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Emerging Critical Role of Molecular Testing in Diagnostic Genitourinary Pathology

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Abstract

Context—The unprecedented advances in cancer genetics and genomics are rapidly affecting clinical management and diagnostics in solid tumor oncology. Molecular diagnostics is now an integral part of routine clinical management in patients with lung, colon, and breast cancer. In sharp contrast, molecular biomarkers have been largely excluded from current management algorithms of urologic malignancies.

Objective—To discuss promising candidate biomarkers that may soon make their transition to the realm of clinical management of genitourologic malignancies. The need for new treatment alternatives that can improve upon the modest outcome so far in patients with several types of urologic cancer is evident. Well-validated prognostic molecular biomarkers that can help clinicians identify patients in need of early aggressive management are lacking. Identifying robust predictive biomarkers that will stratify response to emerging targeted therapeutics is another crucially needed development. A compiled review of salient studies addressing the topic could be helpful in focusing future efforts.

Data Sources—A PubMed (US National Library of Medicine) search for published studies with the following search terms was conducted: *molecular*, *prognostic*, *targeted therapy*, *genomics*, *theranostics and urinary bladder cancer*, *prostate adenocarcinoma*, and *renal cell carcinoma*. Articles with large cohorts and multivariate analyses were given preference.

Conclusions—Our recent understanding of the complex molecular alterations involved in the development and progression of urologic malignancies is yielding novel diagnostic and prognostic molecular tools and opening the doors for experimental targeted therapies for these prevalent, frequently lethal solid tumors.

The completion of the cancer genome project has paved the way to great advances in cancer genetics and genomics that are beginning to affect clinical management and diagnostics in solid tumor oncology. Molecular diagnostics is now an integral part of routine clinical management in patients with lung, colon, and breast cancer. In sharp contrast, molecular biomarkers have been largely excluded from current management algorithms of urologic malignancies. The need for new treatment alternatives and for prospectively validated prognostic molecular biomarkers cannot be overestimated. Furthermore, identifying robust predictive biomarkers that will stratify response to emerging targeted therapeutics is needed. The following discussion focuses on candidate biomarkers that will soon make their transition to daily clinical management of patients with urologic tumors.

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UROTHELIAL CARCINOMA OF URINARY BLADDER

Pathogenetic Pathways in Urothelial Carcinoma: Parallels of 2 Distinct Biologic Phenotypes

Accumulating molecular genetic evidence supports 2 distinct broad pathogenetic pathways for bladder cancer (BC) development that seem to parallel the contrasting biologic and clinical phenotypes of superficial (non–muscle invasive) and muscle invasive urothelial carcinoma. While most invasive urothelial carcinomas are thought to originate through progression from dysplasia to flat carcinoma in situ (CIS) and high-grade noninvasive lesions, superficial urothelial lesions are thought to originate from benign urothelium through a process of urothelial hyperplasia. Progression from superficial to muscle invasive disease accounts for only a small percentage (10%–15%) of the entire pool of noninvasive lesions. Genetic instability is the key to the accumulation of genetic alterations required for progression to muscle invasive bladder cancer (MI-BC).[1–4]

Clinically, a significant proportion of superficial tumors (pTa and pT1) are deemed to recur after transurethral resection of the bladder (TURB) with only a minority of cases enduring progression to high-grade carcinoma that will ultimately progress to MI-BC.

Three primary genetic alterations have consistently been associated with the pathogenesis pathway of superficial non–muscle invasive bladder cancer (NMI-BC). These include tyrosine kinase receptor FGFR-3, HRAS,[5] and PIK3CA.[3,6,7] Alterations in the RAS-MAPK and PI3K-Akt pathways are in large part responsible for promoting cell growth in urothelial neoplasia. Activating mutations in *RAS* lead to activation of mitogen-activated protein kinase (MAPK) and PI3K pathways. Not surprisingly, activating mutations in upstream tyrosine kinase receptor (*FGFR3*) seem to be mutually exclusive with *RAS* mutations, given that both signal through a common downstream pathway in urothelial oncogenesis. *PIK3CA* and *FGFR3* mutations generally co-occur, suggesting a potential synergistic additive oncogenic effect for *PIK3CA* mutations.

The pathogenic pathway for MI-BC primarily involves alterations in tumor suppressor genes involved in cell cycle control, including *p53, p16*, and *Rb*.[1,4,8] As illustrated in Figure 1, progression of the subset of superficial BC into higher-grade muscle invasive disease is similarly based on alterations in *p53* and *Rb* tumor suppressor genes (see Figures 1 and 2).

Prognostic Biomarkers in Superficial Non–Muscle Invasive and Muscle Invasive Urothelial Carcinoma

Established clinicopathologic prognostic parameters for NMI-BC include pTNM stage, World Health Organization (WHO)/International Society of Urological Pathology grade, tumor size, tumor multifocality, presence of CIS, and frequency and rate of prior recurrences.[9] Prognostic parameters that can accurately predict progression in patients with superficial tumors are actively sought to further facilitate identification of those in need of vigilant surveillance and an aggressive treatment plan. The latter is especially pertinent in a disease for which the financial burden and quality of life for patients under surveillance is significant. Per patient, bladder cancer is the most expensive single solid tumor in the United States, with a staggering $3 billion dollars estimated annual cost to our health care system.[10] Furthermore, given the current poor outcome of muscle invasive disease (60% or less overall survival rate), markers that can improve prognostication in this group of patients are equally needed.[11–13]

As our understanding of molecular pathways involved in urothelial oncogenesis increasingly comes into focus, the translational field of molecular prognostication, theranostics, and targeted therapy in BC has sharply gained momentum.[14–32] Evidently, a rigorous validation

process ought to precede the incorporation of such molecular biomarkers in clinical management. Initial retrospective discovery studies need to be confirmed and validated in large independent cohorts. The subsequent crucial step is validating the robustness of the proposed biomarker in a well-controlled, multi-institutional, randomized prospective study. Such a prospective study should support an additive role for the inclusion of the new biomarkers over existing management algorithm(s).[33,34] It is the lack of the latter crucial steps in biomarker development that has hindered the streamlining of clinical utilization of several promising markers in patient management of BC.[35,36]

Chromosomal Numerical Alteration—Chromosome 9 alterations are the earliest genetic alterations in both of the above-described divergent pathways of BC development. They are responsible for providing the necessary milieu of genetic instability that in turn allows for the accumulation of subsequent genetic defects. Several additional structural/numerical somatic chromosomal alterations are also a common occurrence in BC. Among these, gains of chromosome arms 3q, 7p, and 17q and deletions in 9p21 (p16 locus) are of special interest given their potential diagnostic and prognostic value.[37,38] A multitarget interphase fluorescence in situ hybridization (FISH)–based urine cytogenetic assay was developed[39] on the basis of the above numerical chromosomal alterations and is now commercially available and commonly used in clinical management. Initially approved by the US Food and Drug Administration (FDA) for surveillance of recurrence in previously diagnosed patients with BC, the test subsequently gained approval for screening in high-risk (smoking exposure) patients with hematuria. The multicolor FISH assay appears to enhance the sensitivity of routine urine cytology analysis and can be used in combination with routine cytology as a reflex test in cases with atypical cytology. A sensitivity range of 69% to 87% and a specificity range of 89% to 96% have been reported with the multitarget interphase FISH assay.[40] With the exception of 1 study,[41] the multitarget FISH urine assay has been shown to be more sensitive than routine cytology. An additional advantage of urine-based FISH testing could be the anticipatory positive category of patients identified by such assay. This refers to patients for whom the FISH assay detects molecular alteration of BC in urine cells several months before cancer detection by cystoscopy or routine cytology. In the study by Yoder et al,[42] two-thirds of the 27% of patients categorized as “anticipatory positive” developed BC that was detected by cystoscopy up to 29 months later. Such encouraging results point to the great potential of molecular testing in early detection and allocation of vigorous frequent follow-up cystoscopy in at-risk patients.[43–46]

Finally, several recent studies[37,38,47–49] have pointed to the potential prognostic role for multitarget FISH analysis. Maffezzini et al[48] were able to demonstrate that low-risk patients with FISH-positive results, defined as 9p21 loss/chromosome 3 abnormalities, had a higher rate of recurrence as compared to FISH-negative patients. The recurrence rate was even greater for patients with a high-risk positive FISH result (chromosome 7/chromosome 17 abnormality). Both Kawauchi et al,[37] using bladder washings, and Kruger et al,[38] using formalin-fixed, paraffin-embedded transurethral biopsy samples, independently found that loss of 9p21 predicts recurrence but not progression in superficial BC. Furthermore, both Savic et al[47] and Whitson et al[49] found that urine cytology and FISH in post-BCG (Bacillus Calmette-Guerin) bladder washings were predictive of failure of BCG therapy in patients with non–muscle invasive disease. Such a promising prognostic role for multitarget FISH awaits prospective randomized trial before clinical integration into a practice algorithm. Clear guidelines for interpretation and test performance parameters in terms of interobserver reproducibility are also needed.[50]

Receptor Tyrosine Kinases—Recent studies have pointed to the potential prognostic value of evaluating the expression of receptor tyrosine kinases such as FGFR3, epidermal

growth factor receptor (EGFR), and other ERB family members (HER2/*neu* and ERBB3)[4,21,51–61] in superficial and muscle invasive bladder cancer disease.

FGFR3 mutations are a common occurrence in NMI-BC and can theoretically be used alone or combined with *RAS* and *PIK3CA* oncogenes as markers of early recurrence during surveillance. Both Zuiverloon et al[62] and Miyake et al[63] independently developed sensitive polymerase chain reaction (PCR) assays for detecting *FGFR3* mutations in voided urine. A positive urine sample finding by the assay developed by Zuiverloon's group was associated with concomitant or future recurrence in 81% of NMI-BC cases. An even higher positive predictive value of 90% was achieved in patients with consecutive *FGFR3*-positive urine samples. Similarly, Miyake et al[63] were able to detect *FGFR3* mutations in 53% of their 45 patients and found their assay to be superior to cytology (78% versus 0%) in detecting post-TURB recurrence in NMI-BC harboring *FGFR3* mutations in primary tumors.

