Clinically Significant Molecular Markers for Urologic Disease: Focus on Bladder, Kidney, and Prostate Cancer

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Abstract
The most important tools for making therapeutic decisions include stage and grade for bladder and kidney cancer, as well as PSA for prostate cancer. Unfortunately, all tumors of the same stage and grade do not behave the same, indicating the need for additional markers that can provide more accurate prognostic ability. The exponential increase in our understanding of the molecular mechanisms of disease during the past 2 decades has been aided by a large array of bladder, prostate, and kidney cancer biomarkers with potential diagnostic and prognostic value. This article reviews the molecular biomarkers that have shown to be clinically useful in the diagnosis and prognosis of urologic cancers. Ultimately, molecular markers may provide information that can be used to tailor the treatment of individual patients.

Accurate staging information is important to clinicians for providing reliable prognostication regarding recurrence and progression rates and choosing the appropriate treatment for urologic cancers. Currently, most therapeutic decisions are based on cancer stage, grade, and, in the case of prostate cancer, on prostate specific antigen (PSA) levels. Unfortunately, pretreatment nomograms for prostate cancer have at best an area under the ROC curve of 0.75.1-3 As such, additional tools or markers are necessary to improve predictions of which patients are likely to recur or progress. This will help clinicians identify patients at greatest need for adjuvant or neoadjuvant therapy.

The exponential increase in our understanding the molecular mechanism of disease during the past 2 decades has been aided by a large array of bladder, prostate, and kidney cancer biomarkers with potential diagnostic and prognostic value (Table 1). New molecular techniques such as microarray analysis, single nucleotide polymorphism analysis, proteomics, and improved bioinformatics have increased our ability to identify molecular and genetic abnormalities.4 This article reviews the molecular and biochemical biomarkers that have been shown to be clinically useful in the diagnosis and prognosis of urologic malignancies. Ultimately, molecular markers may provide information that can be used to tailor the treatment of individual patients. Molecular markers may also provide new targets for therapeutic intervention.

PSA and Molecular Markers for Prostate Cancer

Prostate cancer is the most common cancer in men. The American Cancer Society estimates that in 2004, 230,110 men will be diagnosed with prostate cancer and 29,900 will die from the disease.5 Classic predictors of prostate cancer recurrence and death include stage, grade, or Gleason score, and serum prostate specific antigen (PSA) level, but these factors are insufficient to adequately predict clinical significance and recurrence of all types of prostate tumors. Moreover, there are several patient management problems associated with prostate cancer that remain unanswered. First, PSA screening results in detection of many prostate cancers that are indolent and unlikely to affect patient survival.6 Second, some patients with clinically localized disease will experience disease recurrence and die from their disease despite adequate local control.2

There is an urgent need for the development of molecular markers to assist in identifying both clinically significant prostate cancers and those with a high propensity for recurrence and progression. For a prognostic marker to be useful, it should provide information that is independent of that provided by standard staging and grading methods. For example, Kattan and colleagues noted that adding novel serum markers such as interleukin-6SR and transforming growth factor-β1 could improve the area under the ROC curve for discriminating between men with and without disease progression using standard parameters from 0.75 to 0.83.7 Several molecular markers associated with prostate cancer have been identified that may be useful clinically to predict outcome or response to therapy, including p16INK4a, P27Kip1, c-Myc, p53, Bcl-2, androgen receptor, E-cadherin, and vascular endothelial growth factor and its receptor.8 These proteins are involved in apoptosis, androgen receptor signaling, signal transduction, cell cycle regulation, cell adhesion, and angiogenesis.

