Clinical Implications of Current Developments in Genitourinary Pathology

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• **Context.**—Several developments in genitourinary pathology are likely to change our understanding and management of some genitourinary cancers considerably.

• **Objective.**—To review 5 stories in genitourinary pathology: (1) fusion in the ETS (E26) gene family in prostatic adenocarcinoma; (2) insulin-like growth factor II messenger RNA-binding protein 3 (IMP3), an important prognostic biomarker for kidney and bladder cancers; (3) translocation renal cell carcinoma; (4) UroVysion fluorescence in situ hybridization test in urine cytology for detection of bladder cancer; and (5) the use of triple immunostaining for diagnosis of prostate cancer.

• **Data Sources.**—Literature review and authors’ personal experiences.

• **Conclusions.**—Many scientific findings have contributed recently to the understanding of the natural pathogenesis and progression of genitourinary cancers. This translational research helps in diagnosing, predicting, and potentially, treating genitourinary cancers.

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**FUSION IN THE ETS GENE FAMILY IN PROSTATIC ADENOCARCINOMA**

In 2005, Tomlins et al discovered recurrent gene fusions between the 5′ untranslated region of the androgen-related transmembrane protease, serine 2 gene (TMPRSS2) and ETS (E26) transcription factors in prostate cancer. The ETS gene family includes ERG, ETV1, ETV4, ETV5, and ELK4. The most common fusion is TMPRSS2:ERG, which has a prevalence of approximately 40% to 70%. At a molecular level, TMPRSS2-ETS fusions occur during an early stage of oncogenesis and are found in high-grade prostatic intraepithelial neoplasia, which is a precursor lesion to prostate adenocarcinoma. This suggests that ETS fusions may play a role in the transition to invasive cancer.

There are several potential clinical utilities of ETS fusions: **Stratifying risk factors among prostate cancer patients.** Currently, the 3 most widely used prognostic factors in prostatic carcinoma are Gleason score, tumor stage, and prostate-specific antigen (PSA) level. Several studies have reported a significant association between TMPRSS2:ERG fusions and higher clinical stage, more aggressive disease, metastatic disease, or decreased survival, which suggests that the presence of an ETS fusion is a predictor of unfavorable prognosis. Tomlins et al showed that urine TMPRSS2:ERG was associated with clinically significant prostate cancer, as indicated by large tumor size, high Gleason score at prostatectomy, and upgrading of Gleason grade at prostatectomy. However, several other studies have not found significant correlations between TMPRSS2:ERG gene fusions and any measures of clinical outcome. Other groups even found an association with a favorable outcome. The lack of concurrence across studies may be because researchers used different endpoints to determine the duration of follow-up (death, metastasis, biochemical failure, or Gleason score). This also suggests that other factors besides the presence of ETS fusions are more important in determining prostate cancer outcomes.

**A diagnostic biomarker for prostatic carcinoma.** Previous studies found that TMPRSS2:ERG gene rearrangement status was highly specific for prostate cancer and was detected in approximately 50% of tissue samples. Recent studies found monoclonal antibodies against ERG immunostaining highly correlated with TMPRSS2:ERG gene rearrangement status. Using ERG immunostaining, He et al and Yaskiv et al showed that approximately 43% of prostate carcinomas are positive for ERG protein expression. In addition, positive ERG staining is not entirely specific for prostate cancer and can be found in 29% of high-grade prostatic intraepithelial neoplasias and 5% of benign glands. Because ERG immunostaining lacks sensitivity and specificity for atypical glands and prostate carcinoma, the utility of ERG immunohistochemistry (IHC) as a diagnostic adjunct for prostate cancer remains to be determined.

**ETS gene fusions in urine.** A potential clinical application of ETS gene fusions lies in detecting fusion transcripts in urine. Of all cancers detected by PSA, about 46% of them contain the TMPRSS2:ERG gene fusion, whereas another 5% to 10% contain one of the less-common fusions. The fusion

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transcript TMPRSS2:ERG was detected after digital rectal examination in urine by reverse transcription-polymerase chain reaction in 42% of men with prostate cancer.24 More-recent studies19,20 of TMPRSS2:ERG detection had sensitivities of 31% to 37% and specificities of 84% to 91%. Theoretically, this test may help detect prostate cancer in the 15% to 20% of patients with prostate cancer who have a PSA less than 4.0 ng/mL, but its maximum sensitivity will be similar to the incidence of TMPRSS2:ERG+ cancers (around 50%).3 Compared with PSA testing, a significant advantage of TMPRSS2:ERG fusion testing is that the ETS gene fusions are specific for neoplastic cells, which is important because prostate cancer tends to be overdiagnosed, partly because of the low specificity of PSA as a screening tool. Whether urine TMPRSS2:ERG fusion testing will be useful for screening or monitoring patients awaits further studies. The sensitivity and specificity of this test need to be established in clinical setting.

