with PCa had higher levels of collagenase and TIMP-1, but lower levels of TIMP-2, than controls as detected by enzyme-linked immunoassays (ELISAs). Collagenase levels were statistically higher in patients with metastases than those without metastatic disease.[94] It seems promising that matrix-degrading enzymes may proven to be useful markers for the aggressiveness of PCa.

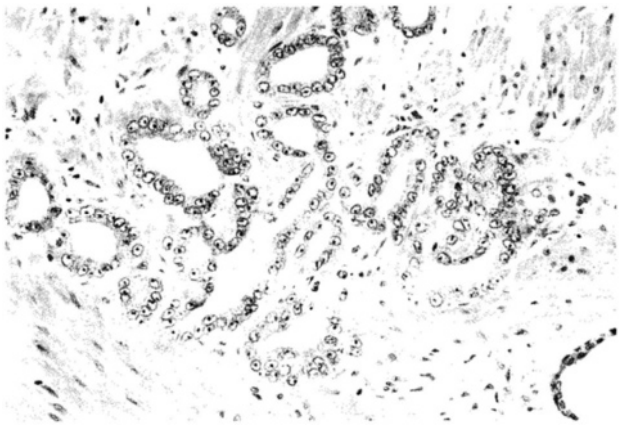

Figure 1. *Prostate adenocarcinoma, Gleason pattern 3, illustrating diagnostic features of malignancy (relatively uniform glands, single cell layer and prominent nucleoli). HE staining; medium power view.*

5. Pathology and Staging of Prostate Cancer

5.1. Diagnostic Criteria

The vast majority of primary carcinomas of the prostate are gland forming (i.e. adenocarcinoma). Considering the large number of needle biopsies being performed in the US, the issue of distinguishing cancer from its mimics is of major importance. Strict application of diagnostic criteria to these small tissue samples is essential in preventing misdiagnosis. Important in this regard are the minimal criteria applied by Gleason for the diagnosis of adenocarcinoma (Gleason grades 1 and 2) which are (i) a relatively uniform proliferation of (ii) small glands (iii) lined by a single layered epithelium (iv) with at least some cells containing prominent nucleoli *(Fig.1)*.[95] These criteria have subsequently been adhered to and advocated by most authorities.[96,97,98]

Of these criteria, the requirement for the presence of at least some "prominent nucleoli" has been challenged.[99] In a study of atypical small gland lesions, the nucleolus was one of the most important features used by a panel of expert pathologists to distinguish between atypical adenomatous hyperplasia (adenosis) and low-grade adenocarcinoma thus highlighting its practical importance[96] In contrast, Kramer and Epstein measured nucleolar size in 113 foci of Gleason grade 1 in 82 carcinomas and compared these with 18 examples of adenosis. They concluded that while nucleoli remain an important diagnostic feature, some cases of carcinoma (8%) contained no prominent nucleoli (defined as being larger than 1.6 microns) and many examples of atypical adenomatous hyperplasia (28%) had occasional or frequent prominent nucleoli.[106] In a similar study, Kelemen and colleagues found the ratio of nuclei with nucleoli larger than 3 microns to all nuclei to be most useful in separating benign from malignant lesions.[108]

In limited biopsy material, one often encounters only a few areas of an infiltrative adenocarcinoma which demonstrate good gland formation (Gleason grade 3). In these cases the few or even single malignant glands, may be present amongst benign glands. In such cases the features most useful in reaching a correct interpretation of adenocarcinoma are the enlarged nuclei when compared with adjacent benign elements, prominent nucleoli, pink acellular or basophilic mucin secretions, amphophilic cytoplasm, prostatic crystalloids and absence of basal cells *(Fig.2)*.[97]

5.2. Ancillary Diagnostic Features

Several additional features are of assistance in recognizing adenocarcinoma. The identification of acidic mucin (wispy basophilic material on HE), has been suggested as a marker of malignancy in the prostate gland.[105] Several studies have shown that luminal acid mucin is frequently present in adenocarcinoma and as such can aid in the diagnosis.[97,104] It is also clear that this finding is not specific to carcinoma but can also occur in atrophy, mucinous metaplasia, basal cell hyperplasia, atypical adenomatous hyperplasia and sclerosing adenosis.[103,104,105] This finding should signal the possibility of carcinoma however the usual histological criteria must still be met to make the diagnosis.

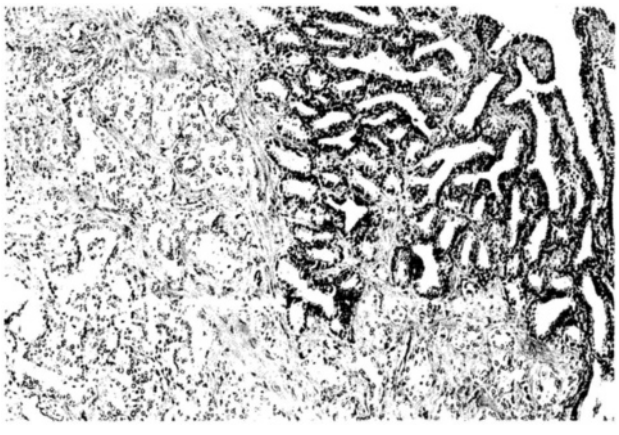

Figure 2. *Limited adenocarcinoma on needle biopsy. There is a uniform proliferation of small glands, note the presence of crystalloids in two of the glands. HE staining; medium power view.*