PSA and ProPSA
Prostate-specific antigen (PSA) is a 33 kDa serine protease secreted by the prostate that functions to liquefy the seminal coagulum. While PSA is the biochemical marker of choice in prostate...
cancer diagnosis and prognosis, its sensitivity and specificity are limited. Using a cut-off value of 4 ng/mL, PSA has shown to have a sensitivity of 71% and specificity of 75% with a positive predictive value of 37% in the detection of prostate cancer.9

For more than a decade, a PSA value of 4.0 ng/mL has been considered the optimal cut-off value for discriminating between men with prostate cancer (PSA >4.0 ng/mL) and those with benign prostate disease or no evidence of malignancy. However, a recent report from the Prostate Cancer Prevention Trial (PCPT) showed that PSA levels of 0 to 4.0 ng/mL were associated with a positive predictive value for prostate cancer between 6.6% and 26.9%. Overall, 14.9% of the men enrolled in this trial with prostate cancer had high-grade disease and 25% between 6.6% and 26.9%. Overall, 14.9% of the men enrolled in this trial with prostate cancer had high-grade disease and 25% of men with a PSA between 3.1 ng/mL and 4.0 ng/mL had prostate cancer.10

In an attempt to improve the diagnostic accuracy of PSA as a screening tool, PSA velocity (PSAV; change in PSA concentration at a 1-year interval) of 0.75 ng/mL per year has been used to improve the diagnostic sensitivity of PSA, while a PSA density (PSAD; PSA concentration/density (in cm³)) of the prostate gland estimated by size measurements by transrectal ultrasonography and mathematical modeling >0.15 has been advocated to improve its specificity.

ProPSA, the precursor of PSA, has been advocated to improve the prognostic ability of PSA in the range of 2 to 10 ng/mL. ProPSA is an inactive 244-amino acid protein secreted by prostate epithelial cells. The proPSA fraction accounts for only 6% to 19% of the free PSA in the serum of patients without prostate cancer, but it represents up to 25% to 95% of the free PSA in serum from men with prostate cancer.11 In the PSA range of 2 to 10 ng/mL, proPSA may be useful to detect prostate cancer and prevent unnecessary prostate biopsies.

**The Cell Cycle Regulators p16INK4A and P27Kip1**

The tumor suppressor gene INK4A encodes the protein p16INK4A, which inactivates the G1–phase cyclin dependent kinases, Cdk4 and Cdk6, and results in reduced phosphorylation of pRb. Overexpression of p16INK4A in prostate cancer tissue has been shown to be an independent predictor of early relapse and poor clinical course in patients treated with radical prostatectomy.12-14

P27Kip1 is a cyclin-dependent kinase inhibitor that negatively regulates cell proliferation by preventing progression of cells from G1- to the S-phase of the cell cycle. Moreover, multivariate analysis demonstrated that low P27Kip1 expression in prostate tissue specimens was an independent predictor of treatment failure.
after radical retropubic prostatectomy. In a separate study, the median recurrence-free interval for patients with prostate tumors that had high, moderate, or low p27 levels was 13.7 years, 8.4 years, and 4.7 years, respectively.

**c-Myc**
Amplification of the proto-oncogene c-myc has been shown to be a potential marker of prostate cancer progression. Increasing levels of c-myc in prostate tissue specimens have been demonstrated in the progression of prostatic intra-epithelial neoplasia (PIN) to localized prostate cancer to metastasis with high Gleason score.

**p53**
The tumor suppressor gene, p53, is one of the most frequently mutated genes in human cancer. p53 plays a role in cell cycle regulation, apoptosis, and angiogenesis. Mutations in the p53 gene result in the accumulation of altered p53 proteins with a prolonged half-life. High level p53 accumulation in prostate tissue specimens has been associated with high tumor grade, increased proliferation rate, and predicted a short progression-free interval and poor survival after radical prostatectomy. p53 has also been shown to be an independent prognostic factor concerning the development of distant metastases, progression-free survival, and overall survival for patients with locally advanced prostate cancer who were treated with radiation therapy. Increased p53 nuclear accumulation has been shown to occur more frequently in metastatic, recurrent, and androgen-insensitive prostate cancer compared with clinically localized disease. However, other studies suggest that p53 mutations in prostate cancer are infrequent and failed to show an association between p53 accumulation and metastases or worse outcome.

**Bcl-2**
Bcl-2 is an apoptosis-suppressing oncoprotein and a member of a family of apoptotic regulators that include the cell death antagonists (Bcl-2, Bcl-xL, and Mcl-1) or agonists (Bax, Bak, Bcl-xS, Bad, and Bid). Formation of dimers between agonists and antagonists determines cell response to these oncoproteins. On multivariate analysis, Bcl-2 expression in prostate tissue specimens has been shown to be an independent predictor of disease-free survival for localized prostate cancer. Bcl-2 expression is also associated with resistance to radiotherapy in prostate cancer. Several studies have shown that increased expression of Bcl-2 in prostate cancer confers androgen resistance, especially in advanced disease.