Kompier et al[7] were recently able to develop a multiplex PCR assay for mutational analysis detecting the most frequent mutation hot spots of *HRAS, KRAS, NRAS, FGFR3,* and *PIK3CA* in formalin-fixed, paraffin-embedded TURB samples. They demonstrated evidence of at least 1 mutation in up to 88% of low-grade NMI-BC samples. Hernandez et al[64] revealed that *FGFR3* mutations were more common among low malignant potential neoplasms (77%) and TaG1/TaG2 tumors (61%/58%) than among TaG3 tumors (34%) and T1G3 tumors (17%). On multivariable analysis, mutations were associated with increased risk of recurrence in NMI-BC.

Van Rhijn et al[65] previously proposed a molecular-grade parameter (mG), based on a combination of *FGFR3* gene mutation status and MIB-1 index, as an alternative to pathologic grade in NMI-BC. Recently, the same group[51] elegantly validated their previously proposed mG parameter and compared it to the European Organization for Research and Treatment of Cancer (EORTC) NMI-BC risk calculator[66] (weighted score of 6 variables including WHO 1973 grade, stage, presence of CIS, multiplicity, size, and prior recurrence rate). The mG was more reproducible than the pathologic grade (89% versus 41%–74%). *FGFR3* mutations significantly correlated with favorable disease parameters, whereas increased MIB-1 was frequently seen with pT1, high grade, and high EORTC risk scores. EORTC risk score remained significant in multivariable analyses for recurrence and progression. Importantly, mG also maintained independent significance for progression and disease-specific survival (DSS), and the addition of mG to the multivariable model for progression increased the predictive accuracy from 74.9% to 81.7%.

Several studies have suggested a negative prognostic role forHER2/*neu* amplification/overexpression in MI-BC.[33,67–69] Most recently, Bolenz et al[59] found that HER2-positive patients with MI-BC were at twice increased risk for recurrence and cancer-specific mortality on multivariable analyses adjusted for pathologic stage, grade, lymphovascular invasion, lymph node metastasis, and adjuvant chemotherapy.

p53, Cell Cycle Regulators, and Proliferation Index Markers—Early studies by Sarkis et al[70–72] revealed p53 alterations to be a strong independent predictor of disease progression in BC (superficial, muscle invasive, as well as CIS). p53 has also been shown to be predictive of increased sensitivity to chemotherapeutic agents that lead to DNA damage.[73–75] Recent studies have further supported the prognostic role of p53[76] in pT1–pT2 tumors of patients after cystectomy, showing an independent role for p53 alteration in predicting disease-free survival and DSS.

Among other G1-S phase cell cycle regulators, cyclins D3 and D1, p16, p21, and p27 have also been evaluated as prognosticators in NMI-BC.[32,75,77–81] Lopez-Beltran et al[77]

confirmed their initial finding[75] of the independent prognostic role of cyclin D3 and cyclin D1 overexpression in predicting progression in pTa and pT1 tumors. Their findings, however, are in contrast to subsequent findings by Shariat et al,[32] emphasizing the need for further validation in multi-institutional large cohorts of patients.

A synergistic prognostic role for combining p53 evaluation with other cell cycle control elements such as pRb, cyclin E1, p21, and p27 is emerging in both NMI-BC and MI-BC.[24,26,73,82,83] In a study by Shariat et al,[26] patients who have NMI-BC, with TURB demonstrating synchronous immunohistochemical alterations in all 4 tested markers (p53, p21, pRb, and p27), were at significantly lower likelihood of sustaining disease-free survival than patients with only 3 markers. The negative predictive effect was decreased with decreasing number of altered markers (3 versus 2 versus 1). Similarly, the same group[83] later found that combining p53, p27, and Ki-67 assessment in pT1 radical cystectomy specimens improved the prediction of disease-free survival and DSS.

A similar synergistic prognostic role for the assessment of immunoexpression of multiple molecular markers (p53, pRb, and p21) was demonstrated by Chatterjee et al[24] in patients undergoing cystectomy for MI-BC. The superiority of multimarker approach compared to prior single-marker approach certainly merits further assessment.[70–72,84] Such multimarker approach of prognostication could soon be integrated in the standard of care in BC management once additional multi-institutional prospective trials confirm the above-mentioned promising findings.

Tumor proliferation index measured immunohistochemically by either Ki-67 or MIB-1 has been consistently shown to be a prognosticator in bladder cancer.[51,65,75,80,85–88] As mentioned above, tumor proliferation index (MIB-1) in NMI-BC plays a prognostic role as one of the elements of the mG parameter forwarded by Van Rihjn et al.[65] The independent prognostic role of proliferation index measured by Ki-67 has also been shown. In the study by Quintero et al,[85] Ki-67 index in NMI-BC TURB biopsy samples was predictive of progression free survival and DSS.

A similar role for proliferation index assessment as prognosticator is established in MI-BC. Building on initial findings of significance in an organ-confined subset of MI-BC by Margulis et al,[86] a recent report of the bladder consortium multi-institutional trial (7 institutions; 713 patients) again confirmed the role of proliferation index, measured in cystectomy specimens.[87] In the later study, Ki-67 improved prediction of both progression free survival and DSS when added to standard prediction models, supporting a role for proliferation index assessment in stratifying patients for perioperative systemic chemotherapy. This has certainly taken Ki-67 assessment a step closer to clinical applicability in MI-BC.

Gene Expression and Genomic Analysis—Several recent gene expression studies have highlighted sets of differentially expressed genes that may play a role in diagnosis and in predicting recurrence and progression in BC.[1,15–17,29,31,89–97] In a landmark study by Sanchez-Carbayo et al,[15] oligonucleotide arrays were used to analyze transcript profiles of 105 cases of NMI-BC and MI-BC. Hierarchical clustering and supervised algorithms were used to stratify bladder tumors by stage, nodal metastases, and overall survival. Predictive algorithms were 89% accurate for tumor staging using genes differentially expressed in superficial versus muscle invasive tumors. Accuracies of 82% (entire cohort) and 90% (MI-BC) were also obtained for predicting overall survival. A genetic profile consisting of 174 probes was able to identify patients with positive lymph nodes and poor survival.

Recently, Birkhahn et al[29] attempted to identify genes predictive for recurrence and progression in Ta category by using a quantitative pathway-specific approach in a set of 24 key genes by real-time PCR in tumor biopsy specimens at initial presentation. They found *CCND3* expression to be highly sensitive and specific for recurrence (97% and 63%, respectively). While *HRAS*, *E2F1, BIRC5/survivin,* and *VEGFR2* were predictive for progression by univariate analysis, on multivariable analysis the combination of *HRAS, VEGFR2*, and *VEGF* expression status predicted progression with an impressive 81% sensitivity and 94% specificity.

In a recent study, Lindgren et al[95] suggested that a combined molecular and histopathologic classification of BC may prove more powerful in predicting outcome and stratifying treatment. The authors combined gene expression analysis, whole-genome array comparative genomic hybridization analysis, and mutational analysis of *FGFR3, PIK3CA, KRAS, HRAS, NRAS, TP53, CDKN2A,* and *TSC1* to identify 2 intrinsic molecular signatures (MS1 and MS2). Genomic instability was the most distinguishing genomic feature of MS2 signature, independent of TP53/MDM2 alterations. Their genetic signatures were validated in 2 independent data sets that successfully classified urothelial carcinomas into low-grade and high-grade tumors, as well as NMI-BC and MI-BC, with high precision and sensitivity. Furthermore, a gene expression signature that independently predicts metastasis and disease free survival was also defined. This clearly supports the role of molecular grading as a complement to standard pathologic grading.

Mengual et al[31] performed gene expression analysis in 341 urine samples from patients with NMI-BC and MI-BC and 235 controls by TaqMan Arrays (Applied Biosystems, Carlsbad, CA). A 12+2 gene expression signature demonstrated a staggering 98% sensitivity and 99% specificity in discriminating between BC and control and 79% sensitivity and 92% specificity in predicting tumor aggressiveness (NMI-BC versus MI-BC). The signature was then validated in voided urine samples and maintained accuracy. In an integrated genetic/epigenetic approach, Serizawa et al[94] prospectively performed mutational screening of a set of 6 genes (*FGFR3, PIK3CA, TP53, HRAS, NRAS,* and *KRAS*) and quantitatively assessed promoter methylation status of 11 additional genes (*APC, ARF, DBC1, INK4A, RARB, RASSF1A, SFRP1, SFRP2, SFRP4, SFRP5,* and *WIF1*) in NMI-BC tumor biopsy specimens and corresponding urine samples from 118 patients and 33 controls. A total of 95 oncogenic mutations and 189 hypermethylation events were detected. The total panel of markers provided a sensitivity of 93% and 70% in biopsy specimens and urine samples, respectively. *FGFR3* mutations in combination with 3 methylation markers (*APC, RASSF1A,* and *SFRP2*) provided a sensitivity of 90% in tumors and 62% in urine, with 100% specificity.

With the impending cost and turnaround time advantages of next-generation sequencing technology, the power of genomic approach in providing a noninvasive diagnostic and predictive tool should be actively pursued in a prospective large cohort.

Epigenetic Alterations—Epigenetic analysis is also gaining momentum in BC as a noninvasive diagnostic tool for screening and surveillance. As a prognostic tool, epigenetic analysis has similarly shown promising potential for patients with BC.[94,98–110]

In an early study by Catto et al,[105] hypermethylation analysis at 11 CpG promoter islands was performed by methylation-specific PCR (MSP) in 116 bladder and 164 upper urinary tract tumors. Promoter methylation was found in 86% of all tumors and the incidence was relatively higher in upper tract tumors than BC. Methylation was associated with advanced tumor stage and higher tumor progression and mortality rates. Most importantly, on

multivariate analysis, methylation at the *RASSF1A* and *DAPK* gene promoters was associated with disease progression independent of tumor stage and grade.

The same group,[110] using quantitative MSP at 17 candidate gene promoters, found 5 loci that were associated with progression (*RASSF1A, E-cadherin, TNFSR25, EDNRB*, and *APC*). Multivariate analysis revealed that the overall degree of methylation was more significantly associated with subsequent progression and death than tumor stage. An epigenetic predictive model developed with artificial intelligence techniques identified the likelihood and the timing of progression with 97% specificity and 75% sensitivity.