Prostatic crystalloids were first described in association with well differentiated adenocarcinoma.[106] Crystalloids are intensely eosinophilic with a glassy appearance and sharp angulated edges giving a false impression of birefringence. Several studies have confirmed a strong association between the presence of crystalloids and carcinoma,[97,107] but also have shown that they may be found in benign glands which are most often PIN and atypical adenomatous hyperplasia.[96,107]

Immunohistochemical staining for high molecular weight cytokeratin has become a valuable tool in the diagnosis of adenocarcinoma. The most commonly used antibody is clone 34βE12 which has frequently (and perhaps inappropriately) been referred to by its commercial catalogue number (CK903). These antibodies are useful because they specifically label basal cells and not secretory cells. Benign mimics of adenocarcinoma are invariably positive with this antibody reflecting the presence of basal cells.[108,109,110] Although negative staining is typical of adenocarcinoma, it is dangerous to base a diagnosis on a negative immunoreaction and so the positive diagnosis of carcinoma should still depend on histological criteria.

5.3. Differential Diagnosis

The differential diagnosis of prostatic adenocarcinoma includes normal tissues such as Cowper's gland, paraganglion tissue and seminal vesicle or ejaculatory duct as well as pathological conditions such as atypical adenomatous hyperplasia, atrophy, basal cell hyperplasia and sclerosing adenosis. Cowper's glands are paired structures located near the prostatic apex. The acini are closely packed, arranged in lobules and lined by a mucin secreting epithelium. Mucinous metaplasia is seen in a variety of prostatic lesions but is not a feature of well differentiated adenocarcinoma. Paraganglion tissue is not rare in the periprostatic connective tissue but is distinctly uncommon in the prostate stroma. Closely packed small cells with clear cytoplasm are arranged in small nests in intimate association with small nerves and blood vessels. The cytology is inconsistent with a high grade prostate adenocarcinoma which might have this architecture. Seminal vesicle or ejaculatory duct epithelium produces difficulty because of the small gland pattern and large bizarre "monster" hyperchromatic nuclei. The latter is in fact an important feature for exclusion of carcinoma which for

practical purposes never contains nuclei of this type. The presence of abundant lipofuscin pigment supports a benign diagnosis.

Atypical adenomatous hyperplasia (adenosis) is a lesion characterized by the proliferation of small acinar structures at the periphery of hyperplastic nodules.[95,96] Although it has frequently been discussed as a possible precursor lesion of transition zone adenocarcinoma, there is little evidence for this possibility other than a similarity of morphology.[111,112] Features helpful in distinguishing atypical adenomatous hyperplasia from cancer are its association with benign glands, pale cytoplasm, absence of basophilic mucin, lack of prominent nucleoli and presence of a partial basal cell layer.[96,113]

Prostatic atrophy begins to appear in the prostate at a relatively early age.[114] It is one of the more frequent lesions resulting in diagnostic problems in needle biopsy specimens. Prostatic atrophy can be divided into three major types; simple lobular, sclerotic and postatrophic hyperplasia.[98] Important clues to its recognition are a lobular arrangement, ectatic central duct, periductal sclerosis and small shrunken epithelial cells. The basal cells may be difficult to identify but can be highlighted by staining for high-molecular weight cytokeratin.[110]

Basal cell hyperplasia usually develops in the transition zone as part of nodular prostatic hyperplasia. It causes confusion in diagnosis because of its "blue" appearance and the presence of nuclear atypia. Important diagnostic features are its arrangement in nodules, the filling of glandular lumina with multiple layers of cells, scant cytoplasm, angulated hyperchromatic nuclei with inconspicuous nucleoli.[104,105]

Sclerosing adenosis is a recently described lesion which is usually found in radical prostatectomy or transurethral resection specimens.[105,115] Characteristic features of this lesion are relative circumscription, a mixture of small and large glands, cellular spindle cell stroma, a thick basement membrane around tubules and the presence of myoepithelial differentiation (S-100 protein and muscle-specific actin immunoreactivity).[105]

5.4. Histological Grading

Adenocarcinoma of the prostate is characterized by a remarkable heterogeneity in terms of its histological differentiation, microscopic growth patterns and biological aggressiveness. Most PCa are multifocal and there are significant variations in tumor grade between anatomically separate tumor foci. Microscopic examination often reveals neoplastic components representing opposite extremes of differentiation in close proximity. These properties of PCa have the potential of exaggerating sampling errors when diagnosis is made on limited tissue specimens.

Although numerous grading systems have been proposed and applied in the past, the Gleason grading system presently has gained almost uniform acceptance.[116,117] It is the most widely used by physicians treating PCa and its utility in predicting tumor behavior has been repeatedly demonstrated.[118,119] It also has been demonstrated that Gleason score in needle biopsy specimens is an important parameter in predicting pathological stage of prostatic adenocarcinoma, including regional lymph node metastasis.[120,121,122,123]

Gleason's grading scheme recognizes five major grades defined by patterns of neoplastic growth. These grades are based on both the degree and architecture of glandular formation and, to a lesser extent, the growth pattern of the periphery of the tumor nodule relevant to the surrounding stroma. This system "quantitates" the neoplastic components acknowledging the most prominent and the second most prominent tumor grades and then adding them together to produce a Gleason's score that ranges between 2 and 10 out of 10.