Therapy applications. The ETS fusions have been the focus of recent studies that target androgen signaling pathways. Abiraterone acetate is an inhibitor of androgen synthesis, which prolongs survival in patients with castration-resistant prostate cancer treated with chemotherapy. Studies by Attard et al21,22 found that a significantly higher percentage of prostate cancer treated with chemotherapy. Studies by which prolongs survival in patients with castration-resistant cancers of recent studies that target androgen signaling pathways.

IMP3—AN IMPORTANT PROGNOSTIC BIOMARKER FOR KIDNEY AND BLADDER CANCERS

In recent years, personalized medicine has emphasized the significance of molecular biomarkers in localized cancers, which may predict disease progression or which, in advanced malignancies, may indicate response to systemic therapy. Studies from our laboratory and others have indicated that IMP3, an oncofetal protein, is an important prognostic biomarker for patients with localized renal cell carcinoma (RCC) and superficial urothelial carcinoma (UC), identifying patients with a high potential to develop progression and who might benefit from early aggressive therapy.24–27

IMP3 protein is a member of the insulin-like growth factor II mRNA-binding proteins that consist of IMP1, IMP2, and IMP3.28 The IMP3 gene is located on band 7p11.2 and is identical to the KH domain–containing protein overexpressed in cancer (KOC) protein.29,30 IMP3 is an oncofetal protein involved in embryogenesis. Recent studies24–27,31–34 have shown that IMP3 is an important cancer-specific gene that is associated with many aggressive and advanced cancers and is specifically expressed in malignant tumors but is not found in benign tissues. Moreover, IMP3 promotes tumor cell proliferation, adhesion, invasion, and metastasis.28,30

IMP3 Predicts Metastasis and Prognosis of Localized RCC

Selecting high-risk patients with localized RCCs to undergo systemic therapy after nephrectomy becomes crucial because new angiogenesis medications have shown promising results for metastatic RCC. Multiple studies of more than 1000 cases of localized RCCs have shown that IMP3, as an independent prognostic marker, can be used at initial diagnosis of RCC to identify patients who have a high potential to develop metastasis and who might benefit from early systemic treatment.24–27,31,36

IMP3 displays several features that make it an attractive prognostic marker for localized RCC. As a biomarker, it is powerful for predicting localized RCC that will subsequently develop metastasis and patient death in the same tumor stage. Our study28 showed that IMP3 is an indicator of decreased 5-year metastases-free survival for patients at TNM stage I (IMP3+ tumors, 44%, versus IMP3– tumors, 98%), stage II (IMP3+ tumors, 41%, versus IMP3– tumors, 94%), and stage III (IMP3+ tumors, 16%, versus IMP3– tumors, 62%). IMP3 expression is also associated with reduced 5-year overall survival for patients at TNM stage I (IMP3+ tumors, 32%, versus IMP3– tumors, 89%), stage II (IMP3+ tumors, 41%, versus IMP3– tumors, 88%), and stage III (IMP3+ tumors, 14%, versus IMP3– tumors, 58%).26 The above findings have been confirmed by a large external-validation study.24

The expression of IMP3 is an independent predictor of tumor metastasis and patients' overall survival. Multivariable analyses showed that the patients with IMP3+ localized tumors were almost 6 times more likely to subsequently develop metastases and 4 times more likely to die than those with IMP3– tumors after adjustment for other well-known clinical variables (eg, age, sex, and tumor stage, size, grade, and subtype).24–27

The hazard ratios of IMP3 status were much higher than those associated with other well-known independent risk factors, such as, tumor stage I and III and tumor size.24–27

Localized RCCs with high levels of IMP3 expression have a much higher chance of developing metastases.25 We used an automated cellular imaging system to quantitatively analyze the expression of IMP3 in localized RCCs. We found that tumors with higher levels of IMP3 progressed more rapidly than did those with lower levels of IMP3 expression.

