Biomarker, Molecular, and Technologic Advances in Urologic Pathology, Oncology, and Imaging

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Urologic pathology is evolving rapidly. Emerging trends include the expanded diagnostic utility of biomarkers and molecular testing, as well as adapting to the plethora of technical advances occurring in genitourinary oncology, surgical practice, and imaging. We illustrate those trends by highlighting our approach to the diagnostic workup of a few selected disease entities that pathologists may encounter, including newly recognized subtypes of renal cell carcinoma, pheochromocytoma, and prostate cancer, some of which harbor a distinctive chromosomal translocation, gene loss, or mutation. We illustrate applications of immunohistochemistry for differential diagnosis of needle core biopsies, intraductal carcinoma of the prostate, and amyloidosis and cite encouraging results from early studies using targeted gene expression panels to predict recurrence after prostate cancer surgery. At our institution, pathologists are working closely with urologic surgeons and interventional radiologists to explore the use of intraoperative frozen sections for margins and nerve sparing during robotic prostatectomy, to pioneer minimally invasive videoscopic inguinal lymphadenectomy, and to refine image-guided needle core biopsies and cryotherapy of prostate cancer as well as blue-light/fluorescence cystoscopy. This collaborative, multidisciplinary approach enhances clinical management and research, and optimizes the care of patients with urologic disorders.


In this review, we summarize the effect of a number of biomarker, molecular, and technologic advances in urologic pathology, oncology, and imaging on routine, day-to-day practice and research at our institution. The objective of this review is not to cover the entire gamut of all advances that we have and use at our institution but, rather, to highlight a few pertinent concepts in our field. Topics that will be covered include biomarkers, molecular testing of selected malignancies (eg, transcription factor E3 [TFE3]/Xp11.2 translocation-associated renal cell carcinoma; p63, high–molecular-weight cytokeratin and racemase cocktail [PIN1]/phosphatase and tensin homolog [PTEN] for some cases of intraductal carcinoma of the prostate; and succinate dehydrogenase subunit B in renal cell carcinoma and pheochromocytoma), amyloidosis of the genitourinary tract, and workup of neoplastic needle core kidney biopsies. In addition, we will report the effect of pathologic, oncologic, and radiologic technologic advances (ie, global transcriptome analysis and RNA biomarkers of prostate cancer; robotic-assisted radical prostatectomy; minimally invasive videoscopic inguinal lymphadenectomy; targeted magnetic resonance imaging [MRI]–guided prostate needle core biopsies; anti-1-amino-3-fluorine 18-fluorocyclobutane-1-carboxylic acid in prostate cancer; cryotherapy for prostate cancer; and blue-light/white-light cystoscopy for bladder lesions) performed at our institution.

XP11 TRANSLOCATION-ASSOCIATED RENAL CELL CARCINOMA

Xp11 translocation-associated renal cell carcinomas (Xp11 RCCs) are characterized by chromosomal aberrations involving the Xp11 breakpoint, resulting in gene fusions of the TFE3 transcription factor gene, which maps to this specific locus.1,2 These tumors have now been incorporated into the larger category of the microphthalmia transcription factor (MiT) family of translocation RCC, which includes TFE3, TFEB, TFC, and MiTF.3 The 2 most common Xp11 RCCs are those bearing the t(X;1)(p11.2;q21) translocation, which fuses the PRCC gene to the TFE3 gene, and the t(X;17)(p11.2q25) translocation, which fuses the ASPSCR1 gene to the TFE3 gene. The latter translocation is the same gene fusion found in alveolar soft part sarcoma.4–6