The reliability of Gleason grading on needle biopsy specimens has been evaluated. Catalona et al[127] correlated the histological tumor grade in the needle biopsy and the subsequent radical prostatectomy specimen in 66 consecutive patients and found that the biopsies were given a lower, a correct and a higher grade in 33, 59 and 8% respectively. Other studies have reported similar rates of discrepancy[124,125] Contemporary series based on ultrasound guided sextant biopsies are limited but suggest a better correlation than found in early reports.[129]

5.5. Staging

Clinical and pathologic stage are accepted as major prognostic indicators in predicting the biologic behavior of PCa.[118,119,127] Although a variety of staging systems have been used, the American Joint Commission of Cancer/Union International Control Cancer Tumor Nodes Metastasis (AJCC/UICC TNM) system has recently gained general acceptance.[128] Limitations of clinical staging have been well recognized, in particular the lack of accuracy in predicting the final pathologic stage.[129,130] In addition there is not presently a pathologic staging system (pT) based on the UICC/AJCC TNM clinical staging categories.[129]

5.5.1. Categories T1a and T1b

T1a and T1b (A1 and A2) refer to PCa diagnosed in the transurethrally resected prostate tissue of patients not suspected of having PCa. The distinction of category T1a from T1b is based on the estimated total percent involvement of the resected tissue by cancer. Tumors with less than or equal to 5% involvement are T1a and with greater than 5% are T1b. With the advent of PSA, this group of tumors is decreasing in frequency as most patients with T1b tumors will have an elevated serum PSA level. There is no pathologic correlate to these categories in the radical prostatectomy specimen.

5.5.2. Category T1c

This category was created to accommodate those patients with prostate carcinoma diagnosed on needle biopsy in which no palpable or visible lesion (by transrectal ultrasonography) is present. The majority of these patients are diagnosed following detection of an elevated serum PSA. Recent studies have highlighted the wide spectrum of pathologic categories represented in this group.[131] The use of needle biopsies to "stage" such patients has been the subject of many recent reports.[121,122,123] Although different methods have been used, results have been consistent in that some quantification of tumor amount and distribution (as well as serum PSA and Gleason score) predict for final pathologic category. There is no pathologic equivalent to category T1c in the radical prostatectomy specimen.

5.5.3. Categories T2a, T2b and T2c

This category includes both palpable and ultrasonographically visible lesions which are clinically judged to be confined to the prostate gland. The subcategories are defined by the degree of involvement of the 2 lobes of the gland. Although the concept of lobes has some utility clinically when evaluating a lesion by digital rectal examination, these are not definable either on gross or histological examination of the resected prostate.[141] Subcategorization by lobes is therefore not practical pathologically. Consideration could be given to defining subcategories of T2 by size or volume. Size cannot be determined accurately as many PCa do not form definable masses.[132] Multifocality further complicates histological evaluation in determining a measurement of size.[132,133]

Volume of cancer has been of reported to be a valuable predictor of stage and therefore prognosis. It could be applied to subcategorize this T-category.[134,135] Methods applied for determining volume include morphometry, grid estimates and surface area estimates, all of which require complete embedding of the gland. This is probably not generally practical outside of academic institutions with specific interest in PCa. What should be included in volume measurement is also problematic i.e. should all foci, only the index lesion, only the lesion which appears to be most biologically significant or some combination be measured? The latter may not necessarily be the largest or the "index" tumor nodule.[136,137]

Once a tumor is confirmed to be pathologically confined to the prostate gland, it may not be necessary to subdivide these any further. Gleason score provides significant prognostic information and the rate of failure in this group is low.[118,138,139]

5.5.4. Categories T3a and T3b

These categories refer to unilateral and bilateral extracapsular extension of tumor respectively. Terminology such as capsular invasion, capsular transgression, capsular penetration, capsular effraction, extracapsular extension and extraprostatic extension has been used to describe invasion of cancer through the capsule of the prostate gland and into the fibrofatty tissue which surrounds the gland.[127] The anatomy and histology of the prostate gland complicate the determination of whether or not extraprostatic spread has occurred as there are no consistent histological landmarks found in the apical region or the anterior surface to define a "capsule."[140] In most cases it is not practical to diagnose extraprostatic extension in these sites. Some groups have equated a positive surgical margin at these locations with T3 disease while others have considered these to be T2 with positive margins, an approach we have favored.[132] Uniform application of one of these alternatives is critical in comparing different institutional series.

The prognostic significance of distinguishing unilateral from bilateral extraprostatic extension is unclear. In most reports these two categories are combined. Of greater interest appears to be quantifying the amount of tumor spread beyond the capsule. The utility of this was demonstrated by Epstein et al, in their "focal" and "established" categories.[118] Unfortunately the criteria used by these authors are difficult to apply and a more reproducible definition needs to be accepted and tested to confirm the significance of this distinction.

5.5.5. Category T3c

Invasion of the muscular wall of the seminal vesicle represents an important negative prognostic indicator in the evaluation of the radical prostatectomy specimen *(Fig.3)*. Ohari et al[109,110] subdivided involvement of the seminal vesicle into 3 types based on the apparent route the tumor followed to get into the seminal vesicle and ascribed to these different prognostic significance. The use of directed needle biopsies to identify seminal vesicle involvement prior to treatment may be a significant development in the staging of PCa patients.[130]

5.5.6. Category T4

This category refers to gross involvement of adjacent structures (urinary bladder, rectum, pelvic side wall) by prostate carcinoma.