Among the studies evaluating the diagnostic role of promoter hypermethylation, the study by Lin et al[99] used MSP assay for 4 genes (*E-cadherin, p16, p14,* and *RASSF1A*) in primary tumor DNA and urine sediment DNA obtained from 57 patients with bladder cancer; MSP detected hypermethylation in the urine of 80% of tested patients. Hypermethylation analysis *of E-cadherin, p14,* or *RASSF1A* in urine sediment DNA detected 85% of superficial and low-grade bladder cancers, 79% of high-grade bladder cancers, and 75% of invasive bladder cancers. The study highlighted the great potential of such tests in detecting NMI-BC. A similar diagnostic role was also found by Cabello et al[101] using a novel technology, methylation-specific multiplex ligation-dependent probe amplification assay, to analyze 25 tumor suppressor genes that have been thought to play a role in BC oncogenesis. The tumor suppressor genes included *PTEN, CD44, WT1, GSTP1, BRCA2, RB1, TP53, BRCA1, TP73, RARB, VHL, ESR1, PAX5A, CDKN2A,* and *PAX6*. The authors found *BRCA1, WT1,* and *RARB* to be the most frequently methylated tumor suppressor genes, with receiver operating characteristic curve analyses revealing significant diagnostic accuracies in 2 additional validation sets.

Finally, assessment of promoter hypermethylation is giving additional insights on BC oncogenesis. Promoter hypermethylation of CpG islands and “shores” controlling microRNA expression is one such example.[103]

Ploidy and Morphometric Analysis—Several studies have pointed to the independent prognostic role of ploidy and S phase analysis in NMI-BC.[88,111–117] Ploidy analysis can be performed by flow cytometry or automated image cytometry and is applicable to urine cytology specimens as well as biopsy supernatant[112] and disaggregated TURB formalin-fixed, paraffin-embedded specimens.[113]

In one of the largest studies assessing DNA ploidy in NMI-BC (377 [test set]; 156 [validation set]), Ali-El-Dein et al[111] found that stage, DNA ploidy, tumor multiplicity, history of recurrence, tumor configuration, and type of adjuvant therapy independently predict recurrence. Recurrence at 3 months, grade, and DNA ploidy were the only predictors of progression to muscle invasion. The constructed “Predictive Index” model successfully stratified patients in a second validation set into 3 risk groups. Likewise, Baak et al[113] were able to show ploidy status and S phase, measured by image cytometry, to be strong independent predictors of recurrence and progression in patients with pTa and pT1 tumors.

Despite all the above-mentioned encouraging data, ploidy analysis still awaits prospective randomized trials to bring image cytometry or flow cytometry technique into current standard management algorithms for NMI-BC.

Emerging Biomarkers—Other biomarkers with encouraging but less robust data on their potential prognostic role in BC include tumor microenvironment markers, such as cell adhesion markers E cadherin and N cadherin,[20,118] and angiogenesis modulators such as HIF-1a, HIF-2, vascular endothelial growth factor (VEGF), CAIX, and

thrombospondin-1.[18,19,119–123] In addition, our group and others have demonstrated a potential prognostic role for mammalian target of rapamycin (mTOR) pathway markers.[20,118,124–126] Other markers such as Aurora-A have also been investigated in this setting.[127,128] Finally, microRNA profile alterations will certainly be a new area of heavy investigation as a noninvasive diagnostic tool and as a prognostic tool in patients with BC.[104,129–131]

Targeted Therapy and Predictive Markers in Bladder Cancer—As illustrated in Figure 1, RTK-HRAS-MAPK, mTOR, as well as angiogenesis pathway of the tumor microenvironment, offer promising opportunities for new targeted treatments of bladder cancer.[7,25,124,126,132–145] Among receptor tyrosine kinases, HER2/*neu* has been targeted in a multicenter phase II trial reported in 2007 by Hussain et al.[146] Forty-four patients with advanced BC who had metastatic disease and evidence of tumor HER2 positivity by either immunohistochemistry (IHC), FISH, or elevated serum extracellular HER2/*neu* domain levels were treated with a combination of carboplatin, paclitaxel, and gemcitabine with the humanized monoclonal anti-HER2 antibody trastuzumab. Approximately 70% of treated patients demonstrated partial (59%) or complete (11%) response with a median overall survival of 14.1 months. A higher response rate was associated with patients with 3+ HER2/*neu* expression by IHC or HER2/*neu* gene amplification by FISH than with those with 2+ HER2/*neu* expression and FISH-negative tumors. Interestingly, in contrast to the strongly correlated HER2/*neu* gene amplification and 3+ IHC HER2/*neu* overexpression usually seen in breast cancer, most HER2/*neu* overexpression in bladder urothelial carcinoma is not associated with HER2/*neu* gene amplification.[147]

A second ongoing randomized phase II trial is evaluating the role of the anti–EGFR recombinant humanized murine monoclonal antibody cetuximab. Patients with metastatic, locally recurrent, or nonresectable disease are treated with standard gemcitabine and carboplatin (GC) chemotherapy with or without cetuximab. By blocking epidermal growth factor binding to the extracellular EGFR domain, cetuximab inhibits downstream signal transduction pathway, accounting for its antiproliferative activity in solid tumors. In BC, a potential added synergistic antiangiogenic effect could be also at play.[148,149] A separate phase II Cancer and Leukemia Group B trial investigated the role of a small molecule inhibitor of EGFR (gefitinb) in patients with advanced bladder cancer. Gefitinib in combination with GC had no survival or time to progression advantage over GC alone.[150,151]

Based on the results of a phase II single-arm trial[152] suggesting a therapeutic advantage for lapatininb (a tyrosine kinase inhibitor targeting both EGFR and HER2) in EGFR- or HER2-positive BC tumors, a phase II randomized trial is underway that is looking at the role of maintenance with lapatininb (versus placebo) for patients with objective response to first-line chemotherapy who test positive for either marker by IHC or FISH studies.

In an attempt to target BC dependence on angiogenesis, monoclonal antibodies and small molecule inhibitors of angiogenesis are under investigation in advanced disease. An initial phase II trial evaluating the role of bevacizumab—a recombinant humanized monoclonal anti-VEGF antibody—in combination with GC as a first-line therapy in metastatic BC revealed objective response in two-thirds of patients, with 6 of 43 patients showing complete response albeit with significant treatment-related toxicity.[153] A CALBG (Cancer and Leukemia Group B) phase III randomized trial for GC, with and without bevacizumab, for metastatic urothelial carcinoma is now underway, as well as other phase II trials for bevacizumab in combination with other chemotherapeutic agents such as M-VAC (methotrexate, vinblastine, adriamycin, and cisplatin).[154]

The role of multitarget tyrosine kinase inhibitors in BC has also been investigated with mixed results. While sorafenib (inhibits Raf kinase, *PDGFR-B, VEGFR-2,* and *VEGFR-3*) phase II trials have failed to show significant objective response, sunitinib (inhibits *VEGFR-2* and *PDGFR-B*) has shown a more promising effect in a recent phase II trial involving 77 patients at Memorial Sloan-Kettering Cancer Center (New York, New York) in which clinical benefits were observed in almost one-third of the patients. A subsequent randomized double-blind phase II trial is underway, investigating the efficacy of sunitinib in delaying progression as a maintenance agent in patients with initial response to standard chemotherapy.[155] Finally, given the recent evidence suggesting the presence of mTOR pathway alterations in BC, a phase II trial evaluating the potential role of everolimus, an inhibitor of mTOR pathway, in advanced BC is underway.[124–126]

In summary, as our understanding of the complex molecular mechanisms involved in BC development has come into a sharper focus, our approaches to diagnosis and management of bladder cancer continue to evolve. In the not-so-distant future, the current paradigm of clinicopathologic-based prognostic approach to predicting progression in superficial BC[90,156–158] is to be supplemented by a molecular-guided approach based on some of the markers listed in Table 1.* Several new targeted therapy agents are under investigation in randomized trials in combination with standard chemotherapy agents, either as first-line treatment or on a maintenance basis to prolong response in patients with advanced bladder cancer.

PROSTATE ADENOCARCINOMA

The continuous debate on whether current serum prostate-specific antigen (PSA)–based screening strategies are potentially leading to “overtreatment” of a subset of patients with prostate cancer (PCa) has further fueled the interest in pursuing clinicopathologic and molecular parameters that may help identify patients with biologically “significant” prostate cancers.[162,163] A parallel pursuit of clinicopathologic algorithms and criteria that can accurately predict “insignificant” PCa tumors is also gaining momentum. The latter are generally defined as tumors that lack the biologic potential to affect disease-specific mortality and morbidity within a given patient life expectancy. As alternative PCa management approaches, such as “proactive surveillance,” are increasingly offered, accurate identification of insignificant PCa becomes more pressing.

Meanwhile, prostate needle biopsy continues to be the gold standard for establishing the diagnosis of PCa in patients with elevated serum PSA levels and/or positive digital rectal examination results. Established clinicopathologic parameters including clinical stage, pathologic stage, histologic Gleason grade, and serum PSA levels are the sole guiding tools of prognostication and disease management in PCa.[164–166]

Given the existing need to improve upon the prognostic and predictive power of the above-established parameters, an extensive list of molecular biomarkers have been evaluated in the last decade for their potential role in enhancing our ability to predict disease progression, response to therapy, and survival.[167–171] These research efforts have been greatly facilitated by the wealth of information garnered from gene expression array studies and by sophisticated bioinformatics tools evaluating the overwhelming data sets generated from genomic, transcriptomic, and proteomic studies. These genomic technologies continue to yield new markers that can in turn be evaluated for clinical utility in a high-throughput manner with IHC and FISH-labeled tissue microarrays and state-of-the art image analysis systems.[172–174]

*References 9, 11, 24, 35, 36, 83, 105, 110, 121, 123, 124, 126, 159–161.