The Xp11 RCCs were first described in the 2004 World Health Organization renal tumor classification and are typically recognized by their histologic features. Morphologically, these tumors have a varied spectrum of findings, but the most distinct pattern is that of a papillary architecture with clear, epithelioid cells. Some, but not all, cases show abundant psammomatous calcifications and areas mimicking other RCCs to include mixtures of solid and nested growth, anaplastic/pleomorphic giant cells, and cystic, sarcomatoid, oncocytic, tubular, and/or trabecular patterns.7,8 Certain morphologic features can be associated with the particular translocation, in that the ASPSCR1–TFE3 RCCs show large tumor cells with voluminous cytoplasm,
discrete cell borders, vesicular chromatin, and prominent nucleoli; a prominent alveolar or pseudopapillary architecture can also be present as well as extensive psammomatous calcifications. In contrast, PRCC-TFE3 RCCs tend to have less-abundant cytoplasm with fewer psammomatous calcifications and more compact and nested growth patterns. The most common morphologic mimicker of Xp11 RCC is clear cell RCC with focal papillary or pseudopapillary features, in which the carbonic anhydrase IX (CAIX) immunohistochemical (IHC) stain is helpful in the differential diagnosis because it demonstrates diffuse, membranous staining in clear cell RCC. Papillary RCC with clear cell features can be differentiated from Xp11 RCC by its diffuse labeling with cytokeratin 7 and because clear cell areas in a true papillary RCC tend to be focal and associated with degenerative changes. Diffuse CD117 staining can help to favor chromophobe RCC, rather than Xp11 translocation RCC.3

Immunohistochemically, even though Xp11 RCCs tend to underexpress the more-common epithelial markers, such as cytokeratins, epithelial membrane antigen, and vimentin, they consistently express CD10 and RCC as well as the kidney specific markers PAX2 and PAX8. The Xp11 RCCs occasionally express melanocytic markers, such as Melan-A and HMB-45. Strong nuclear expression (with adjacent negative staining in renal tubules) with the TFE3 antibody is the most sensitive and specific IHC finding to support a diagnosis of Xp11 RCC. However, the technical difficulty associated with the IHC of this tumor, including adequate overnight incubation and dependence on optimal fixation, leads to difficulty in interpreting patchy and/or weak-to-moderate intensity staining in tumor cells and in internal control tissue (Figure 1, A through D).

The TFE3 fluorescence in situ hybridization assays with break-apart probes have been extremely useful in detecting TFE3 gene fusions in these lesions because they are much less susceptible to the technical issues associated with IHC. Cathepsin K IHC can also be helpful in the diagnosis of these tumors. In a series of cytogenetically confirmed cases of Xp11 RCCs, 60% labeled with cathepsin K (PRCC-TFE3 > ASPSCR1-TFE3), in contrast to the absence of staining in the conventional clear cell, papillary, chromophobe RCCs or oncocytomas, or in any adjacent nonneoplastic renal tissue.

Outcome data on this entity are still relatively premature and highly variable, with some patients having an extremely
aggressive course and others a relatively indolent one. Overall, survival is similar to that seen in patients with clear cell RCC, and among patients with PRCC-TFE3 RCC and ASPSCR1-TFE3 RCC, only advanced stage and older age at diagnosis independently predicted death in a multivariate analysis of the 2 tumors.9

In summary, Xp11 RCC should be considered when assessing any RCC, in both children and adults, that has clear cell and papillary morphology and IHC features that are difficult to classify. Cytogenetic studies with fluorescence in situ hybridization can provide a definitive diagnosis, and TFE3 IHC in combination with cathepsin K IHC can aid in identifying lesions that should be triaged for cytogenetic testing.

**PIN4/PTEN EXPRESSION IN INTRADUCTAL CARCINOMA OF THE PROSTATE**

Historically, the term *intraductal carcinoma of the prostate* (IDCP) was used variably to describe prostatic acinar adenocarcinoma, prostatic ductal adenocarcinoma, and urothelial carcinoma showing extension into prostatic ducts and acini.13 Now the term IDCP is primarily used in reference to prostatic adenocarcinoma showing extension and proliferation within preexisting prostatic ducts.14,15 By definition, the proliferation of malignant epithelial cells filling large acini and prostatic ducts must be contained, at least focally, within a preserved layer of basal cells that can be identified by an IHC cocktail (PIN4) containing one or more basal cell markers and racemase (AMACR/P504S).16 Intraductal carcinoma of the prostate is associated with adverse pathologic findings, including high Gleason score (grade group), a high volume of primary (invasive) tumor in the prostate, and a greater likelihood of extraprostatic extension.16 The intraductal component itself should not be assigned a Gleason score, but the associated invasive component is typically significant prostatic adenocarcinoma with Gleason scores of 7 to 10 (grade groups, 2–5).17 The architectural pattern of IDCP includes solid growth, a dense cribriform or micropapillary pattern with marked nuclear atypia, and occasional comedonecrosis. The morphologic similarity to high-grade prostatic intraepithelial neoplasia (HGPIN), including the required presence of at least a focally intact basal cell layer, makes differentiating between IDCP and HGPIN a common diagnostic dilemma.