5.6. Surgical Margins

Involvement of the surgical resection margins is known to be an important predictor of therapeutic failure and reduced survival in patients treated by radical prostatectomy.[118,119,134] Recent studies have indicated that quantification of the amount of involvement may be prognostically important;[118] in order for this parameter to be tested, a reproducible method of quantification

needs to be agreed upon. The pattern of involvement in relation to predicting tumor biology also need to be explored. Categories such as surgically induced (incision of the gland), bulging positive margins and tumor extending to the limit of the surgical resection have been suggested.[132] Finally the concept of positive margins in organ confined (T2) tumors has been accepted by some groups. The validity of this category needs further clarification.[132,139]

6. BIOLOGICAL ASPECTS OF PROSTATE CANCER

6.1. Apoptosis and Prostate Cancer

Apoptosis, or programmed cell death, is characterized by distinct morphological and biochemical features. It has been proposed to be involved in multiple biological and pathological processes.[143,144,145] Although apoptosis is generally considered a genetically regulated process as seen in physiological events, it can be triggered by physical and toxic exposures, cellular effects of hormones, growth factors, and cytokines, viral infection, and immunologic mechanisms.[146] Numerous factors have been implicated in regulating/modulating apoptosis, which include: oncogenes/tumor suppressor genes; hormones, cytokines and growth factor/growth factor receptors; intracellular signal transducers; cell cycle regulators; reactive oxygen species or other free radicals; extracellular matrix regulators/cell adhesion molecules; and specific endonucleases.[147,149,150,151,152,153] Many of these apoptosis regulators have been associated with various human malignancies. For example, studies on human tumors have demonstrated an overall positive correlation between increased expression of Bcl-2 (or Bcl-X_L) or decreased expression of Bax and uncontrolled tumor cell growth, and, in some cases, with tumor progression and a poor prognosis of cancer patients.[154,155,156] Another example is p53, a phosphoprotein known to modulate gene transcription, police cell cycle checkpoints, control DNA replication and repair, and maintain genomic stability. Wild type p53 also positively regulates apoptosis. p53 gene mutations have been linked to attenuated apoptosis in multiple cancers represented by Wilms' tumor, colon cancer, cervical carcinoma and breast cancer.[157,158,159] Since apoptosis plays a critical role in multiple steps of tumorigenesis, many chemoprevention and therapeutic regimens attempting to manipulate apoptotic process have been proposed to aid in the clinical treatment of cancer patients.[160,161,162]

Apoptosis is closely involved in the initiation, progression and metastasis of human PCa. Prostate as an organ is very similar to the breast of females in that both organs depend on circulating hormones for their growth. Therefore, androgens play a paramount role in regulating the

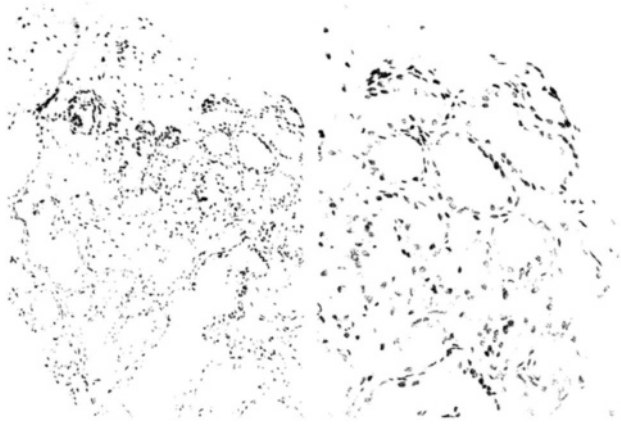

Figure 3. Example of a T3c prostatic adenocarcinoma with tumor glands (left) invading the muscular wall of the seminal vesicle (right). HE staining, medium power view.

growth of normal as well as malignant prostate epithelial cells.[163] Early work done in John Issacs's lab and many other labs with castrated rats has revealed a very important relationship between androgens and growth of prostate glandular epithelial cells. Ablation of androgen results in: 1) a rapid increase in the transcription of multiple genes normally repressed by testosterone in intact prostate. These include testosterone repressed message 2 (TRMP-2),[164] immediate early response genes exemplified by c-Fos and c-Myc, HSP70, TGFβ, and glutathione S-transferase;[165,166,167] 2) a rapid and sustained elevation in the $[Ca^{2+}i]$, leading to the activation of a Ca^{2+}/Mg^{2+}-dependent endonuclease[168] and subsequent DNA fragmentation, which are observed within day 1 after castration;[169] 3) all of the above changes continue to persist and appear to maximize by day 4. Simultaneously, all of the morphological characteristics (chromatin margination and condensation, membrane blebbing, nuclear disintegration and formation of apoptotic bodies) of apoptosis can be observed by day 2 and become progressively more prominent with time. By day 21 after castration, all androgen-dependent epithelial cells have died and apoptotic cells and bodies have been cleared;[163,169] and 4) androgen ablation-induced apoptosis is independent of cell proliferation and occurs at the interphase.[163] Subsequent experiments with in vitro organ cultures recapitulated some of the results described above, e.g., the role of Ca^{2+} in the induction of apoptosis.[170] These observations clearly indicate that androgens are critical survival factors for normal prostate epithelial cells. The same notion also holds true for the androgen-dependent human prostatic cancer cells. For example, androgen-dependent PC-82 human prostate carcinoma xenografted into nude mice rapidly involutes following castration.[171]