**Androgen Receptor**
Androgen receptor (AR) expression is associated with progression, recurrence, lymph node metastases, and androgen resistance. In multivariate analysis, after controlling for Gleason score and pre-operative PSA level, AR overexpression in prostate tissue specimens predicts poor cancer-specific survival in patients with prostate cancer and lymph node involvement. In addition, overexpression of AR correlates with mutation/amplification of the androgen receptor and with androgen resistance. In patients with androgen-insensitive prostate cancer, mutations in the androgen receptor gene at codon 877 predicted the response of PSA to withdrawal of flutamide. Moreover, AR mutations were shown to occur in response to selective pressure from AR antagonist treatment and these mutations were shown to contribute to prostate cancer progression after androgen ablation therapy.

**E-cadherin**
E-cadherin is a cell adhesion molecule that is involved in cell-cell interactions. Low/altered expression of E-cadherin in prostate tissue specimens has been associated with more aggressive prostate cancer and poor outcome. Multivariate regression analysis showed that the prognostic value of E-cadherin was independent of tumor grade but not of metastasis.

**Vascular Endothelial Growth Factor**
The cytokine vascular endothelial growth factor (VEGF) is an important factor in stimulating cell proliferation and angiogenesis. The VEGF expression in endothelial cells and prostate cancer cells correlates with increased Gleason grade. High expression of VEGF predicts biochemical relapse (ie, increased PSA level prior to pathologic evidence of prostate cancer disease progression) after radical retropubic prostatectomy and the likelihood of death from prostate cancer in patients with prostate cancer managed expectantly, and in patients with hormone-refractory prostate cancer. Moreover, elevated serum levels of VEGF were predictive of earlier disease progression after radiation therapy and radical retropubic prostatectomy.

**Summary on Prostate Cancer Markers**
While there are numerous potential serum and tissue markers that may be useful in predicting recurrence and the malignant potential of prostate cancer, it is too early to begin widespread use of these markers outside of a clinical trial setting. Only prospective evaluation will allow identification of those markers that will withstand the test of time with regard to their clinical utility in the diagnosis, treatment, and follow-up of men with prostate cancer. Moreover, the absence of highly effective adjuvant therapies for treating prostate cancer limits the utility of identifying patients at high risk for early treatment failure.

**Molecular Markers of Bladder Cancer**
In the United States, bladder cancer is the fourth most prevalent cancer in males and the ninth most prevalent cancer in females with 63,210 cases, and 13,180 deaths estimated for 2005. Survival rates for bladder cancers are stage and grade dependent and 5-year survival for bladder tumors confined to the mucosa are significantly higher than for cancers that are muscle-invasive or metastatic. Approximately 25% of bladder tumors are diagnosed beyond the superficial cancer stage. The 2 main potential uses of molecular markers, including urine-based bladder tumor markers (UBBTMs), are detection and surveillance of tumors, while serum or tissue markers are useful for predicting increased risk of disease recurrence and progression. An array of UBBTMs have been applied to the diagnosis and surveillance of bladder cancer including nuclear matrix protein (NMP) 22, bladder tumor antigen (BTA), immunocyt, and Urovysion (a fluorescent in situ hybridization assay).

Several other molecular markers of bladder cancer which have potential diagnostic and prognostic value in the diagnosis, treatment, and follow-up of patients with bladder cancer include p53, E-cadherin, p120, and telomerase.
Urine-Based Bladder Tumor Markers