In the last decade, steps detailing the many genetic alterations involved in the progression of PCa have been unveiled (Figure 3). The discovery by Tomlins et al[175,176] of a recurrent chromosomal rearrangement in more than one-half of their analyzed PCa cases is ranked as one of the most notable in solid tumor biology, given the shear prevalence of PCa. The recurrent chromosomal rearrangements lead to a fusion of the androgen-responsive promoter elements of the *TMPRSS2* gene (21q22) to 1 of 3 members of the *ETS* transcription factor family members–*ERG, ETV1,* and *ETV4*–located at chromosome bands 21q22, 7p21, and 17q21, respectively. Although the prognostic role of assessing *TMPRSS2-ETS* rearrangements in PCa tissue samples has been called into question by recent, well-designed large cohort studies,[177,178] the discovery will no doubt have great implications in terms of furthering our understanding of the steps involved in the development and pathogenesis of PCa and will provide a new marker for molecular diagnosis and potential target(s) of therapy in PCa.[179–188] The potential diagnostic and prognostic role of detecting *TMPRSS2-ERG* in post–prostate massage urine samples requires further investigation.[189–191] Figure 4 (A and B) depicts a commonly used FISH split apart–based approach for the evaluation of *ERG* gene fusion.

Recently, commercial anti-ERG monoclonal antibodies became available that make it possible to use IHC for evaluating ERG protein expression as a surrogate approach to detecting *TMPRSS2-ERG* fusion by FISH. We, and others, have demonstrated a strong correlation between ERG overexpression by IHC and *ERG* fusion status with rates greater than 86% for sensitivity and specificity (see Table 2 and Figure 5 [A through F]). Immunohistochemistry may offer an accurate, simpler, and less costly alternative for evaluation of ERG fusion status in PCa on needle biopsy and radical prostatectomy samples.[192,193]

The perceived need to identify “objective” markers to supplement, or conceivably supplant, the more “subjective” established histologic parameters has been a major driving force behind biomarker discovery efforts. It is crucial to recognize and account for the potential variability that can exist even with the new molecular parameters. Sources of variability include differences in molecular technique methodologies, tissue fixation and processing, interobserver and intraobserver variability (in IHC-based biomarkers), and differences in cutoff points.[194] Furthermore, illustration of statistical significance for a particular biomarker does not alone assure its utility for a given patient. Therefore, a promising prognostic or therapeutic target biomarker should endure a rigorous “evidence-based” analysis and be validated in large, prospective clinical trials before transition into standard practice.[195]

Emerging Prognostic Factors

Currently pursued prognostic molecular biologic markers in PCa are categorized as College of American Pathologists Consensus Category III Prognostic Factors.[196] Category III prognostic factors are those still needing additional studies to assure their prognostic utility before undergoing further clinical trials. In contrast, Category I factors are considered as proven to be useful in clinical practice and include preoperative PSA, TNM stage, Gleason grade, and surgical margins. Category II factors are factors that have been extensively studied but await statistically robust trials and include parameters such as tumor volume, histologic type, and DNA ploidy analysis.

The wide array of molecular–based PCa markers include proliferation index (Ki-67), microvessel density, nuclear morphometry, tumor suppression genes (eg, p53, p21, p27, NKX3.1, phosphatase and tensin homolog [PTEN], and retinoblastoma gene [Rb]), oncogenes (eg, Bcl2, c-myc, EZH2, and HER2), adhesion molecules (CD44, E-cadherin), PI3K/akt/mTOR pathway,[197,198] apoptosis regulators (eg, survivin and transforming growth

factor β 1), androgen receptor status, neuroendocrine differentiation markers, and prostate tissue lineage-specific marker expression (PSA, prostate-specific alkaline phosphatase, and prostate-specific membrane antigen).[199–201]

Proliferation Index—A single study[202] has so far found proliferation index, as measured by Ki-67 and percentage of cells in S phase and G2M, to be superior to Gleason score in predicting biochemical recurrence after radical prostatectomy. Two additional studies[203,204] have shown a similar role for Ki-67 index measurement as an independent prognosticator in prostatectomy specimens. Conflicting reports have been furthered by others.[205–208]

Angiogenesis—The mean number of microscopic blood vessels in tissue is higher in PCa and prostatic intraepithelial neoplasia than normal prostate tissue. In a study evaluating microvessel density (MVD) on needle biopsy, the authors[209] found that MVD, when combined with Gleason score and preoperative PSA, provided improved ability to predict extraprostatic extension at radical prostatectomy. Although MVD was significant in the multivariate analysis, Gleason score and serum PSA levels were much more powerful predictors of extraprostatic disease. Three additional studies[210–212] revealed a prognostic role for MVD in prostatectomy specimens. Others,[213–215] however, failed to confirm such a role. Differences in vascular antibodies used and topography of vessel measurements could account for the variable results. It appears that MVD will have a marginal adjunctive role, if any, to established current parameters.

Tumor Suppressor Genes and Oncogenes—Among tumor suppressor genes, there is mounting evidence to support a role for p53 expression in predicting prognosis in PCa. Brewster et al found[216] p53 expression and Gleason score in needle biopsy to be independent predictors of biochemical relapse after radical prostatectomy. Another study[217] found p53 status on prostatectomy but not needle biopsies to be predictive, raising the issue of sampling. Many studies evaluating prostatectomy specimens found p53 to be of prognostic significance, independent of grade, stage, and margin status.[207,215,218–223] The results of these studies suggest that p53 evaluation could become a clinically used parameter, at least in prostatectomy specimens, once standardization of cutoffs and immunostaining methodologies are achieved in large prospective studies. Most studies of another tumor suppressor gene, p27, a cell cycle inhibitor, have also supported a correlation with progression after prostatectomy.[208,224]

Several recent studies have demonstrated that the PTEN/PI3K/mTOR pathway plays an important role in cell growth, proliferation, and oncogenesis in prostate cancer.[225–231] Phosphatase and tensin homolog is a negative regulator of this pathway. Loss of PTEN tumor suppressor gene activity and the ensuing mTOR pathway activation appear to be associated with poor prognosis in prostate cancer. The mTOR pathway is also a potential target for prostate cancer treatment, and several rapamycin analogs are currently being tested as potential therapeutic agents for PCa.[197,230] We recently reported the results of a pilot study evaluating the pharmacodynamic efficacy of neoadjuvant rapamycin therapy in PCa.[197] Using IHC analysis, we found a significant decrease in Phos-S6 protein, the main downstream effector of mTOR pathway, in patients receiving neoadjuvant therapy.[197]

While less robust evidence exists for the prognostic role of p21,[232] a downstream mediator of p53, and transcription factors such as NKX3.1,[172,233] preponderance of evidence supports a prognostic role for Bcl2[203,216,218,220,222] and myc oncogenes[234,235] as potential adjuncts to histologic prognostic parameters.

Despite great interest in HER2 and its potential use as a target of therapy, the data on its relation to prognosis in PCa are conflicting, with 1 study by Veltri et al[236] showing HER2 to

be an independent prognosticator and more recent studies using both IHC and FISH assessment showing lack of its utility in predicting progression.[237]

Genomic Data—In an elegant gene expression profiling study using cDNA microarrays containing 26 000 genes, Lapointe et al[238] identified 3 subclasses of prostate tumors by distinct patterns of gene expression. High-grade and advanced-stage tumors, as well as tumors associated with recurrence, were disproportionately represented among 2 of the 3 subtypes, one of which also included mostly lymph node metastases. Furthermore, 2 surrogate genes were differentially expressed among tumor subgroups by IHC. These included MUC1, a gene highly expressed in the subgroups with "aggressive" clinicopathologic features, and AZGP1, a gene highly expressed in the favorable subgroup. The 2 surrogate markers were strong predictors of tumor recurrence, independent of tumor grade, stage, and preoperative PSA levels. Such study suggests that prostate tumors can be classified according to their gene expression patterns; these tumor subtypes may provide a basis for improved prognostication and treatment stratification.

In another study, Tomlins et al[239] used laser-capture microdissection to isolate 101 cell populations to illustrate gene expression profiles of PCa progression from benign epithelium to metastatic disease. By analyzing expression signatures in the context of more than 14 000 "molecular concepts," or sets of biologically connected genes, the authors generated an integrative model of progression. Molecular critical transitions in progression included protein biosynthesis, E26 transformation-specific (ETS) family transcriptional targets, androgen signaling, and cell proliferation. Known prognostic markers, such as grade, could be ascribed to noted attenuated androgen-signaling signature seen in high-grade cancer (Gleason pattern 4), similar to metastatic prostate cancer, which may reflect dedifferentiation and explain the clinical association of grade and prognosis. Taken together, these data show that analyzing gene expression signatures in the context of a compendium of molecular concepts is useful in understanding cancer biology.

Lapointe et al[240] complemented their above-mentioned gene expression findings by looking for associated copy number alterations with array-based comparative genomic hybridization. They were able to identify recurrent copy number genetic aberrations[240] corresponding to 3 prognostically distinct groups of PCa: (1) deletions at 5q21 and 6q15 deletion group, associated with favorable outcome; (2) a 8p21 (NKX3-1) and 21q22 (resulting in TMPRSS2-ERG fusion) deletion group, and (3) gains in 8q24 (MYC) and 16p13, and loss at 10q23 (PTEN) and 16q23 groups, correlating with metastatic disease and aggressive outcome.

Finally, in a recent genome-wide analysis of PCa, Taylor et al[241] elegantly illustrated how detailed annotation of PCa genomes can affect our understanding of the disease and its treatment strategy. Assessing DNA copy number, messenger RNA expression, and focused exon resequencing in 218 prostate cancer tumors, the authors identified the role of nuclear receptor coactivator NCOA2 as a novel oncogene in 11% of PCa cases. TMPRSS2-ERG fusion was associated with novel prostate-specific deletion at chromosome band 3p14, which may implicate FOXP1, RYBP, and SHQ1 as potential cooperative tumor suppressors. Most intriguing was their ability to define clusters of lowrisk and high-risk disease beyond that achieved by Gleason score by using DNA copy number data. As shown in Figure 6 (A and B), six clusters of PCa tumors are identified by unsupervised hierarchical clustering with distinct risk for biochemical recurrence.

Genomic studies suggest that prostate cancers develop via a limited number of alternative preferred genetic pathways. The resultant molecular genetic subtypes provide a new

framework for investigating PCa biology and explain, in part, the clinical heterogeneity of the disease.