Unfortunately, PCa in patients, at the time of clinical diagnosis, mostly present themselves as heterogeneous

entities, i.e., mixtures of androgen-dependent and -independent tumor cells. Therefore, as can be expected, the approach of androgen ablation will not be able to eradicate those androgen-independent cancer cells (see below). To make it worse, cells initially dependent on androgens for their growth and survival may evolve to become androgen-independent. So the key to the cure of PCa appears to lie in the elimination of androgen-dependent cancers by hormonal ablation, eradication of androgen-independent cancer cells with very effective chemotherapeutic drugs or radiotherapy, and prevention of transition of androgen-dependent cells to androgen-independent cells (see below). The difficulty in achieving these end points results from our lack of appropriate therapeutic strategies to eradicate androgen-independent cells. There is still hope, though, since androgen-independent PCa cells, unresponsive to androgens, still retain the apoptotic machinery which can be activated under certain circumstances. For example, androgen-independent Rat Dunning AT-3 prostatic cancer cells undergo apoptosis when treated with thymidine analogs 5-FU or trifluorothymidine.[171] Also, increasing intracellular Ca^{2+} concentrations by ionophore can induce the apoptotic death of AT3 cells even when the latter are not proliferating.[172]

These observations, in conjunction with previous discussions on various regulators of apoptosis, suggest that it is possible to manipulate these individual signal transduction pathways in order to enhance cell killing by inducing apoptosis of prostatic cancer cells since it has been observed that apoptotic indices appear to parallel the biologic activity of PIN (prostate intraepithelial neoplasia) and malignant prostatic tissue.[173]

6.1.1. Androgen ablation by castration or biochemical antagonists

This represents the most frequently adopted (or standard) clinical procedure for PCa patients due to the well-documented effects of androgens in promoting PCa cell survival. Indeed, androgen ablation has been shown to increase the apoptotic index in prostatic cancer patients[174] and castration also promotes apoptotic death of human prostate carcinoma grown in mice.[171,175] Recent studies also have demonstrated that castration therapy may induce apoptosis as well as decrease cell proliferation, although these may be observed in different patients.[174] The molecular mechanism(s) for androgen ablation-induced glandular epithelial cell death are not very clear, but cell proliferation, DNA repair, and the p53 function do not appear to be involved.[177] It may involve, instead, a TGFβ1-dependent mechanism since, as described earlier, the TGFβ1 gene expression is rapidly induced following castration[166] and TGFβ1 can induce apoptosis of multiple tumor cells[178,179] including prostate epithelial cells.[180] Hormonal ablation can rarely cure the patients due to the presence and constant generation of androgen-independent cancer cells, which may evade apoptosis induction due to some defects in their autocrine production of and/or their response to TGFβ1.[163] Very recent experimental results from Kim et al[181] have provided strong evidence for this possibility. These authors observed that although TGFβ1 dose-dependently inhibits the proliferation of PC-3 and Du145 cells, another human PCa cell line isolated from metastatic loci (LNCaP) has completely lost its response to this cytokine. Further examinations reveal that LNCaP cells have a genetic change in their type I TGFβ receptor (TβR-I),[181] an absolutely required component for the TGFβ1 binding and signaling. This observation also has provided an important clue to the previously observed paradox that TGFβ1 in general is growth inhibitory for tumor cells, however, a variety of tumor cells have been found to overexpress TGFβ1. Some new therapeutic protocols such as combination therapy of castration with administration of estrogens[182,183] or chemotherapeutic drugs[184] have been proposed to target androgen-independent prostatic cancer cells.

6.1.2. Apoptosis induction by chemotherapeutic drugs or other natural/synthetic chemicals

Although androgen-independent prostatic cancer cells are refractory to apoptosis induction by androgen ablation, they still may respond to other apoptotic inducers since the apoptotic machinery in these cells appears to be functional, if not intact. This hypothesis has gained support from recent observations that PC3 cells, a p53-negative, androgen-independent human PCa cell line, are triggered to undergo notable apoptosis upon treatment with several conventional chemotherapeutic drugs including cisplatin, camptothecin, tenoposide, vincristine and lovastatin.[185] The apoptosis induced by these drugs does not appear to depend on macromolecular synthesis. Similarly, "thymineless" death of hormone-independent human PCa cells has been observed with cytotoxic drugs such as 5-FU.[186] Since human prostatic cancer cells (including androgen-independent, metastatic cells) have an extremely low proliferation rate,[187] it can be predicted that patients with androgen-independent, metastatic prostatic cancer cells will not respond very well to most of those chemotherapeutic drugs currently available in clinical use. Thus new chemicals or natural products have to be developed which do not depend on cancer cell proliferation for their apoptosis-inducing effects. Linomide, a reported angiogenesis inhibitor with a quinoline-3-carboxamide structure, has recently been shown to inhibit the angiogenesis in TSU and PC-3 androgen-independent human prostate carcinoma xenotransplanted into SCID mice.[188] The hypoxia due to blocked blood vessel forma-

tion also induces dramatic apoptotic death of transplanted tumor cells.[188] b-lapachone (3,4-dihydro-2,2-dimethyl-2*H*-naphthol[1,2-*b*] pyran-5,6-dione), a simple plant product isolated from *Tabebuia avellanedae* and a novel inhibitor of topoisomerase I, has recently been shown to strongly induce apoptosis of androgen-independent human PCa cells (PC-3, Du145 and LNCaP).[189,190] These observations suggest that functional apoptotic machinery does exist in androgen-independent, metastatic human PCa cells. Therefore, it is possible to induce their apoptotic death by new chemicals/natural products of novel mechanisms of action.