Urinary-based bladder tumor marker assays use varying strategies to identify changes in the level of urinary markers associated with bladder cancer. These assays are based on biochemical detection of proteins (eg, NMP22) with increased expression in cancer cells, detection of cellular antigens by immunohistochemistry or cytochemistry (Immunocy), or the detection, using fluorescence in situ hybridization (FISH) techniques, of genetic alterations in genes encoding proteins associated with bladder cancer. The Immunocy assay uses antibodies labeled with fluorescent markers for a mucin glycoprotein and carcinoembryonic antigen (CEA). The Urovysion assay detects aneuploidy in chromosomes 3, 7, 9, or 17 via FISH. The bladder tumor antigen, NMP22, Urovysion, and Immunocy assays are commercially available. Classically, cytology has been used along with cystoscopy for the detection of bladder cancer. However, a meta-analysis showed that for grade 1 and 2 bladder tumors, UBBTMs have better sensitivity compared with cytology but are inferior to cytology regarding specificity.48 For grade 3 bladder tumors, most of the UBBTMs had equivalent sensitivity but were not superior to cytology. Currently, most urologists are not routinely using UBBTMs due to their lower specificity as compared with cytology. Nevertheless, there have been reports showing that the use of UBBTMs is cost effective in the surveillance of bladder cancer to lengthen the period of time between cystoscopies.49

Recently, the Food and Drug Administration (FDA) approved the use of several UBBTMs, including Bladdercheck (NMP22) for the diagnosis of bladder cancer in high-risk patients. Bladdercheck is a point-of-care test that provides immediate results and does not require sending a urine specimen to a clinical laboratory for evaluation. A multi-institutional study by Grossman and colleagues utilized NMP22 to evaluate urine specimens from a large cohort of patients at elevated risk for bladder cancer due to factors such as age, history of smoking, or hematuria.50 They found bladder cancer in 6% of their cohort and NMP22 rendered a sensitivity, specificity, and PPV of 55.7%, 85.7%, and 19.7%, respectively. Prospective trials are needed to evaluate the clinical utility of using UBBTMs for screening patients at high risk for bladder cancer.

Markers for Invasive Bladder Cancer

Several markers have been evaluated to identify the risk of disease recurrence and progression in patients with invasive bladder cancer. Approximately 25% of patients with muscle-invasive bladder cancer have lymph node involvement that is not detected by modern imaging techniques.51,52 Patients who receive neoadjuvant chemotherapy prior to cystectomy have an improved survival rate compared to patients who undergo cystectomy alone.53 However, if all patients received chemotherapy, many patients without metastatic disease would undergo “unnecessary” therapy. Unfortunately, there are many patients with organ-confined bladder disease and negative lymph nodes who still recur after cystectomy.51 Identifying those patients at greatest risk for disease progression would allow tailored therapy that may maximize the benefits of chemotherapy. Currently, most molecular markers used in the assessment of bladder cancer provide information on mutations/defects in tumor suppressor genes, oncogenes, and DNA-repair mechanisms.

The Cell Cycle Regulatory Proteins: p53, pRb, p21, p16

Mutations in cell cycle regulatory genes are common findings in human malignancies. Alterations in the p53 and pRb tumor suppressor genes play an important role in the development of bladder cancer. p53 induces cell cycle arrest after DNA damage occurs. Mutations in p53 disrupt its function and prolong its half-life. The accumulation of p53 protein has been shown to correlate with the p53 gene mutation. Nuclear accumulation of p53 in bladder cancer specimens has been shown to be an important prognostic indicator of bladder cancer progression, and an independent predictor of recurrence, and decreased survival on multivariate analysis in patients with T1 disease undergoing radical cystectomy and neoadjuvant chemotherapy.54-56 Additionally, p53-positive tumors may be more chemoresistant.57 Esrig and colleagues found a significant difference in the recurrence and survival rates of 243 patients who were treated by radical cystectomy based on nuclear p53 reactivity.54 In patients with cancer confined to the bladder, the rates of recurrence for stage P1, P2, and P3a tumors that had no detectable nuclear p53 reactivity at 5 years were 7%, 12%, and 11%, respectively, compared to 62%, 56%, and 80%, respectively, for tumors that had p53 immunoreactivity. In a multivariable analysis stratified according to tumor grade, pathological stage, and lymph-node status, nuclear p53 status was an independent predictor (and in cancer confined to the bladder, the only independent predictor) of recurrence and overall survival (P<0.001).54