Emerging Early Detection Markers and Targets of Therapy—Markers of PCa detection that can be applied to blood, urine, or prostatic secretion fluid (ejaculate or prostate massage fluids) are of great interest and have been the focus of active research. Markers that have been investigated in the urine or prostatic secretions include gene promoter hypermethylation profile assays[242–245] and differential display code 3 (DD3), also known as PCA3 (Figure 7). DD3 is a gene that expresses a noncoding RNA and was initially identified by Bussemakers et al[246] as one of the most specific markers of PCa. Quantitative real-time reverse transcriptase PCR assay detecting PCA3 can be applied to blood, urine, or prostatic fluid.[247]

Evaluation of PCA3 in postattentive prostate massage urine samples with transcription-mediated amplification technology has shown to be superior to serum PSA determination in predicting biopsy outcome, with sensitivity and specificity approximating 70% and 80%, respectively, and a negative predictive value of 90%[248–251]; it is currently under evaluation for FDA approval in the United States. Encouraging data from the REDUCE trial support a role for evaluation of PCA3 in postattentive prostate massage urine sample in predicting positive prostate needle biopsy findings in immediately subsequent, as well as future, biopsies after an initial negative biopsy result. PCA3 may also have a role in predicting the risk for higher Gleason score and larger tumor volume on radical retropubic prostatectomy. If confirmed, the latter could be of great value in treatment option algorithms and in delineation of candidates for active surveillance.[252–255] Multiplex urine assays to include PCA3, TMPRSS-ERG, SPINK1, and GOLPH2 are also under evaluation, with recent data suggesting an improved performance of such assays compared to PCA3 alone.[256]

Finally, several markers are being investigated as potential targets of therapy for prostate cancer. The list includes tyrosine kinase receptors (eg, EGFR), angiogenesis targets (eg, VEGF),[257] fatty acid synthase,[258] PI3K/akt/mTOR,[197,230,259] endothelin receptors,[260,261] and prostate-specific membrane antigen,[262–265] to name a few.

RENAL CELL CARCINOMA

Current established prognostic parameters in renal cell carcinoma (RCC) include pTNM stage, Fuhrman grade, histologic subtype, and clinical parameters such as the Eastern Cooperative Oncology Group (ECOG) performance status, hemoglobin level, and lactate dehydrogenase levels, among others.[266–268] Continuous refinements of staging criteria and development of nomograms to integrate the factors listed above promise to yield better prognostic and management discriminators.[269]

A large number of biomarkers are under current intense investigation for their potential utility as prognosticators and/or therapy predictors in RCC.[269–276] Table 3 lists some of these markers. Kim et al[271] evaluated a set of immunohistochemical markers including Ki-67, CAIX, CAXII, p53, PTEN, gelsolin, EpCAM, and vimentin in combination with established parameters. Their study suggested that a new combined molecular and clinicopathologic prognostic model (CAIX, vimentin, p53, pTNM, ECOG performance status) is superior to prior models of clinicopathologic parameters alone, including the commonly used University of California Los Angeles integrated staging system model.

Pantuck et al[277,278] recently highlighted a promising prognostic role for mTOR pathway members. Their study revealed an independent negative prognostic role for PTEN loss and

phos-S6k overexpression. The same study showed that an increase in phos-Akt cytoplasmic expression and loss of phos-Akt nuclear expression were negative predictors of survival.

Bui et al[279] demonstrated that both low expression of CAIX and high Ki-67 proliferation index were independent negative predictors of survival in clear cell renal cell carcinoma (ccRCC). Interestingly, CAIX overexpression predicted response to interleukin 2 immune therapy in metastatic RCC, a finding also documented in the study by Atkins et al[280] and Stillebroer et al.[281] A prognostic role for angiogenesis pathway has been revealed in RCC. In a study by Jacobsen et al,[282] VEGF expression appeared to correlate with tumor size and pTNM stage in RCC. The authors found high VEGF expression to be a negative prognosticator for survival on univariate but not multivariate analysis. Separately, Kluger et al[272] analyzed tissue microarrays containing 330 ccRCCs and papillary renal cell carcinoma (PRCC), using a novel method of automated quantitative analysis of VEGF and VEGF receptor expression by fluorescent IHC. Unsupervised hierarchical clustering classified tumors by coordinated expression of VEGF and VEGF receptors. The authors found that high expression of VEGF and VEGF receptors was associated with poor survival. Finally, a study by Lidgren et al[283] revealed that high expression of hypoxia-inducible factor 1 α (HIF1A) was an independent negative prognosticator in ccRCC.

Among cell cycle control molecules, p27 (Kip1) and cyclin D1 appear to have a promising prognostic role in ccRCC. Migita et al[284] found loss of p27 expression to be an independent predictor of poor DSS. Similar p27 findings were also documented by Hedberg et al.[285,286]

Genomic and Theranostic Applications

A tight correlation is maintained between morphologic phenotype and underlying genetic alterations in renal tumors. Lessons learned from the relatively rare familial renal cancer syndromes have helped unlock the complex molecular mechanisms involved in sporadic RCC tumorigenesis. As a result, many potential new targets of therapy are now under investigation in the heretofore unsuccessful endeavor of treating advanced RCC. The achieved understanding of the molecular basis of von Hippel-Lindau disease (VHL) best illustrates the great therapeutic and theranostic potentials of uncovering molecular mechanisms of oncogenesis.[287] von Hippel-Lindau syndrome is a rare autosomal dominant familial cancer syndrome (retinal angiomas, hemangioblastomas, pheochromocytomas, ccRCC). Patients with VHL are born with a germline (constitutive) VHL gene mutation affecting all their cellular elements. Inactivation or silencing of the remaining wild-type allele in renal tissues facilitates the formation of ccRCC. The fact that similar defects in the VHL gene are found to be responsible for approximately 60% of sporadic ccRCCs[288,289] has greatly widened the implications in understanding the molecular mechanisms of VHL inactivation.

HIF1A is a transcription factor that can be thought of as a cellular oxygen sensor. Under normoxic conditions, HIF1A interacts with normal VHL protein that, in turn, facilitates its elimination through ubiquitinization.[290] Under hypoxic conditions, HIF1A escapes destruction by VHL and is allowed to exert its crucial role in triggering the transcription of angiogenesis factor (VEGF); cell growth factors (transforming growth factor α [TGFα] and transforming growth factor β [TGFβ]); and factors involved in glucose uptake and acid-base balance (GLUT-1 and CAIX, respectively). A defective VHL function in ccRCC leads to abnormal accumulation of HIF1A even under normal conditions, in turn resulting in the overexpression of the above-mentioned proteins that are normally inducible only during hypoxia. The overexpressed VEGF, platelet-derived growth factor β, and TGFβ act on neighboring vascular structures to promote tumor angiogenesis. The augmented tumor vasculature provides additional nutrients and oxygen to promote the growth of tumor cells.

Furthermore, TGFα acts in an autocrine manner on tumor cells by signaling through EGFR, which promotes tumor cell proliferation and survival.

Several of the above-mentioned proteins are currently being investigated as targets of therapy for advanced ccRCC.[291–298] A randomized phase II trial involving patients with metastatic ccRCC investigated the efficacy of bevacizumab, a humanized anti-VEGF antibody.[299] Although the treatment resulted in only a few months extension of time to tumor progression, it provided a key "proof of principle" of the efficacy of antiangiogenic therapy. Inhibitors of VEGF receptor tyrosine kinase activity alone, and in combination with other tyrosine kinases, are also under study. The multitargeted kinase inhibitors sunitinib and sorafenib have shown great promise in phase II and phase III trials, with at least stabilization of disease in as many as 70% of patients with cytokine refractory disease.[287,298]

Another growth factor target is TGFα, which promotes ccRCC growth through its interaction with EGFR. A human monoclonal antibody against human EGFR (panitumumab [ABX-EGF]), as well as small molecule inhibitors of the EGFR tyrosine kinase activity (gefitinib and erlotinib), are 2 strategies being tested to target the TGFα/EGFR axis.[292,294–296]

Other options being pursued include the use of temsirolimus (CCI-779), a selective inhibitor of mTOR. Partial responses were noted in 7% of patients and minor responses in 26%. The median survival rate was 15 months. The notable activity of the drug for patients with poor prognostic features prompted a phase III trial.[280,293] Finally, agents targeting HIF1A and CAIX, including anti-CAIX radiolabeled monoclonal antibody, are also under development.[289,300]

CONCLUSIONS

A wide array of molecular markers may be used in the near future as adjuncts to currently established prognostic parameters in urologic malignancies. For bladder cancer, molecular grade as a combination of proliferation index and *FGFR3* mutation; multitarget FISH analysis for chromosomes 3,7,17, and chromosome band 9p21; and cell cycle and apoptosis control markers (p52, Rb, p21, p27, and p16) are among the leading markers that will soon enter routine use. In prostate adenocarcinoma, biologic markers that can refine early detection (eg, PCA3 and *ERG*) and serve as prognostic markers and potential targets of therapy (mTOR pathway) are expected to be among the first to enter the clinical arena. Finally, our detailed understanding of the complex molecular alterations involved in the development of renal cell carcinomas is yielding novel diagnostic and prognostic molecular tools and yielding many new targets of therapies including mTOR, VEGF, EGFR, HIF1A, and CAIX.