6.1.3. Induction of programmed cell death by manipulating apoptosis-related oncoproteins

As discussed before, in response to castration, a gene termed TRPM-2 is rapidly induced.[164] TRPM-2, also known as sulfated glycoprotein-2 (SGP-2) or clusterin, is a ubiquitous protein with multiple biological functions.[191] Androgen ablation has consistently been shown to induce this gene[192] and some apoptosis inducers such as a ribonucleotide reductase inhibitor (MDL 101,731) also elevate TRPM levels,[193] suggesting that TRPM-2 may be associated with the induction of apoptosis. Indeed, subsequent work reveals that TRPM-2 gene is induced in many tumor cells undergoing apoptosis.[194] Therefore the induction of this gene expression has once been used as a marker for apoptosis. However, recent work has suggested that the hypothesized role of TRPM-2 in apoptosis may be an oversimplification. Treatment of LNCaP cells with TNFα induces an initial transient elevation of TRPM-2 followed by the depletion of the protein which temporally precedes the initiation of apoptosis.[195] More interestingly, transfection of antisense oligo directed against TRPM-2 to LNCaP cells significantly increased cell death while overexpression of the protein by stable transfection of an expression vector carrying the TRPM-2 cDNA results in resistance to TNFα-induced cytotoxicity.[195] These results suggest that maybe the depletion of TRPM-2, rather than its expression, is associated with cell death.

In contrast to TRPM-2, Bcl-2 has been unequivocally linked to the development in prostatic cancer cells of resistance to apoptosis induction. Although normal prostatic secretory epithelial cells do not express Bcl-2, human PCa cells endogenously express various amounts of Bcl-2, whose levels further increase as these cancer cells become androgen-independent and refractory to androgen-ablation.[196] Also androgens have been shown to induce expression of Bcl-2 protein in hormone-sensitive LNCaP cells,[197] which may represent one of the mechanisms whereby hormone-dependent prostatic cancer cells evolve into androgen-independent cells. Indeed, enforced overexpression of Bcl-2 in LNCaP cells make these cells highly resistant to androgen ablation as well as apoptosis induced by androgen ablation and many other stimuli such as serum deprivation and phorbol esters.[198] LNCaP cells overexpressing Bcl-2 also form earlier and bigger tumors when s.c inoculated into male nude mice and they are the only cells (compared with untransfected or control vector-transfected) that give rise to tumors when xenotransplanted into castrated nude mice.[198] These important experimental observations suggest that Bcl-2 overexpression also confers on androgen-dependent cells the ability to form hormone-refractory prostate tumors *in vivo*.[198] On the other hand, downregulation of Bcl-2 expression with gene-specific antisense oligos has been shown to abolish the Bcl-2-conferred resistance to apoptosis induction as well as androgen-protected apoptosis induction of LNCaP cells by etoposide.[197] The above observations, taken together, suggest that Bcl-2 can be a prime target for our interference to prevent generation of androgen-refractory prostatic cancer cells. Blocking Bcl-2 expression with unmodified or phosphorothioate antisense oligonucleotides[199] may also provide beneficial effects for combined hormonal ablation and cytotoxic chemotherapy, a treatment regimen advocated by many prostate oncologists.[200,201]

p53 tumor suppressor gene is the most frequently mutated gene found in multiple human malignant tumors including PCa.[202] p53 mutations have been observed in a small percentage (10-20%) of advanced, high Gleason PCa patients.[203,204] The importance of p53 in PCa also may stem from the fact that p53 regulates the gene expression of Bcl-2 and Bax,[205] two critical proteins implicated in apoptosis, and that wild type p53 mediates apoptosis induced, e.g., by irradiation and genotoxic drugs. In general, no correlation between p53 mutations and early-stage PCa has been noticed. Since p53 has an important role in introducing the G_0/G_1 check point, abrogation or attenuation of p53 activity after DNA damage has been hypothesized to be an early event in prostate oncogenesis, leading to increased probability of a cell accumulating the genetic alterations necessary for transformation. This hypothesis has gained partial support from the experiments by Girinsky et al[206] in which they have observed that primary cultures of human prostatic epithelial cells, in contrast to similarly prepared stromal cells, do not accumulate p53 as well as $p21^{WAF-1}$ in response to irradiation. These observations suggest that targeted introduction of p53 into prostate cells may help prevent transformation of normal prostate epithelial cells. On the other hand, enforced expression of p53 targeted to prostate may induce apoptotic cell death in advanced-stage prostate carcinomas. The validity of this rationale is supported by recent observations that expression of p53 (transfected by replication-defect adenovirus) in a p53-defective, androgen-independent human PCa cell line (Tsu-pr1) results in the induction of apoptosis and suppression of tumor formation in nude mice.[207]

In addition to Bcl-2 and p53, other proto-oncogenes known to be linked to apoptosis such as ras, c-myc and c-jun also may be involved in regulating apoptosis of human PCa cells. Increased expression of these oncoproteins have been observed in PC-3 variant cells resistant to chemotherapeutic drug-induced apoptosis such as by VP16 and cisplatin.[208] Enforced expression of ras has been shown to confer on cells the androgen independence.[209] Based on these observations, it is also possible, at least from the theoretical point of view, to target these molecules to induce or enhance apoptosis of human prostatic cancers.