The retinoblastoma (Rb) tumor suppressor gene also plays an important role in bladder cancer development. The protein encoded by this gene, pRb, inhibits cell cycle progression at the G1- to S-phase checkpoint. pRb binds to 3 types of cellular proteins: cyclins, cyclin-dependent kinase inhibitors, and the E2F family of transcription factors. Altered pRb expression in bladder specimens, due to mutation(s) in the Rb gene, was associated with significantly decreased 5-year survival in patients with muscle-invasive bladder cancer.58

The protein, p21, is a p53-inducible and p53-independent cyclin-dependent kinase inhibitor that can arrest cell replication at the DNA level.59 The protein, p16, also plays a role in tumor progression and loss of p16 expression has been associated with alterations in the RB gene.60 A study by Shariat and colleagues found that altered expression of p53, p21, pRb, and p16 in bladder cancer specimens was common in patients with muscle-invasive bladder cancer and each was independently associated with disease progression and survival.61

Due to the multiple studies identifying p53 as a poor prognostic finding, there is a Phase III randomized study of Methotrexate, Vinblastine, Doxorubicin, and Cisplatin treatment versus observation alone based on p53 gene status in patients with organ-confined transitional cell carcinoma of the bladder who have undergone radical cystectomy and bilateral pelvic lymphadenectomy.62

The Oncogenes: c-ras, mdm-2

Overexpression of the c-ras oncogene product, p21, has been implicated with early recurrence in patients with superficial bladder cancer.53 Multivariate analysis of data from patients with superficial bladder cancer demonstrated that simultaneous mdm-2 and p53 expression in bladder cancer specimens were independent predictors of disease progression and survival.54,65

The Proliferation-Associated Antigen: Ki67

The Ki67 protein is only expressed in the nuclei of proliferating cells. Ki67 expression in bladder cancer specimens has been shown to correlate with tumor progression and shorter recurrence-free intervals in patients with superficial bladder cancer.66,67
The Angiogenic Factors

The angiogenic factors are acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and thrombospondin-1. Acidic fibroblast growth factor has been associated with cell proliferation and angiogenesis. Urinary aFGF has also been shown to correlate with tumor stage.68 Similarly, urinary bFGF has also been correlated with muscle invasive disease.69 Vascular endothelial growth factor (VEGF) induces tumor angiogenesis. Urinary levels of VEGF correlate with bladder tumor recurrence rates and tumor grade.70

Thrombospondin-1, an extracellular matrix glycoprotein, is the only inhibitor of angiogenesis that has been studied in human bladder cancer. Bladder cancer cell lines isolated from high and low grade tumors express low levels of thrombospondin-1 in vitro.71

The Cell Adhesion Molecules: E-cadherin, p120, α-β-, and γ-catenin

The cadherins are the most important cell adhesion molecules. The loss of membranous E-cadherin in bladder cancer specimens correlates with high grade (grades 2 and 3) and advanced tumor stage (stages T3 and T4) and lower 5-year survival.72 The p120 protein binds to the cytoplasmic portion of E-cadherin and is a substrate of receptor tyrosine kinases. Loss of p120 expression has been shown to occur in greater than 80% of bladder cancer specimens and is correlated with high grade, stage, and overall poor prognosis.73 Loss of α-, β-, and γ-catenin immunoreactivity in bladder cancer specimens was correlated with advanced tumor grade, stage, and loss of membranous γ-catenin was associated with a worse prognosis in patients with advanced bladder cancer.74

Telomerase

Telomeres are repetitive sequences at the end of chromosomes. Chromosomes lose nucleotides from their telomeres until a set length remains and the cell undergoes apoptosis. However, the telomeric sequence can be reattached by the enzyme telomerase. Telomerase is not normally expressed in cells. However, in cancer cells, telomerase can be expressed at high levels. The levels of mRNA in bladder cancer specimens encoding the catalytic component of telomerase correlates with stage and grade of differentation in bladder cancer.75

Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Metalloproteinases (TIMPs)

Matrix metalloproteinases are enzymes that degrade the extracellular matrix and basement membrane, and play a role in the migration and invasion of cells. Tissue inhibitors of metalloproteinases inhibit the action of MMPs. Multivariante analysis of data in patients with urothelial cancer with muscular invasion or lymph node metastasis who underwent complete resection demonstrated that high serum levels of MMPs were independent predictors of disease recurrence.76

Summary of Bladder Cancer Markers

Currently, bladder cancers are staged based on tumor-node-metastasis (TNM) criteria; however, molecular markers such as p53 will be used increasingly in the future to identify those patients at highest risk for disease progression. Although urine-based molecular markers have a current limited role in surveillance for bladder cancer, in the future, greater use of these markers will allow less invasive monitoring for bladder cancer and potentially serve as screening tools in identifying high-risk patients.