Interestingly, our data show that patients at TNM stage I and II with high levels of IMP3 in their tumor have a much higher chance of developing metastases compared with patients at stage III without IMP3 expression.23

IMP3 is also an independent prognostic biomarker for patients with localized papillary and chromophobe RCC.27 Although relatively rare, patients with localized papillary and chromophobe RCC can develop metastasis after nephrectomy, and IMP3 can be used to help identify subgroups of patients with high potential for developing metastases after surgery.27

IMP3 Predicts Aggressive Superficial UC

Most UCs of the bladder are superficial tumors, including TNM categories of Ta, noninvasive papillary tumors; Tis, flat carcinoma in situ; and T1, tumors invading the lamina propria.27,34 The biologic behavior of these superficial UCs is considerably variable, ranging from the relatively benign, noninvasive papillary tumor to a highly aggressive tumor with a significant mortality rate.28 An important clinical question is how to accurately assess individual risk of progression and to stratify patients for treatment. Currently, the identification of patients with aggressive superficial UC and determination of their treatment are mainly based on the tumor grade and stage.28 However, tumor grade and stage have limited ability to predict tumor progression. Therefore, there is a great need for biomarkers that can accurately distinguish superficial tumors with a high
probability of progression from those that will remain indolent. Many tumor markers (Ki67, p53, pRB, p21, and p27) have been studied for their potential use in assessing the prognosis of UC. However, a recent international consensus panel on prognostic markers for bladder cancer concluded that, although certain markers, such as p53, appear promising in predicting the progression of bladder cancer, the data were still heterogeneous.

IMP3, as a good prognostic marker, shows several attractive aspects for superficial UCs. The expression of IMP3 is correlated with other known aggressive indicators for superficial UC. Our data31 showed that the expression of IMP3 was strongly related to higher tumor grade and stage and to tumor recurrence.

The expression of IMP3 in biopsies of superficial UCs is an independent predictor of tumor progression.31 We found significantly increased tumor progression in patients with superficial UCs expressing IMP3 as compared with those without IMP3 expression. This was independent of tumor grade and stage. In a multivariable Cox analysis, patients with IMP3 expression in their superficial UCs subsequently developed invasive lesions or metastasis at a rate more than 6 times greater than did patients without expression of IMP3, adjusting for other well-known clinical variables.31 Therefore, IMP3 status in the initial tumor biopsies is a potentially important new risk factor that may be used in addition to tumor stage, grade, size, multiplicity, and coexistent carcinoma in situ to guide the decision of adjuvant courses of intravesical therapy after tumor resection.

IMP3 expression is associated with metastasis of UC. The expression of IMP3 was significantly increased in patients with primary T1 tumors who subsequently developed metastatic disease. Sixty percent of patients with IMP3 positivity in their primary T1 UCs developed metastases, whereas none of the patients without expression of IMP3 in their primary T1 tumors developed metastases.31 Currently, the decision as to whether to attempt bladder conservation with intravesical therapy or to perform a cystectomy is the most difficult issue in the management of superficial bladder cancer. Patients who undergo cystectomy have an unpleasant lifestyle, and in the absence of better prognostic tools, many patients who would not have progressed are subjected to these potential side effects.40 Therefore, accurately identifying the high-risk patients, particularly those with T1 tumors with poor prognosis, becomes an important clinical issue. The ability of IMP3 to identify patients presenting at stage T1 that will progress and present with metastatic disease would provide important clinical information. The results need to be confirmed and verified by additional studies with a large cohort of patients.

**TRANSLOCATION RCC**

Xp11.2 translocation renal cell carcinoma is a recently identified subtype of renal cancer defined by a breakpoint at Xp11.2 with gene fusions between the transcription factor E3 (TFE3) gene and 6 other genes. The most common fusion partners are the papillary renal cell carcinoma (PRCC, 1q21) gene and alveolar soft part sarcoma chromosome region, candidate 1 (ASPSCR1, 17q25) gene. The same ASPSCR1-TFE3 gene fusion is seen in alveolar soft part sarcoma, a rare pediatric tumor.42 Another renal translocation carcinoma exhibits the t(6;11)(p21;q12) translocation with a gene fusion between the alpha gene and the transcription factor EB (TFEB) gene.43,44 Both TFEB and TFE3 are members of the MiTF/TFE family of transcription factors.45 As both the Xp11 and the t(6;11) translocation carcinomas share the same clinical, genetic, morphologic, and immunohistochemical features, Argani et al.46 proposed that they should be grouped together as MiTF/TFE renal translocation carcinomas.