6.1.4. Modulation of intracellular signal transducers to induce apoptotic cell death

Calcium has been shown to play an essential role in activating endonucleases and cleaving internucleosomal DNA and thus in apoptosis.[148] Androgen ablation induces a rapid elevation in the $[Ca^{2+}i]$ in androgen-dependent cells, however, it fails to do so in androgen-independent prostatic cancer cells.[163] Therefore, manipulations aimed to increasing intracellular calcium concentration may induce apoptosis or enhance the cells' response to apoptotic inducers. Thapsigargin, a sesquiterpene γ-lactone which selectively inhibits the Ca^{2+}-dependent ATPase pumps in sarcoplasmic and endoplasmic reticulum, induces prominent apoptotic cell death of androgen-independent PCa cells.[208] The apoptosis-inducing effect of thapsigargin does not depend on intracellular pH and does not require proliferation, but requires a sustained elevation in the intracellular calcium.[210] TPA, a well-known diacylglycerol analog that activates protein kinase C, has been shown to induce apoptosis of LNCaP cells.[210] This effect appears to be dependent on PKC-mediated induction of the early response transcription factors NGFI-A (nerve growth factor induced gene A) and c-fos.[211] Also, the steady state mRNA for c-jun and the orphan steroid receptor *nur77* also are rapidly induced following TPA treatment.[212] In contrast, androgen induction of human kallikrein-1 and c-myc mRNA is repressed by TPA.[212] The TPA induced apoptotic death of LNCaP cells could be inhibited by staurosporine, a known PKC inhibitor.[211,212] These results collectively suggest that PKC may be a negative growth regulator for human PCa cells due to its involvement in triggering apoptosis.

Many other signal transducers such as protein kinases, phosphatases, eicosanoids and ceramide[149,150] have been demonstrated to regulate cell survival and death in many tumor systems. However, their involvement in human PCa have not been explored. There is no question that, with our increased understanding of the molecular mechanisms regulating apoptosis, some novel mechanism-based, apoptosis-targeted treatment regimens may be developed. Already, gene therapy targeted to Bcl-2 and p53 in an attempt to induce or enhance apoptosis of human prostatic cancer cells has provided novel therapeutic approaches.

6.2. Prostate Cancer Metastasis

Cancer metastasis, like oncogenesis, is an extremely complicated pathological process that involves extensive tumor-host interactions.[213,214] For a transformed cell to form a successful metastatic colony, it must in general complete all or most of the well-defined steps that comprise the "metastatic cascade".[213,214,215] The first step is uncontrolled cell proliferation, characteristic of both benign and malignant tumor cells. Intrinsic or acquired genetic instability, together with various epigenetic factors, generate tumor cell variants which acquire unique phenotypic characteristics which dissociate them from the parent tumor population and thus allow these variants to escape from the "social" constraints imposed by the host. This step confers on these "mutated" tumor cells invasive or metastatic capabilities and is generally considered to be the first step leading to site-specific metastasis. In the next step, tumor cells, in response to various chemoattractants and cytokines derived from the host and/or tumor cells, migrate towards neighboring vasculature or intravasate into the vasculature of the tumor and thus enter the hematogenous or lymphatic circulation. Subsequently, tumor cells travel to and arrest in the capillary by specific adherence to the endothelial cells of the target organ.[213,215] Thereafter, tumor cells induce endothelial cell retraction, exit from circulation (extravasation), interact with the organ-specific extracellular matrix, proliferate in response to local growth factors, and finally form a metastatic colony. Failure at any one of these steps generally will abort the metastatic process. Completion of every step of the metastatic cascade is subject to a multitude of variable influences, an apparent example being the requirement of angiogenesis for the growth of both primary and secondary tumors.[216,217]

Multiple genetic and epigenetic factors have been implicated in the oncogenesis of PCa although the molecular mechanisms for the disease remain largely unknown (see above). Metastasis of human PCa occurs early and represents the major hurdle to a successful therapy. More than 100 biomarkers (diagnostic and prognostic) have been proposed (see above),[218,219] most of which, unfortunately, are useless in terms of the practical clinical applications. Some of these markers such as *c-erb*B-2/*neu* oncogene and HGF/*c-met* have been associated with the progression and metastasis of PCa.[220] The frustration in the clinical management of PCa is derived not only from the fact that no single gene or molecule can serve as a reliable marker but also from the reality that an effective therapeutic regimen is still lacking. Vigorous search for diagnostic/prognostic markers and development of novel mechanism-based therapeutic protocols are therefore urgently needed.

Adhesion process plays a pivotal role in mediating tumor cell-tumor cell, tumor cell-platelet, tumor cell-