Recent studies have shown that IDCP is genetically different from HGPIN in terms of greater loss of heterozygosity and, occasionally, a greater frequency of *ERG* rearrangements than found in invasive cancer.18–22 In addition, *PTEN* is one of the most frequently inactivated tumor suppressors in prostate cancer, and it remains one of the most powerful, single-gene prognostic indicators in the disease.23 Lotan et al23 found that, in a panel of 58 cell lines, PTEN IHC was 100% sensitive and 97.8% specific for more basal cell markers and racemase (AMACR/P504S).16 The intraductal component itself should not be assigned a Gleason score, but the associated invasive component is typically significant prostatic adenocarcinoma with Gleason scores of 7 to 10 (grade groups, 2–5).17 The architectural pattern of IDCP includes solid growth, a dense cribriform or micropapillary pattern with marked nuclear atypia, and occasional comedonecrosis. The morphologic similarity to high-grade prostatic intraepithelial neoplasia (HGPIN), including the required presence of at least a focally intact basal cell layer, makes differentiating between IDCP and HGPIN a common diagnostic dilemma.

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Despite the poor prognosis of IDCP, its morphologic appearance alone has overlap specifically with the non-cribriform and cribriform variants of HGPIN, although both entities can be seen in association with invasive carcinoma and HGPIN does not have the same association with aggressive outcome.25 Given the clear difference in treatment and outcome, IHC studies beyond demonstration of basal cells at the periphery of either lesion are required for an accurate diagnosis. Combined immunostaining for PTEN and basal cell markers with PTEN and PIN4 IHC (with or without associated staining with the ERG antibody) can reliably distinguish IDCP from HGPIN morphologically; the presence of basal cells, coupled with the loss of PTEN staining in epithelial cells (and retained ERG staining) is diagnostic of IDCP. In contrast, the presence of basal cells with retention of PTEN (and absent staining with ERG) suggests HGPIN (Figure 2, A through D).25 Only a few studies have shown *PTEN* deletion in HGPIN lesions,27–28 and in most of those cases, the HGPIN was found to be closely adjacent to invasive adenocarcinoma, indicating that some HGPIN lesions adjacent to high-grade prostatic adenocarcinoma may actually represent IDCP not fully meeting morphologic criteria as such.23

In summary, IDCP is an exceedingly specific marker of clinically aggressive disease that is almost invariably associated with high-grade disease in radical prostatectomy specimens, so much so, that when it is identified in isolation on needle core biopsies without associated invasive tumor, patients should still undergo definitive treatment.23 As such, accurate identification and differentiation from the most common morphologic mimicker (HGPIN) is paramount, with IHC demonstrating loss of PTEN in epithelial cells and retention of basal cells a useful and effective measure, particularly on needle core prostate biopsies.