Approximately one-third of pediatric RCC cases involve gene translocations, whereas the translocation accounts for a lower percentage of RCCs in the adult population. However, there are actually more cases of translocation RCC in adults because RCC itself is much more commonly found in older adults. Ross and Argani47 estimate that there are 1260 new adult cases of translocation RCC per year, compared with 8 pediatric cases per year. The incidence of translocation RCC in adults may be underestimated, because of morphologic overlap and misclassification, as conventional RCC.

Like other types of RCCs, translocation RCCs are typically discovered incidentally during routine abdominal imaging for another purpose, and the patient is usually asymptomatic at discovery. The only known predisposing risk factor is cytotoxic chemotherapy treatment during childhood, which was found in about 15% of patients. The chemotherapeutic agents are thought to cause DNA damage, which then induces repair mechanisms that foster a translocation.41

Histologically, Xp11.2 translocation RCC is characterized by mixed alveolar and papillary growth of voluminous tumor cells with clear or eosinophilic cytoplasm. Psammomatous calcifications are often present in the stroma. There is substantial morphologic overlap with other subtypes of RCC; hence, IHC studies are typically used to confirm the diagnosis. The lack of reactivity of keratins and vimentin, which is distinct from conventional RCC and papillary RCC, is typical for Xp11.2 translocation RCC. The best positive IHC marker for Xp11.2 translocation RCC is strong nuclear staining for TFE3, which is very sensitive and specific for TFE3 gene fusions and thus for the diagnosis of Xp11.2 translocation RCC. However, excessive antigen retrieval can lead to false-positive results because native TFE is ubiquitously distributed (nonneoplastic tissue is a convenient internal negative control), and a negative TFE3 IHC cannot completely exclude a diagnosis of Xp11.2 translocation RCC.46 Interestingly, a recent study47 adds more complexity to this picture. Macher-Goeppinger et al.47 showed that TFE3 activation is not limited to Xp11.2 translocation RCC but may also be observed in a subset of nontranslocation RCCs associated with aggressive clinical behavior and poor survival. Hence, IHC by TFE3 will give us most, but not all, of the answers. Another good IHC marker for translocation RCC is cathepsin K.48 Like the Xp11 translocation carcinomas, the t(6;11) tumors are also focally positive or negative for pancytokeratin but are positive for HMB-45 and Melan-A. The fusion gene product TFE3 antibody is specific for the t(6;11) translocation carcinomas.44

Molecular diagnostic methods have also been employed to diagnose translocation RCC, including fluorescence in situ hybridization (FISH) and reverse transcription-polymerase chain reaction. The FISH assay uses break-apart probes that cannot detect each partner of the specific translocation but which show the presence or absence of a specific gene translocation.49 The FISH assay may be a useful complement to IHC because TFE3 staining may be falsely positive or negative. Currently, the use of FISH and reverse transcription-polymerase chain reaction is mostly
limited to research, but they may be incorporated into patient care in the near future.

As translocation RCC is a rare tumor, clinical outcome data are still premature.\textsuperscript{,}42,50 The prognosis depends on the age of the patient and the pattern of spread at diagnosis. Children with nodal metastasis but without hematogenous spread have a more-favorable, short-term prognosis.\textsuperscript{42} Perlman\textsuperscript{46} examined 6 different studies of pediatric patients and found that some studies reported a good prognosis even with lymph node metastasis, whereas others reported a poor outcome regardless of stage. Adults often present with aggressive tumors with widespread, systemic metastases, and those patients have a poor clinical outcome.\textsuperscript{52,50} Researchers\textsuperscript{53} studied a cohort of 54 patients with translocation RCC, the largest group studied so far, and found that being older than 25 years and lymph node status were independent prognostic variables. Tumor progression occurs in approximately 45% of tumors with either nodal or distant metastasis at diagnosis, and in only 15% of tumors without metastasis at diagnosis.\textsuperscript{46} Thus, Xp11.2 translocation RCC may be intrinsically more aggressive in adults than it is in children. However, the studies are limited by relatively short follow-up and by bias inherent in nonconsecutive case series, which precludes a definitive statement.