endothelial cell, and tumor cell-matrix interactions, which are essential steps in the "metastatic cascade".[214-216] Various adhesive interactions are mediated primarily by five families of adhesion molecules, i.e., carbohydrates, integrins, cadherins, immunoglobulins, and selectins. Most of these adhesion molecules have been implicated in tumor progression and metastasis. For example, transformed and malignant cells tend to lose the α5β1 integrin, a prototypical fibronectin receptor.[217] In contrast, tumor cells may demonstrate enhanced expression of some integrin receptors such as α4β1 in metastatic melanoma cells,[218] α6β4 in metastasizing 3LL cells,[219] and β2 integrins in human small cell lung carcinoma cells.[220] Transfection of cloned α2 subunit cDNA into human rhabdomyosarcoma cells resulted in enhanced tumor cell adhesion to collagen as well as increased experimental and spontaneous metastasis.[221] Similarly, a strong correlation has been observed among down-regulation of E-cadherin expression and tumor invasion and metastasis. Loss of cell-cell adhesion in carcinoma cells has long been hypothesized to play a role in the acquisition of an invasive, metastatic phenotype. Downregulated E-cadherin expression has now been shown to be involved in this loss of cell-cell contact. An inverse relationship between E-cadherin expression and differentiation level of many carcinomas has been noted.[222,223] Transfected E-cadherin could restrict or reverse the invasive behavior of epithelial tumor cells.[228,229] These distinct properties of E-cadherin make it a perfect metastasis suppressor gene. By analogy with oncogenic tumor suppressor genes (e.g., p53), loss of metastasis-suppressive E-cadherin will facilitate tumor cell dissociation and subsequent dissemination. Certain immunoglobulin super-gene family adhesion molecules may also be involved in tumor cell invasion and cancer metastasis. Two gene products, CEA (carcinoembryonic antigen) and DCC (Deleted in Colon Carcinoma) appear to function as dominant and recessive metastasis-related oncogenes, respectively. CEA, a widely used human tumor marker, mediates Ca^{2+}-independent, homotypic aggregation of cultured human colon carcinomas and colon carcinoma cell adhesion to collagen. It is also localized to cell-cell contact sites *in situ*.[230] A direct positive correlation also has been observed between serum CEA levels and tumorigenicity in nude mice of excised human colorectal carcinoma cells.[230] Human PCa cells express multiple adhesion receptors including integrins, immunoglobulin family members, cadherins and carbohydrate ligands. Compared to the normal prostate epithelial cells, PCa cells demonstrate a variety of quantitative and qualitative alterations with respect to the expression/function of these adhesion molecules. For instance, although normal prostate epithelial cells express α2, α3, α4, α5, α6, αv, β1, and β4 integrin subunits, the expression of β4 is completely lost in carcinoma cells.[231] Similarly, PCa cells very frequently demonstrate decreased E-cadherin expression/function, which has been linked to either mutations in α-catenin (a cytoplasmic linker for cadherin and cytoskeleton) or to hypermethylation in the 5' region of the gene.[232,233] Another adhesion molecule termed C-CAM, which also is a potential tumor suppressor, has also demonstrated a significantly lowered expression in PCa.[234] Sialyl Lewis^x antigen, a carbohydrate ligand for adhesion molecules selectins, has been shown to be significantly increased in its expression in metastatic PCa,[235] suggesting that cell surface carbohydrate antigens may be important for PCa cell metastasis. To support this hypothesis, it has been shown that exogenous carbohydrate ligands such as galectins rich in galactosyl residues could inhibit spontaneous metastasis of rat MAT-LyLu prostate carcinoma. These observations suggest that, by manipulating the expression/function of some of these adhesion molecules, it might be possible to retard the progression of human PCa. It can anticipated, for example, that transfection of functional E-cadherins and C-CAM cDNAs may slow down the dissemination of metastatic PCa cells.

Tumor cell invasion is mediated by various proteolytic enzymes represented by metalloproteinases and lysosomal proteases such as cathepsins. Human prostate carcinomas and prostate intraepithelial neoplasia express matrilysin, gelatinase A and B, although it appears that only the former exists in the active form.[236] Matrilysin also appears to be highly expressed in prostatic ducts and atrophic glands.[237] It's not known whether these enzymes are associated with PCa progression. On the other hand, hyaluronidase, a matrix-degrading enzyme, has been demonstrated to be expressed at an elevated level and is found to be associated with PCa progression.[238] Prostate specific antigen is generally increased in PCa, however PSA is also detectable in many non-prostate cells such as breast, ovarian, and lung epithelial cells.[239,240,241,242] Recently, PSA has been shown to be a serine protease which activates single-chain urokinase-type plasminogen activator and thus facilitates PCa cell invasion.[243] Also human prostate carcinoma cells have been observed to express urokinase-type plasminogen activator;[244] therefore, inhibitors for uPA may prove to be useful for metastatic PCa.[245]

Angiogenesis, the process of neovascularization, is absolutely required for the growth of solid tumors, which generally cannot grow above 2 mm in diameter without the formation of new blood vessels. A variety of angiogenic factors have been reported and many potential angiogenic inhibitors have been discovered. The growth and progression of human PCa have been associated with the extent of angiogenesis. Linomide, a reported angiogenesis inhibitor with a quinoline-3-carboxamide structure, has recently been shown to inhibit the angiogenesis in TSU and PC-3 androgen-independent human prostate carcinoma xenotransplanted into SCID mice.[246-248] Plus, the hypoxia

due to blocked blood vessel formation also induces dramatic apoptotic death of transplanted tumor cells.[245] These observations suggest that anti-angiogenesis might represent a rational approach for the treatment of PCa.

Adhesion, invasion, and angiogensis are distinct biological processes that are interlinked by various signal transduction pathways. Thus, adhesion responses, via signaling functions, could trigger tumor cell invasion and could also regulate angiogenesis.[246,247] The central importance of cell signaling in connecting all these steps of the metastatic cascade is best illustrated, for example, by 12(S)-HETE [12(S)-hydroxyeicosatetraenoic acid], an eicosanoid derived from the lipoxygenase metabolism of arachidonic acid. This fatty acid is biosynthesized by a wide variety of solid tumors including prostate carcinomas. In many cases the ability of tumor cells to generate 12(S)-HETE has been correlated with their metastatic potential.[248,249] 12(S)-HETE is a physiological signaling molecule which, via activating both protein kinase C and protein tyrosine kinases,[250,251] mediates a wide-spectrum of biological activites such as modulating adhesion molecule expression in both tumor cells and vascular endothelial cells, inducing a non-destructive retraction of endothelial cells, reorganizing tumor cell cytoskeleton leading to an enhanced spreading of tumor cells on matrix, facilitating the release of proteolytic enzyme cathepsin B, promoting tumor cell migration and invasion, and positively regulating angiogenesis.[248,249,252] Furthermore, 12-lipoxygenase, the enzyme responsible for the conversion of arachidonic acid to 12(S)-HETE, has been found to be elevated at the mRNA levels in human PCa.[253] These observations suggest that blocking the positive signaling transduced by molecules such as 12(S)-HETE (for example, using 12-lipoxygenase-specific inhibitors) may help prevent the progression and metastasis of human PCa.

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