**SDHB MUTATIONS IN PHEOCHROMOCYTOMA AND RENAL CELL CARCINOMA**

Pheochromocytoma and paraganglioma are relatively uncommon tumors of neuroendocrine origin that arise from neural crest–derived chromaffin cells of the adrenal medulla and sympathoadrenal and parasympathetic paraganglia, respectively.29,30 Originally described by Gimenez-Roquelpo et al31 genetic variants in the B, D, and C subunits of the mitochondrial complex II–succinate dehydrogenase (*SDHB*, *SDHD*, and *SDHC*) have recently been associated with the hereditary paraganglioma–pheochromocytoma syndromes (PGL4, PGL1, and PGL3).32–34 The SDH or respiratory complex II is an enzyme complex that catalyzes the oxidation of succinate to fumarate in the Krebs cycle and participates in the electron transport chain. It is composed of 4 subunits encoded by the corresponding genes: *SDHA*, *SDHB*, *SDHC*, and *SDHD*.35–38 Inactivating mutations in 1 of the 4 genes lead to accumulation of succinate, formation of reactive oxygen species, stabilization of HIFα, and activation of hypoxia-dependent pathways.29,39 Defects in *SDHA* cause metabolic neurodegenerative disorders, such as congenital Leigh syndrome and late-onset optic atrophy, ataxia, and myopathy.38 *SDHC* and *SDHD* mutations are typically associated with head and neck paragangliomas.40 *SDH* mutations are more frequently associated with extra-adrenal sites, recurrence, and malignancy of these lesions; however, a significant number of *SDHB*-mutated tumors involve the adrenal gland (and kidney) and, therefore, may be submitted as a specimen from the genitourinary tract.33,41–43 Because malignant pheochromocytomas are currently incurable, with a 5-year survival of approximately 50%, additional studies are needed to identify tumors that harbor germline succinate dehydrogenase mutations.29,39 Definitive clinical or pathologic criteria to determine malignancy (before metastasis) are largely lacking, but identification of
these mutations, when present, can significantly contribute to better prognostication with guidance of surveillance and can have a significant effect on therapeutic measures.\textsuperscript{40} SDHB mutations are present in approximately 1.7\% to 6.7\% of patients with “sporadic” pheochromocytomas having a penetrance of approximately 25\% to 40\% and a high frequency of malignancy.\textsuperscript{35} Approximately 20\% of mutation carriers will develop malignant disease, and up to 50\% of patients with malignant pheochromocytomas and paragangliomas harbor a germline SDHB mutation.\textsuperscript{44–47} One study showed negative immunoreactivity (loss) with the SDHB antibody in 8\% (n = 13) of moderately or poorly differentiated pheochromocytomas or paragangliomas, and 10 of those 13 patients (77\%) had developed metastases, suggesting that routine IHC staining with SDHB might be useful for prediction of metastasis in these tumors.\textsuperscript{48} In another study detailing 33 bilateral adrenal pheochromocytomas and 26 abdominal, but extra-adrenal sympathetic paragangliomas, no SDHB mutations were found in any of the bilateral pheochromocytomas; however, 3 SDHB mutations were found in the 26 sympathetic paragangliomas, of which, 1 was metastatic at the time of the study.\textsuperscript{30}

Detection of the mutation in surgical pathology specimens can be performed by IHC because SDHB protein expression is a sensitive and specific tool for detecting patients with germline SDHA, SDHB, SDHC, or SDHD (SDHx) mutations. The SDHB protein is lost in all SDHx-mutated tumors, irrespective of the mutated gene.\textsuperscript{49} In contrast, SDHB\textsuperscript{+} (retained) immunostaining (characterized by a mitochondrial-specific granular labeling pattern) is detected in other, non-SDHx, inherited forms and in sporadic tumors. A few sporadic tumors with no SDHB staining (loss) have been described, rendering SDHB IHC a predictive criterion for detection of the mutation, with a specificity of approximately 84\%.\textsuperscript{36,49} Negative staining (loss) either corresponds to the presence of a mutation or to a false-negative result in a sporadic tumor. In either case, a recommendation for genetic testing should ensue, particularly because even SDHB\textsuperscript{−} tumors by IHC, without an associated SDHx mutation, have been shown to be associated with pheochromocytoma-paraganglioma syndrome.\textsuperscript{50}