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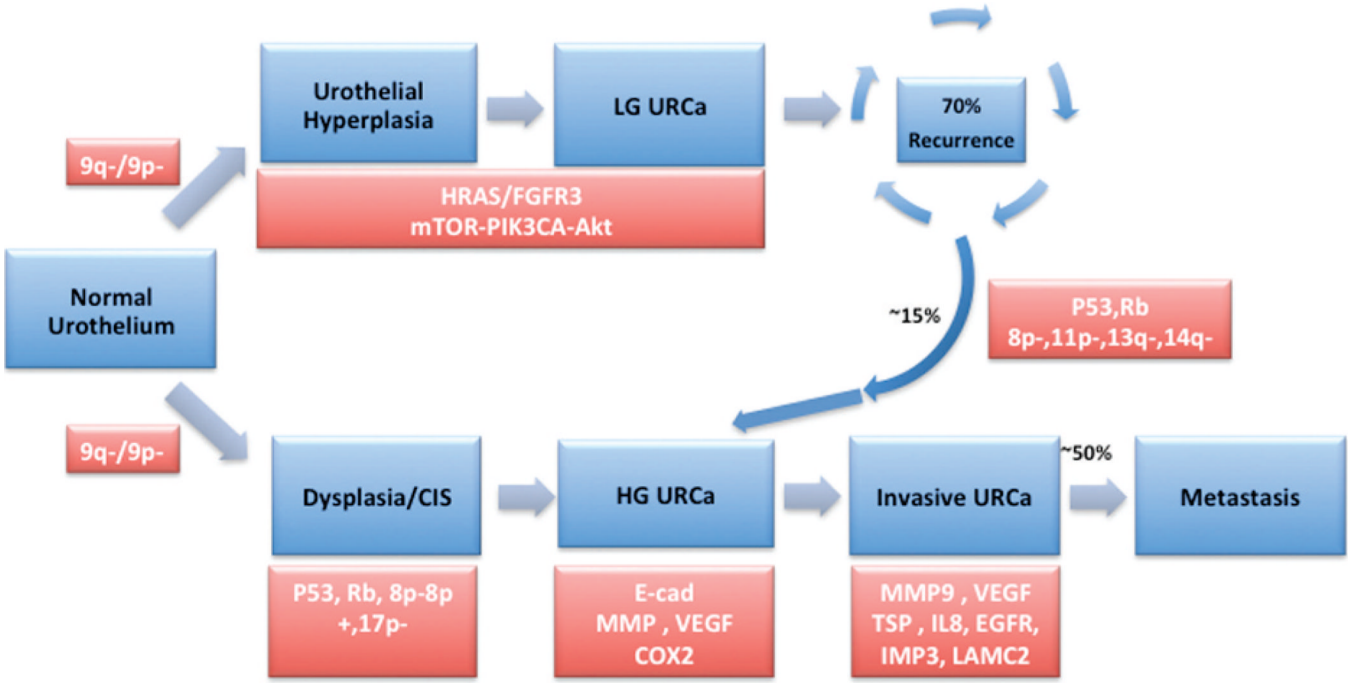

Figure 1.

Divergent molecular pathways of oncogenesis in superficial and muscle invasive urothelial carcinoma of urinary bladder. Genetic alterations are depicted in key stages of disease progression. Abbreviations: CIS, carcinoma in situ; EGFR, epidermal growth factor receptor; HG URCa, high-grade urothelial carcinoma; LG URCa, low-grade urothelial carcinoma; mTOR, mammalian target of rapamycin; URCa, urothelial carcinoma; VEGF, vascular endothelial growth factor.

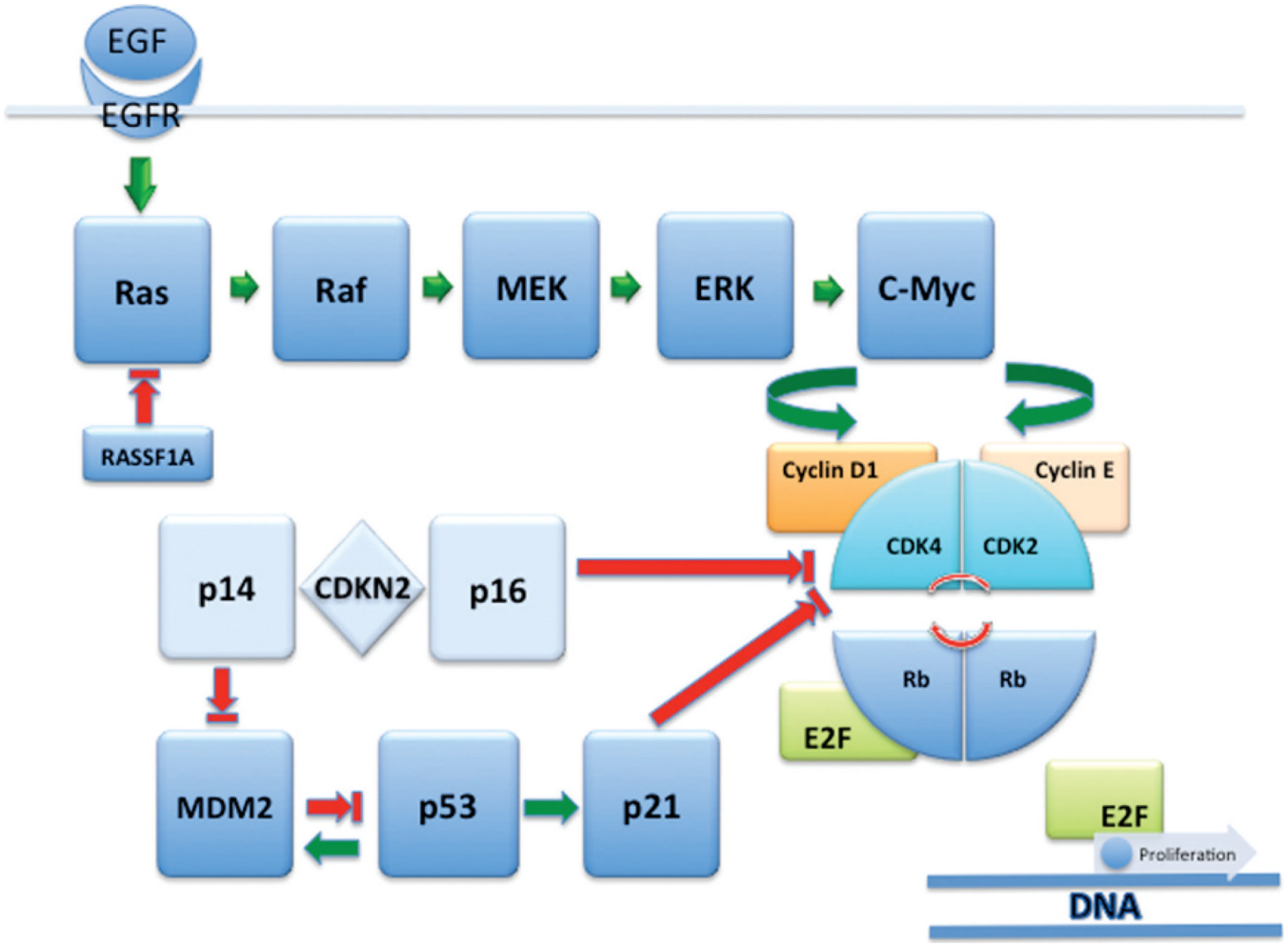

Figure 2.
Receptor tyrosine kinase (EGFR/Ras/Mek/ERK) and cell cycle regulator (p14, p16, p53, p21, cyclin D1, cyclin E, and Rb) pathways in urothelial carcinoma. Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor.

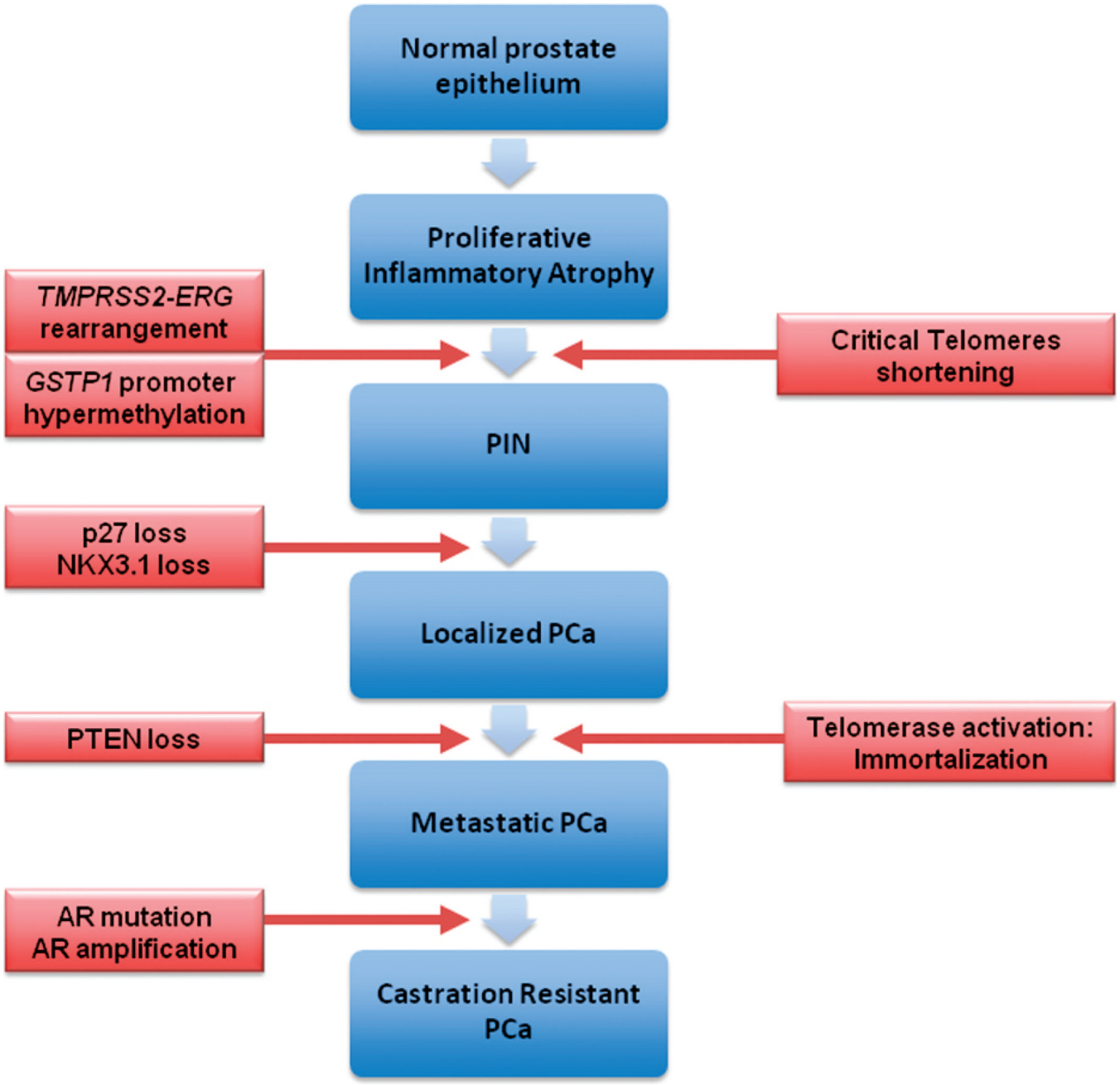

Figure 3.
Molecular alterations involved in oncogenesis and progression of prostate cancer. Abbreviations: PCa, prostate adenocarcinoma; PIN, prostatic intraepithelial neoplasia; PTEN, phosphatase and tensin homolog.