The best treatment of translocation RCC has yet to be determined. Many patients have received immunotherapy, which is the only standard treatment of patients with advanced-stage clear cell RCC. Malouf et al\textsuperscript{52} studied the benefit of targeted therapy using vascular endothelial growth factor receptor (VEGFR)–targeted agents and/or mammalian target of rapamycin (mTOR) inhibitors in patients with translocation RCC. They found that the effects of targeted therapy in this patient population were similar to that in patients with clear cell RCC and that sunitinib appeared to be more effective than cytokines (interferon-\(\alpha\) and interleukin 2). There is also emerging evidence that MET tyrosine kinase may be a potential therapeutic target for future research. The inhibition of MET tyrosine kinase or RNA interference of MET diminishes the growth of an ASPC1-TFE3 cell line in vitro.\textsuperscript{59} More work remains to be performed before this research can become useful clinically.

**UROVYSION FISH TEST IN URINE CYTOLOGY FOR DETECTION OF BLADDER CANCER**

Urine cytology is the most commonly used screening method to detect UCs. In recent years, many biomarkers have been employed to improve sensitivity in detecting UC in urine cytology. Examples include proteomic assays, BTA stat (Polymedico, Inc, Cortlandt Manor, New York) and nuclear matrix protein 22 (NMP22, BladderChek, Alere, Waltham, Massachusetts); the immuno cytochemical assay, ImmunoCyt (DiagnoCure, Inc, Saint-Quebec City, Quebec, Canada); and the UroVysion bladder cancer kit (Abbott Molecular Inc, Des Plaines, Illinois). Currently, UroVysion is widely used in clinical practice.

UroVysion is a multitarget multicolor FISH assay that examines 4 chromosomal abnormalities that commonly occur in UC. The assay is done on exfoliated urothelial cells using centromeric fluorescent denatured chromosome enumeration probes for chromosomes 3, 7, and 17, as well as a locus-specific identifier probe for 9p21. Normal cells are diploid or possess 2 copies of each chromosome. The types of genetic abnormalities observed by FISH include gains (3 or more copies) of one or more chromosomes, monosomy (1 copy), or deletions (no copies). The abnormalities that have been found to be associated with UC are polysomy (including tetrasomy), trisomy, and 9p21 deletion.\textsuperscript{54}

In an important trial conducted in 2002, which led to the US Food and Drug Administration (FDA) approval of UroVysion for the detection of UC in patients with a history of UC, the sensitivity of FISH was found to be 71% compared with 26% for cytology.\textsuperscript{53} In 2006, the FDA also approved the use of FISH for the new diagnosis of UC in patients without a previous history of UC. The FISH assay detected 69% of biopsy-proven UC cases, whereas cytology diagnosed only 38%.\textsuperscript{56} The FDA-approved criteria for a positive FISH result are (1) 4 or more cells with polysomy (3 or more copies of 2 or more chromosomes), or (2) 9p21 deletion in 12 or more cells. A minimum of 25 morphologically abnormal cells need to be scanned.

A meta-analysis done in 2008 from 14 studies totaling 878 patients and 2477 FISH tests showed that, although cytology is highly specific (96%), it has a low negative predictive value (42%). The sensitivity and specificity of FISH were found to be 72% and 83%, respectively. Both positive and negative FISH results affect posttest probability of disease. However, the differences in sensitivity and specificity were only present when superficial cancer cases (Ta tumors) were excluded from the analysis.\textsuperscript{57}

An issue has arisen about the interpretation of the test because different groups have modified the FDA criteria to define a positive test result. This may be the cause of published differences in test performance. The authors\textsuperscript{58} of the study that first introduced a set of positivity criteria used receiver operating characteristic curve analysis to determine different cutoff points, and most groups use various modified versions of those criteria. There remains a need for a larger-scale study with receiver operating characteristic curve analysis to assess and fine-tune the other criteria that have been proposed.

How clinicians can best make use of the UroVysion test is important. UroVysion has been used as a surveillance test for patients with a positive history of bladder cancer and for targeted screening in patients who present with symptoms that increase their risk of bladder cancer, such as hematuria. In cases of frankly positive cytology results for UC, FISH does not appear to be necessary. However, FISH has been useful in cases of equivocal cytology to help clinicians make decisions such as whether cystoscopy and biopsy are needed.

The advantages of UroVysion are improved sensitivity, compared with cytology, and its noninvasive nature compared with cystoscopy. A good surveillance tool should have high sensitivity and high negative predictive value.\textsuperscript{59} The FISH assay satisfies the former criterion but not the latter. If a patient has negative cytology results and a negative FISH result, cystoscopy may reasonably be avoided or postponed. If a tumor were to be present, it would most likely low grade, that is, slow growing and highly treatable.