In addition to pheochromocytomas and paragangliomas, SDHB mutations are also associated with RCC, which can have an aggressive phenotype in young women. Therefore,

\begin{figure}[h]
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\caption{A, A single gland with cribriforming architecture, focal comedonecrosis, and relatively banal-appearing epithelial cells. B, Staining with PIN4 by immunohistochemistry demonstrating an intact basal cell layer. C, Absence of cytoplasmic staining with the PTEN antibody. D, Strong-intensity nuclear staining with ERG (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×200 [B through D]).}
\end{figure}
it is recommended that patients with SDHB mutations be offered surveillance screening for RCC.\textsuperscript{50–53} SDHB-mutated RCCs also lack IHC labeling (loss) for the SDHB antibody, and many of the lesions reported to date have a distinct morphology, characterized by sheets of uniform cells with eosinophilic or oncocytic cytoplasm containing cytoplasmic vacuoles or flocculent inclusions and, occasionally, intratumoral mast cells (Figure 3, A through D).\textsuperscript{51} Williamson et al\textsuperscript{51} studied 11 of these tumors from 10 patients that displayed some of the above distinctive morphologic features and loss of the SDHB antibody by IHC. Next-generation sequencing of DNA extracted from paraffin-embedded tissue showed an SDHB mutation and loss of the second in 5 of 6 tumors studied. A recent report by Gill et al\textsuperscript{54} described 36 cases of succinate dehydrogenase–deficient RCCs, 21 of which were previously unreported. This study demonstrated the presence of the described morphologic features in 94\% of the tumors studied and estimated that 0.05\% to 0.2\% of all RCCs are SDH deficient with a slight male predominance, bilaterality in 26\%, and a strong association between high-grade nuclei and subsequent metastasis.

In summary, reports of succinate dehydrogenase–deficient tumors are increasing as awareness is increasing, and the lesion can occasionally be encountered as a surgical pathologic specimen from the genitourinary tract. Liberal use of the SDHB IHC antibody on pheochromocytomas, intra-abdominal paragangliomas, and RCCs with the morphologic features described above (particularly when bilateral) can help in further identifying these tumors and direct patients and their families to appropriate genetic counseling.

\textbf{AMYLOIDOSIS OF THE GENITOURINARY TRACT}

Amyloidosis is a pathogenetically diverse disease that can be localized to particular organs or spread systemically among several organs. It originates from soluble globular proteins that undergo a poorly understood conformational change (fibrillar proteins in a $\beta$-pleated sheet conformation), leading to deposition in tissues and associated functional disturbances.\textsuperscript{55} Surgical pathologic and autopsy evaluation of amyloidosis has historically been limited to confirmation of the substance by Congo red birefringence; however, characterization of amyloid deposits with various amyloid-related IHC stains and with mass spectrometry may serve as additional steps in the workup of these patients.

Occasionally, localized amyloidosis of the genitourinary tract can be the first symptomatology of systemic disease,
rendering proper diagnostic characterization crucial. The most common locations of amyloid deposits in the genitourinary tract are in the seminal vesicle, bladder (Figure 4, A through E), upper urinary tract (ureter and renal pelvis), glans penis, and rarely, within the prostatic stroma. Seminal vesicle amyloidosis (SVA) is typically found at radical prostatectomy and, less commonly, on needle core biopsies, with deposit in a bandlike, subepithelial location. Historically, it was believed that SVA was associated with prior hormonal treatment that acted as a stimulant of the epithelium of the seminal vesicles to produce amyloid. However, more recent studies have found SVA to be an incidental finding on surgical pathology specimens that did not correlate with prior treatment or the presence or grade of prostatic adenocarcinoma but that could be associated with hematospermia. Very few studies with IHC characterization have been performed, and results have been conflicting. A single study on a few cases (n = 5) has shown that the predominant subtype in this location, upon characterization with mass spectrometry, is senogelin type 1; however, this number largely underestimates the total number of cases of SVA in the literature and easily in existence because SVA is not consistently reported on surgical pathology reports.

Only about 200 cases of primary bladder amyloidosis have been reported worldwide. Primary (or secondary) amyloidosis of the urinary tract commonly masquerades as malignancy, indicating the clinical importance of the location. Patients typically present with grossly painless hematuria and lower urinary tract symptoms in the fifth to seventh decades of life with an equal sex distribution. The diagnosis is difficult to make by cytologic examination of urine specimens because deposition within the bladder wall shows poor correlation with precipitation in urine. The most common subtype of amyloid involving the bladder is amyloid light chain (AL), with conflicting reports on the predominant light chain (λ versus κ). A few cases of ATTR (transthyretin) have also been reported; however, none of those cases had been confirmed characterized by mass spectrometry.