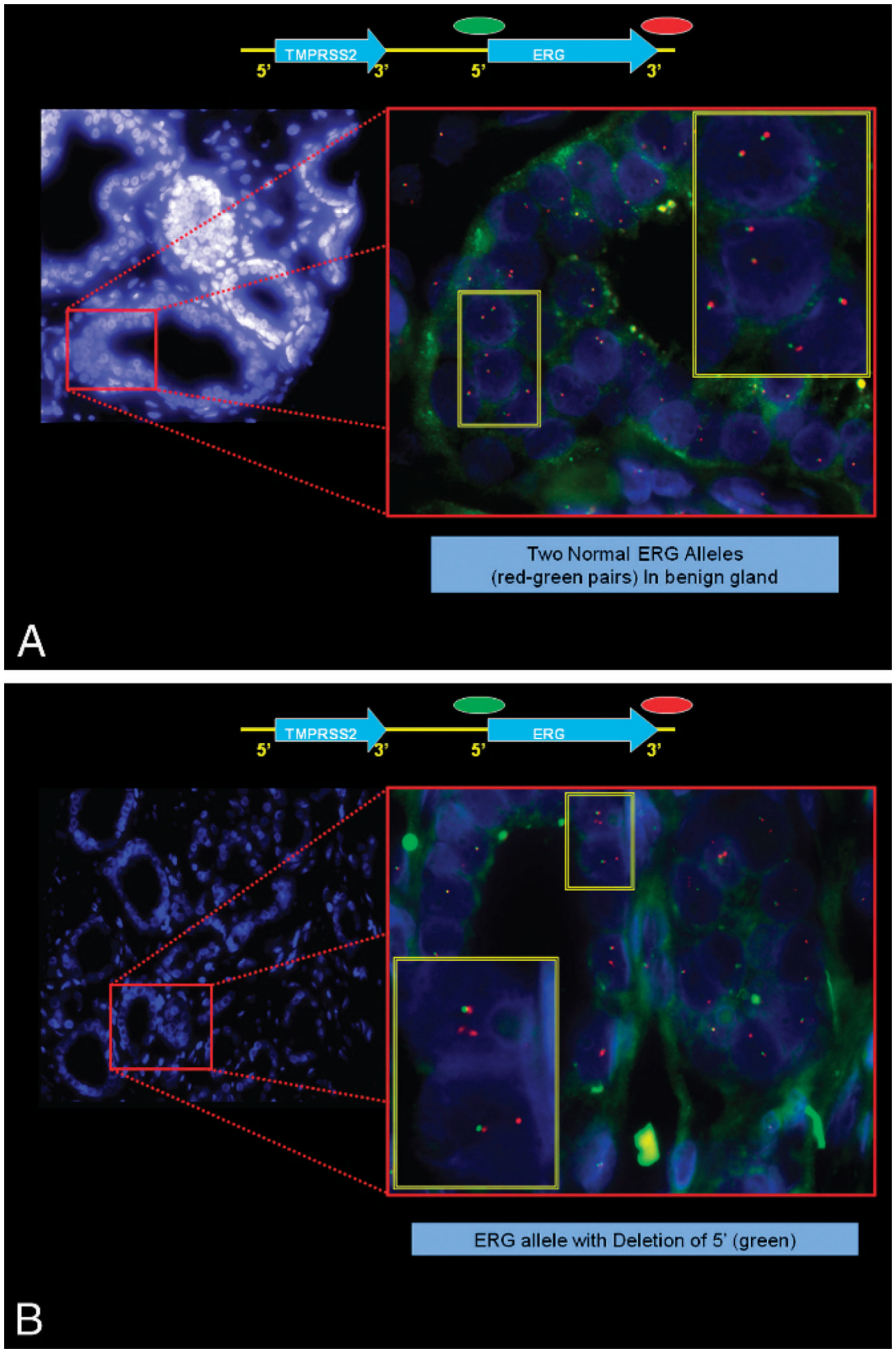

Figure 4.

Fluorescence in situ hybridization–based evaluation of *ERG* gene fusion in prostate carcinoma. A, A normal *ERG* allele will hybridize with the *ERG* 3′ and the *ERG* 5′ probes, leading to the formation of juxtaposed green-red signals or a yellow overlap signal. The 2 *ERG* rearrangements associated with *TMPRSS2-ERG* fusion lead to either a loss of 5′ (green) signal or to a split of the *ERG* 5′ (green) and *ERG* 3′ (red) signals. B, Example of prostate adenocarcinoma showing *ERG* fusion by deletion.

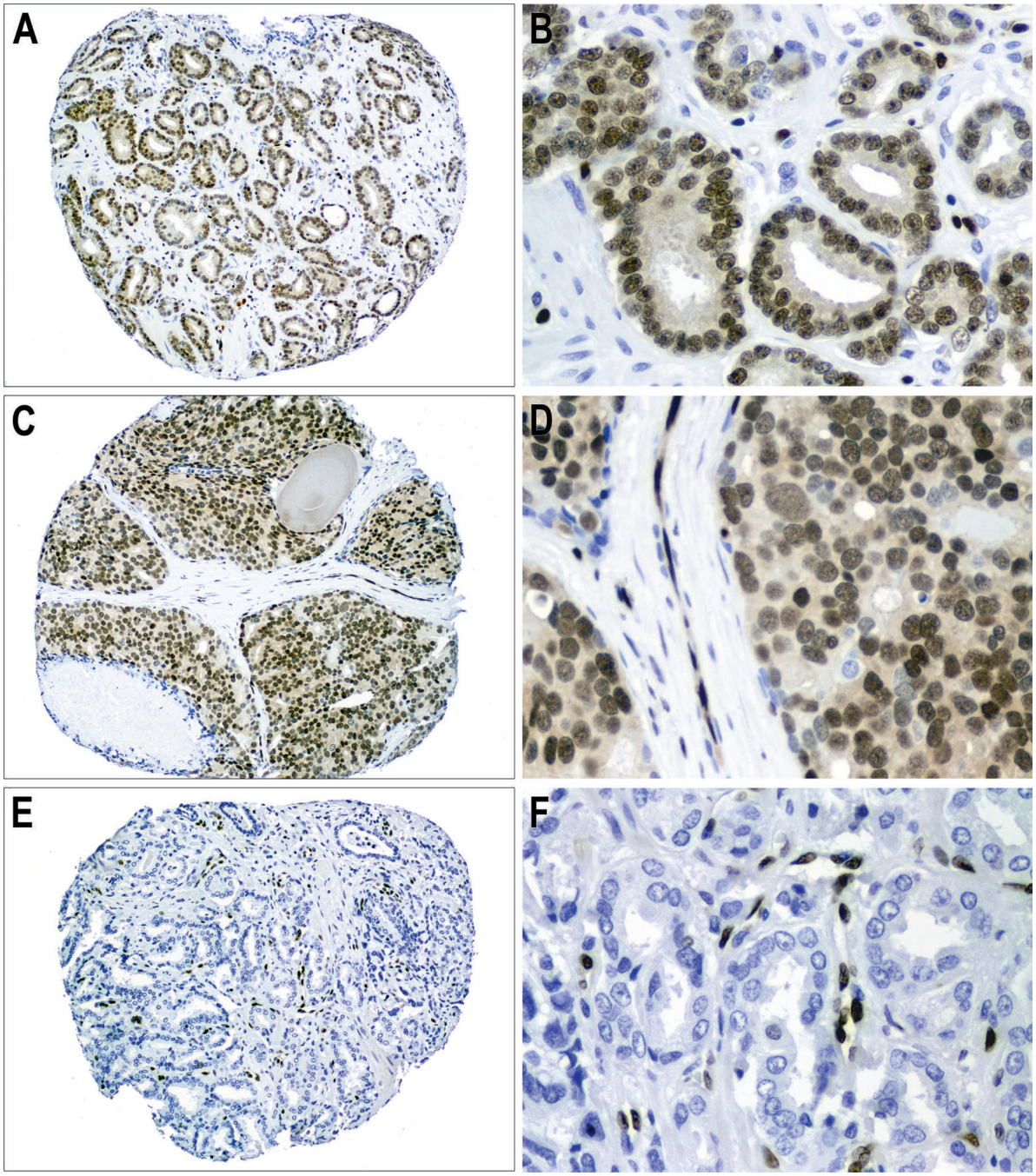

Figure 5.
Overexpression of ERG, as demonstrated by immunohistochemistry, is a simple surrogate method for evaluating *TMPRSS2-ERG* fusion in prostate adenocarcinoma. Positive expression of ERG in Gleason grades 6 and 8 cases that were also positive for *TMPRSS2-ERG* fusion by fluorescence in situ hybridization (FISH) are shown in A and B and in C and D, respectively. Lack of ERG expression in a Gleason grade 6 tumor that lacked *TMPRSS2-ERG* fusion by FISH is shown in E and F (ERG immunostain, original magnifications ×100 [A, C, and E] and ×200 [B, D, and F]).