Renal Cell Carcinoma Biomarkers

Cancers of the kidney or renal pelvis will account for approximately 36,000 new cases, and 12,600 deaths in the United States in 2005.46 Of these patients, 20% to 30% will present with metastatic disease and approximately 30% will present with clinically localized disease that will recur after undergoing a nephrectomy.77 Patients with recurrence or metastases are treated with immune-based therapies; but response rates range between 15% to 30%, and there is no benefit to adjuvant immune-therapy in high-risk patients.78

Traditionally, stage, grade, and performance status (overall patient health) have been used for prognostication in renal cell carcinoma (RCC).79 There have been many molecular markers identified that are associated with RCC including markers for angiogenesis/hypoxia, proliferation, cell-cycle regulation, and cell adhesion.79 A thorough discussion of each is beyond the scope of this review but it is important to note significant advances in the understanding of angiogenesis/hypoxia pathways that is leading to the introduction of multiple potentially useful adjuvant therapies.

The molecular mechanisms of the hypoxia-inducible pathway which affects angiogenesis, glucose transport, glycolysis, apoptosis, cell migration, and proliferation may be responsible for the radio- and chemotherapy resistance of RCC.80 The steps in the hypoxia-inducible pathway link genetic abnormalities such as the Von Hippel Lindau gene, growth factors and their receptors (VEGF and EGF), carbonic anhydrase IX (CA IX), and the Ras/Raf signaling pathway.79

Carbonic anhydrase IX is one of the best markers for clear cell RCC. It is expressed in tissue from patients with clear cell RCC but not in normal tissues.81 Carbonic anhydrase IX is a member of the carbonic anhydrase family that is thought to play a role in pH regulation during periods of hypoxia. Low CA IX staining by IHC was shown to be an independent prognostic indicator of poor survival in patients with metastatic RCC.82 High CA IX staining is associated with IL-2 response, which may explain why papillary and chromophobe tumors (which do not express CA IX) respond poorly to immune-therapy.79

Many drugs/agents have been introduced to target steps in the hypoxia-inducible angiogenesis pathways, including antibodies to CA IX and epidermal growth factor receptor (EGFR), inhibitors of EGFR tyrosine kinase, thalidomide (an anti-angiogenesis agent), inhibitors of VEGFR, and inhibitors of small molecules in the pathway such as rapamycin.79

Assessing Prognosis in Patients With Renal Cell Carcinoma Using a Protein Expression Profile Nomogram

A large number of molecular markers are currently being investigated to predict patients at increased risk for disease recurrence and progression. Identifying patients at higher risk of recurrence will allow better targeting of therapies and improve patient selection for adjuvant therapy trials. Increased immunostaining of Ki-67, p53, vimentin, and gelsolin, and decreased immunostaining of CA IX, CA XII, phosphatase, tensin homologue deleted on chromosome 10 (PTEN), and epithelial cell adhesion molecule (EpCAM) correlate with worse survival.83 On multivariate analysis of data from patients with RCC, CA IX, vimentin, and p53 were statistically significant predictors of
survival independent of clinical predictors including tumor stage and grade, performance status, and presence of metastasis. A nomogram using these protein expression profiles for clear cell RCC performed better than standard clinical predictors in predicting disease-free survival.83

Conclusion

There has been an explosion in the number of molecular markers of prostate, bladder, and kidney cancer over the last 20 years that provide additional prognostic information to stage and grade in the treatment and follow-up of patients with these types of cancers. Ultimately, molecular markers may be useful in predicting which patients will benefit from adjuvant therapies. Moreover, certain molecular markers will allow tailored therapy with specific drugs that will target the appropriate aberrant pathway for each tumor affecting the prostate, bladder, and kidneys.1M