Less commonly, amyloidosis is seen in the glans penis. Approximately 12 cases have been reported in the literature, with the common presentation of asymptomatic, nodular, tan/yellow, and rubbery deposits on the skin of the penis. Of the 3 cases in a report characterized by IHC, 2 were serum amyloid-associated protein, and the other was an AL-λ amyloid; the remaining cases were unable to be fully characterized by IHC or mass spectrometry. None were found to be definitively associated with systemic disease. A single report of prostate stromal amyloid deposition preceding a diagnosis of systemic AL amyloidosis has been reported.

In summary, amyloid deposition within the genitourinary tract (excluding renal biopsies for medical disease) is a relatively rare occurrence that has the most significant effect when it involves the upper and lower urinary tract. Definitive characterization of more cases of SVA by mass spectrometry, which can affect the treatment regimen, may be considered, particularly when deposition in the genitourinary tract is the initial presentation of systemic disease.

**Biomarker Workup of Needle Core Biopsies from Renal Neoplasms**

Needle core biopsies of renal masses have become more frequent in the past decade at our institution because of the number of incidentally detected masses, as well as advances in the procedural techniques that make them a relatively low-risk procedure that can yield valuable information.

Most needle core biopsies are diagnostic and relatively easy to subtype because most will fall into 1 of the 4 most common categories of renal neoplasms: clear cell RCC, papillary RCC, chromophobe RCC, or renal oncocytoid neoplasm. A few needle core biopsies represent uncommon lesions, an unusual presentation of the 4 common subtypes of renal neoplasms, or secondary/metastatic tumors and, therefore, require an especially diligent approach when reviewing such cases.

**Establishing a Diagnosis of Primary Renal Origin**

In general, most clinically detected renal masses represent primary renal cell neoplasms; however, it is important to initially assess whether the clinical setting and radiologic findings point toward a secondary/metastatic tumor. In a patient with suspected metastatic disease or hematopoietic neoplasms, it is easier to know this information up front to rule in or out this possibility. PAX8 is considered a reliable universal marker for tumors of renal origin. PAX8 is a nuclear stain that is positive in most primary renal neoplasms, although loss of expression may occur in dedifferentiated primary renal tumors. However, tumors from other organ systems can also be positive for PAX8, including gynecologic tract tumors and thyroid tumors. Some cases of urothelial carcinoma arising from the pelviccalyceal system may also be positive for PAX8, highlighting the necessity of using this marker as part of a panel dictated by the histologic features on hematoxylin-eosin stain.

Once a primary renal origin is established, masses should be grouped into 1 of 4 major histologic categories: (1) tumors predominantly composed of clear cells, (2) tumors with a predominant papillary architecture, (3) tumors with eosinophilic/oncocytic cytoplasm, or (4) tumors with a spindled appearance.

**Tumors With Clear Cytoplasm**

Most RCCs with clear cytoplasm are conventional clear cell RCCs; however, other subtypes enter the differential diagnosis, such as clear cell papillary (tubulopapillary) RCC, papillary RCC with predominance of clear cells, and translocation-associated RCC. In general, the differential diagnosis can be resolved with the help of a panel of IHC stains.