On the other hand, UroVysion is an expensive, time-consuming assay that uses nonstandard criteria. The high cost limits its use as a screening tool, which is clearly not cost effective. Like cytology, FISH also requires the expertise of a pathologist to interpret the results, as well as extensively trained technicians to perform the assay.

The FISH assay is especially good for detecting carcinoma in situ, a flat, high-grade lesion often not visible on cystoscopy. However, most UCs are low grade, and more than two-thirds of these tumors tend to recur.
cytology nor FISH have been very sensitive at detecting low-grade superficial bladder cancers. In this function, cystoscopy has been more effective because most low-grade tumors are papillary lesions that can be seen and biopsied. More recently, a combination of cytology and FISH has been suggested to yield the best test for UC by first using bright-field microscopy to select abnormal-appearing cells that are then targeted for FISH. The pathologist is able to compare the bright field to fluorescent views of the same cell to use morphology as guide when deciding whether a cell appears truly abnormal.

With its higher sensitivity and ability to detect occult disease, FISH has found a niche in patient care, during the past decade, to support cystoscopy and/or cytology. Much more work remains to be done to determine a definitive role of UroVysion FISH in the detection of UC. There has yet to be a prospective, randomized trial that can help assess the true potential of this promising test.

**TRIPLE IMMUNOSTAINING OF DIAGNOSIS OF PROSTATE CANCER**

In the era of PSA screening of asymptomatic men, more prostate biopsies have resulted in more diagnoses of a small focus of prostate cancer on needle core biopsy. Prostate cancer and its mimics may be difficult to differentiate on needle core biopsy. In some cases, differentiation is not possible, and the equivocal category of atypical small acinar proliferations has emerged to classify needle biopsies with small clusters of glands suggestive of cancer but without sufficient changes for a definitive cancer diagnosis. The differential diagnosis for atypical small acinar proliferation includes prostate carcinoma, high-grade prostatic intraepithelial neoplasia (HGPIN), adenosis, atrophy, and crowded benign glands, among others. Immunohistochemistry is important in clarifying a diagnosis in these cases. Single chromagen immunostains for high-molecular-weight cytokeratin (34BE12) and p63 as negative markers for detecting basal cells were initially used to help differentiate prostate cancer from its mimics. More recently, the positive biomarker α-methylacyl coenzyme A racemase (AMACR) or P504S, which is highly expressed in prostate cancer, has been used to help clarify equivocal foci. Three antibodies are now frequently combined into a *triple cocktail* (PIN4) with 2 chromogens (AMACR red; p63/34BE12, brown) to better facilitate the workup of small, atypical foci.

P63 and 34BE12 are 2 basal cell–associated markers used to detect the presence of a basal cell layer. In invasive prostate cancer, the basal cell layer is typically absent, and absence of staining supports a diagnosis of malignancy. The monoclonal antibody 34BE12 targets cytoplasmic high-molecular-weight cytokeratin filaments in basal cells of the benign prostatic glands, namely CK1, CK5, CK10, and CK14. P63 is a nuclear stain for the p63 nuclear protein, which selectively stains basal cell nuclei and may provide greater specificity compared with 34BE12, which is more likely to have nonspecific reactions. When used together, p63 and 34BE12 highlight both the cytoplasm and the nuclei of basal cells and allow better evaluation of the basal cell layer. As discussed below, basal cells may be discontinuous or even absent in certain benign lesions; thus, the lack of basal cells is not, by itself, diagnostic of cancer. Because a negative reaction for basal cells is not diagnostic for prostate cancer, using a positive marker in combination with basal cell markers can help improve accuracy.