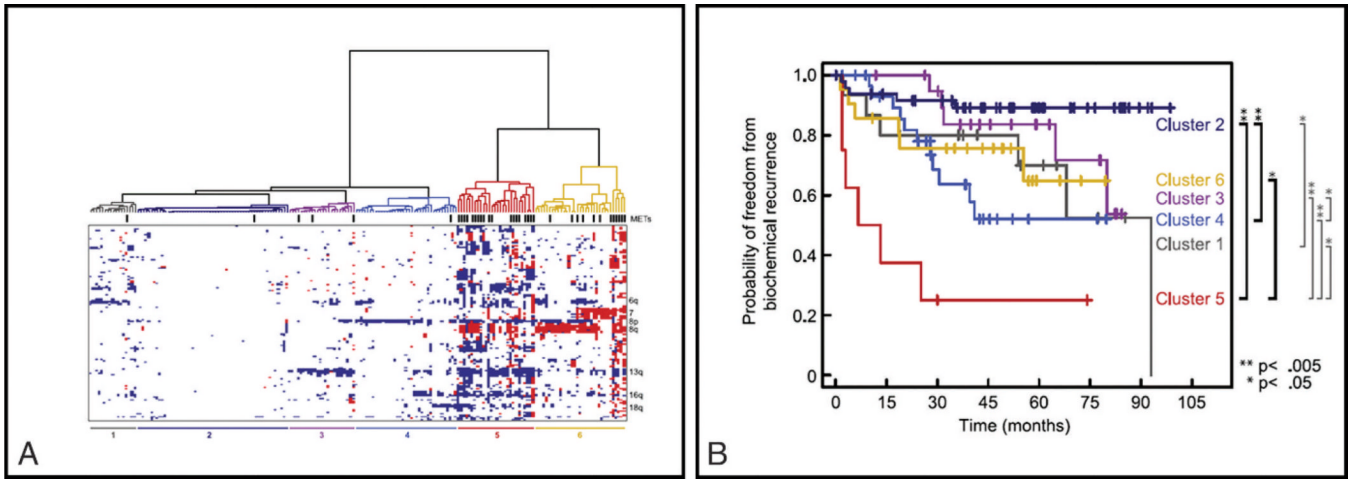

Figure 6.

Clinically distinct groups of prostate cancer are identified by genomic alterations. A, Unsupervised hierarchical clustering of copy number alterations identified 6 groups (clusters) of prostate cancers. B, Statistically significant differences in freedom from biochemical recurrence are found among the 6 groups. Adapted from Taylor et al[241] with permission from Elsevier.

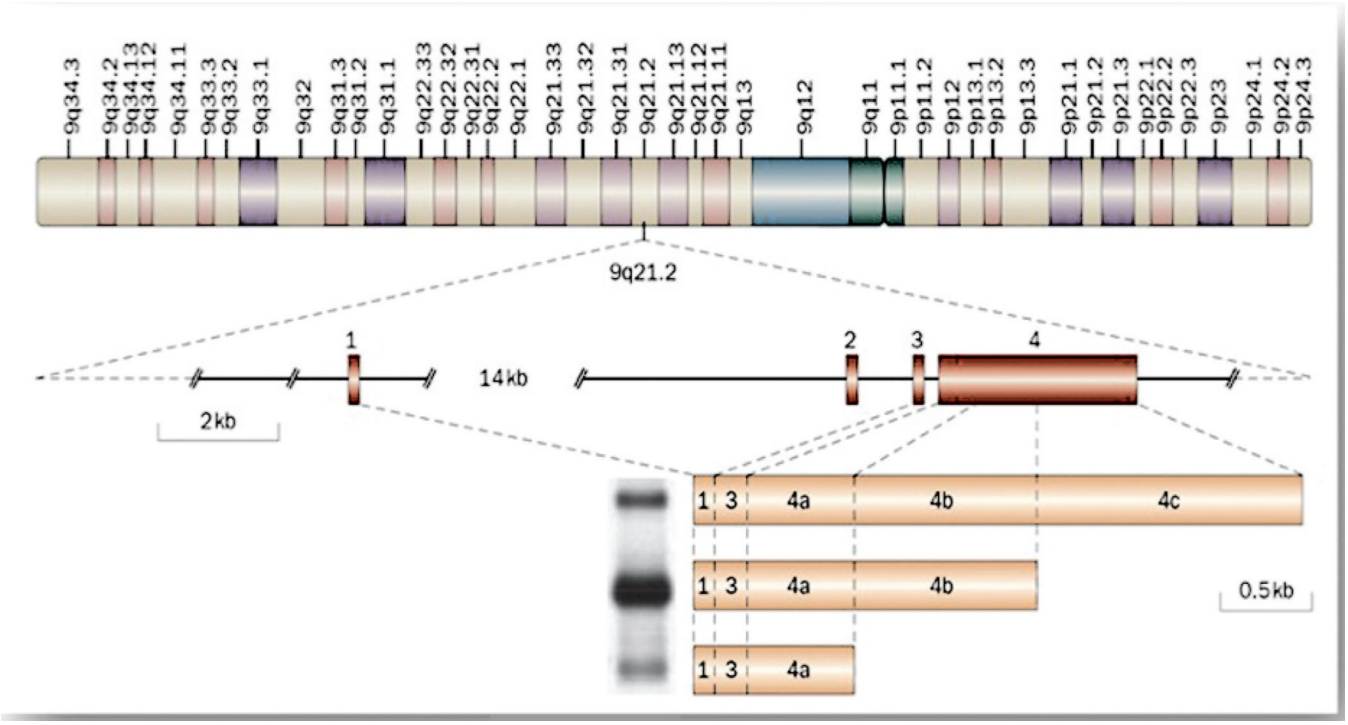

Figure 7.

Structure of the *PCA3/DD3* gene. The gene, which expresses a noncoding RNA, was mapped to chromosome 9q21–22 and consists of 4 exons. Alternative polyadenylation at 3 different positions in exon 4 (indicated as 4a, 4b, and 4c) gives rise to 3 different-sized transcripts. The most frequently found transcript contains exons 1, 3, 4a, and 4b.

Table 1

Established Clinicopathologic and Potential Molecular Prognostic Parameters in Superficial and Muscle Invasive Urothelial Carcinoma of Bladder

Clinicopathologic Prognostic Parameters in Urothelial Carcinoma	
Superficial Urothelial Carcinoma	**Muscle Invasive Urothelial Carcinoma**
WHO/ISUP grade	pTNM
pT stage	LVI
Presence of associated CIS/dysplasia	Resistance to neoadjuvant chemotherapy
Disease duration	
Time to and frequency of recurrences	Divergent histology
Multifocality	Micropapillary
Tumor size (>3 cm)	Osteoclast rich
Failure of prior BCG therapy	Undifferentiated/giant cell
Presence of LVI	Plasmacytoid
Depth of lamina propria invasion	
Emerging Molecular Prognostic Markers	
Superficial Non–Muscle Invasive Urothelial Carcinoma (NMI-BC)	**Muscle Invasive Urothelial Carcinoma (MI-BC)**
Proliferation index (Ki-67, MIB-1, S phase)	p53 inactivation/accumulation
FGFR3 mutation/overexpression (protective)	Alterations of Rb expression
mG (FGFR#/MIB-1)	Loss of p21 expression
p53 inactivation/accumulation	Alteration of p16 expression
DNA ploidy status	Loss of E-cadherin
Multitarget FISH	RTK
HRAS	*EGFR* overexpression
ERBB3, ERBB4 overexpression (protective)	HER2/*neu* overexpression/amplification
Loss of E-cadherin	
	Angiogenesis markers
Cell cycle control	VEGF overexpression
Down-regulation of Rb expression	HIF1A overexpression
Down-regulation of p21 expression	TSP1 overexpression
Down-regulation of p27 expression	
Cyclin D3 overexpression	mTOR-Akt pathway
Cyclin D1 overexpression	mTOR
	Phos-S6 expression (protective)
Multimarker immunoexpression analysis	
p53, p27, Ki-67, Rb, p21	Genomic and gene expression array panels
Angiogenesis markers	Epigenetic alterations
VEGF overexpression	RASSF1 promoter hypermethylation
HIF1A overexpression	E-cadherin promoter hypermethylation
TSP1 overexpression	EDNRB promoter hypermethylation

Clinicopathologic Prognostic Parameters in Urothelial Carcinoma	
Superficial Urothelial Carcinoma	**Muscle Invasive Urothelial Carcinoma**
Genomic and gene expression array panels	
Epigenetic alterations	
RASSF1 promoter hypermethylation	
DAPK promoter hypermethylation	
APC promoter hypermethylation	
E-cadherin promoter hypermethylation	
EDNRB promoter hypermethylation	

Abbreviations: BC, bladder cancer; BCG, Bacillus Calmette-Guerin; CIS, carcinoma in situ; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; ISUP, International Society of Urological Pathology; LVI, lymphovascular invasion; mG, molecular grade parameter; mTOR, mammalian target of rapamycin; RTK, receptor tyrosine kinase; VEGF, vascular endothelial growth factor; WHO, World Health Organization.

Table 2

Immunohistochemistry Expression of ERG Protein Strongly Correlates With *TMRSS2-ERG* Fusion Regardless of Mechanism of Fusion[a]

FISH	No. Cases	No ERG Expression, No. (%)	ERG Expression, No. (%)	*P*
Esplit				<.001
	108	17 (15.7)	91 (84.3)	
	319	218 (68.3)	101 (31.7)	
Edel				<.001
	158	21 (13.3)	137 (86.7)	
	269	214 (79.6)	55 (2.4)	
2Esplit				.008
	6	0 (0)	6 (100)	
	421	235 (55.8)	186 (44.2)	
2Edel				<.001
	24	1 (4.2)	23 (95.8)	
	403	234 (58.1)	169 (41.9)	
Any fusion				<.001
	195	28 (14.4)	167 (85.6)	
	232	207 (89.2)	25 (10.8)	

Abbreviations: Edel, deletion; Esplit, insertion leading to FISH signal split; FISH, fluorescence in situ hybridization.

[a]Reprinted from Chaux et al[193] with permission from Lippincott Williams & Wilkins.

Table 3

Current and Emerging Prognostic Parameters in Clear Cell Renal Cell Carcinoma (ccRCC)

Current Prognostic Parameters in ccRCC
Patient factors
Age
Sex
Pathologic factors
pTNM
Histologic type
Fuhrman grade
LVI
Tumor necrosis
Clinical factors
ECOG performance status
Hgb level
Serum LDH
Emerging Potential Molecular Prognostic and Predictive Parameters in ccRCC
Hypoxia inducible
HIF-1
CAIX
CAXII
CXCR4
VEGF/VEGF-R
ILGF1
Cell adhesion markers
EpCAM
E-cadherin
α-Catenin
Catenin-6
Proliferation markers
Ki-67
MCM2
Cell cycle regulators
Cyclin
p27
Apoptosis regulators
p53
Bcl2
Smac/DIABLO
mTOR pathway
PTEN

Current Prognostic Parameters in ccRCC
akt
Phos-S6k an

Abbreviations: ccRCC, clear cell renal cell carcinoma; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; Hgb, hemoglobin; LVI, lymphovascular invasion; PTEN, phosphatase and tensin homolog; VEGF, vascular endothelial growth factor.