The AMACR gene product of AMACR is overexpressed in prostate cancer using complementary DNA (cDNA) library subtraction combined with high-throughput microarray screening and, in recent years, has been used as a positive marker for prostate cancer. Because negative staining for basal cell markers in a few atypical glands may not be sufficient for a definitive diagnosis of malignancy, AMACR as a positive diagnostic marker for prostatic adenocarcinoma may enhance our ability to diagnose limited prostate cancer. Jiang et al studied 73 prostate needle biopsies with a small focus (<1 mm in diameter) of prostatic carcinoma and 69 benign prostates. The AMACR immunoreactivity was found in 69 of 73 cases (94.5%) of carcinoma but not in any benign prostates (0 of 69; 0%) or benign glands adjacent to malignant glands. Many studies have demonstrated that AMACR has a sensitivity for prostate carcinoma, ranging from 82% to 100%, and a specificity of 79% to 100%, making it the ideal positive marker to be used in conjunction with the basal cell markers. However, using AMACR alone may lead to diagnostic errors, because AMACR is expressed in high-grade prostatic intraepithelial neoplasia, atypical adenomatous hyperplasia, postatrophic hyperplasia, and nephrogenic adenoma. In one study, in hormonally treated prostate cancers, 29% of tumor cases did not express AMACR. Likewise, the pseudohyperplastic variant of prostate carcinoma and “foamy gland” carcinoma do not always react to AMACR. Indeed, studies show that a combination of markers improves accuracy when one marker alone may lead to a misdiagnosis.

Several studies published in recent years using 2-antibody cocktails (a basal cell marker plus AMACR), support the use of concurrent positive and negative markers together to improve diagnostic accuracy for ambiguous prostate cancer cases. The AMACR/P63 cocktail is also useful in evaluating involvement of the seminal vesicles by prostate cancer. For the triple cocktail (AMACR, p63, and 34BE12), Jiang et al found the triple-antibody cocktail to be 95% sensitive and 100% specific for prostate cancer using 2 chromogens. Ng et al achieved a sensitivity of 93.8% and specificity of 100% using a single chromogen for all 3 antibodies. Another advantage of the triple-antibody cocktail is maximizing the use of small biopsy specimens, reducing the risk of exhausting all the tissue available for subsequent studies. Performing multiple stains simultaneously on one level may also save time, and it allows scrutiny of a small focus that may be lost if multiple levels are required.

Although the use of a double- or triple-antibody cocktail is clearly advantageous in improving diagnostic accuracy, these tests are far from perfect and should always be used as an adjunct to, and not in lieu of, light microscopy. Despite its high accuracy, a positive AMACR stain does not always mean malignancy, and a negative stain does not always rule out malignancy, as previously discussed. Similarly, basal cell markers may show absent or focal/weak staining in benign mimics of cancer, such as HGPIN, glandular atrophy, adenosis, or posthypertrophic hyperplasia. For example, HGPIN often shows only patchy staining, and some tangential sections of glands containing HGPIN may not stain for basal cell markers. Another common misinterpretation of basal cell staining is rendering a suspicious diagnosis because of a few, small, minimally atypical glands with absent staining admixed with similar-appearing glands with a patchy pattern of basal staining. This is a common pattern in benign mimics of cancer and thus overreliance or...
overuse of the triple immunostain may increase the “atypia” rate. Outpouchings of HGPIN and nephrogenic adenoma may both have results that are positive for AMACR and negative for basal cell markers, adding to the complexity of this picture.8,9,10 The AMCACC stain retains its properties as a good positive marker of prostate cancer in patients after radiation therapy; in most cases, it highlights the cancerous glands, which may be almost impossible to see on routine hematoxylin–eosin stain sections.11

A current issue is whether the triple-antibody cocktail should be routinely performed in all prostate needle biopsy specimens, before initial review by a pathologist. In a recent study by Tolonen et al,12 the authors recommended routine triple immunostaining of all prostate needle biopsies, which would increase diagnostic sensitivity and reduce workload for the pathologist, who is able to save time by the simultaneous review of the IHC and hematoxylin–eosin slides. Other authors do not recommend routine application of IHC to all prostate biopsies because they are helpful only in a small subset of difficult specimens, and in certain cases, IHC may even mislead the pathologist in diagnosis.13,14

Routine use of IHC is also not cost effective. Another related issue is the potential for overuse of the triple-antibody cocktail by pathologists who are either inexperienced with prostate biopsies or who are financially motivated to overuse the test. We have observed increased use of the triple cocktail in general pathologists, as compared with genitourinary pathologists (ongoing study at University of Massachusetts Medical Center, Worcester). The recent focus on health care inflation and the inevitable transition to capitated-reimbursement schedules may force a critical assessment of this disparity.

In summary, the triple-antibody cocktail has proven a powerful tool in the diagnosis of the small focus of prostate carcinoma. We strongly recommend the use of the triple cocktail in the study of problematic cases suspicious for focal carcinoma. However, pathologists must interpret the results judiciously in conjunction with their findings on hematoxylin–eosin slides.

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