Clear cell RCC is the most-common subtype of RCC. Clear cell RCC is positive for CAIX, RCC, vimentin, and CD10. CAIX is the most recent and reliable marker for clear cell RCC, where it usually shows a diffuse, strong cytoplasmic and membranous staining pattern. Sometimes, papillary RCC presents with patchy or predominantly clear cell cytoplasm, and similar to classic papillary RCC these tumors are usually positive for CK7 and racemase (AMACR/p504S). Clear cell papillary (tubulopapillary) RCC is a separate subtype of RCC with a good prognosis, which is characterized by low-grade nuclei, reverse polarity, and “picket-fencing” of the nuclei, in addition to a tubular and papillary architecture. Clear cell papillary (tubulopapillary) RCCs are positive for CAIX; however, unlike clear cell RCC, the positivity is “cuplike” with an absence of staining toward the lumen (clear cell RCC shows boxlike positivity, with positivity on all sides of the cells, including luminal...
Figure 4. A bladder biopsy specimen with amyloid deposition, characterized by mass spectrometry as λ light chain (AL) associated. A, Normal urothelium overlying stromal and vascular infiltration by pink amorphous material. B, Bright Congo red staining, which was polarizable to apple green. C, Strong-intensity staining in the deposits with the P fragment of amyloid (amyloid P immunohistochemical stain). D and E, λ Light chain (E) predominant staining over κ light chain (D) (hematoxylin-eosin, original magnification ×100 [A]; original magnification ×100 [B through E]).
staining). Clear cell papillary (tubulopapillary) RCCs are also positive for CK7 (an unusual finding in clear cell RCC) and can be positive for high–molecular-weight cytokeratin. Translocation-associated RCC can show foci that are indistinguishable from clear cell RCC and should be suspected when the cytoplasm is voluminous or when dealing with a younger patient. Translocation-associated RCCs can be negative for pancytokeratin and are usually positive for cathepsin K and TFE3. The TFE3 IHC stain is a technically difficult stain, and we recommend performing TFE3 fluorescence in situ hybridization studies to confirm the diagnosis of this entity. A small subset of translocation-associated RCCs can be positive for HMB-45. Although chromophobe RCC can sometimes have focal cytoplasmic clearing, the nuclear hallmarks of chromophobe RCC (raisinoïd nuclei, binucleation, and perinuclear halos) are preserved and are helpful. Chromophobe RCC is usually diffusely positive for CK7 and CD117.

**Tumors With Papillary Architecture**

The most common tumor with papillary architecture is papillary RCC.74 Papillary RCC represents about 10% of all RCCs and can be bilateral and/or multifocal. Papillary RCC is subdivided into types 1 and 2, based on cytologic features. Type 2 papillary RCC shows nuclear enlargement, eosinophilic cytoplasm, and nuclear pseudostratification and can lack the characteristic foamy macrophages within the fibrovascular cores (Figure 5, A through D). Type 1 papillary RCCs are thought to have a better prognosis and are usually positive for CK7 and racemase, whereas type 2 papillary RCCs have a worse prognosis. Although type 2 papillary RCCs are usually positive for CK7 and racemase, they can occasionally demonstrate negativity for CK7. Although papillary RCCs in most cases present with papillary architecture, in a few cases it may present with a solid architecture. Papillary adenomas are differentiated from papillary RCC by a size cutoff between 0.5 and 1.5 cm (recommended by the International Society of Urological Pathologists). If the specimen represents a targeted biopsy of a lesion identified on imaging, the size of the lesion is usually addressed radiologically. However, when a papillary neoplasm is incidentally identified on a medical renal biopsy, it is important to include papillary adenoma within the differential diagnosis.

Clear cell papillary (tubulopapillary) RCC should also be considered in the differential diagnosis of a tumor with papillary architecture on needle core biopsy. As addressed above, the tumor is usually positive for CAIX in a cuplike fashion. It is also positive for CK7 and high–molecular-weight cytokeratin and shows characteristic histologic features. Translocation-associated RCCs are also part of the differential diagnosis (discussed above). Perhaps the most significant differential diagnosis on needle core biopsy is between papillary RCC and collecting duct carcinoma, considering the aggressive behavior of the latter. Collecting duct carcinoma is usually a high-grade tumor with variable histologic features, including papillary, tubular, glandular, and solid patterns, and is embedded within a desmoplastic stroma with an inflammatory response. The IHC pattern of collecting duct carcinoma is not specific but is usually positive for CK7 and can be variably positive for other markers. Lectins, *Ulex europaeus* agglutinin-1, peanut, and soybean agglutinin are also helpful when positive.

**Tumors With Eosinophilic Cytoplasm**

The most-common renal tumors with eosinophilic cytoplasm are oncocytoma and chromophobe RCC.75 Oncocytomas are benign neoplasms that represent approximately 10% of renal tumors. Histologically, they are composed of nests of bland oncocytic cells in a hypocellular and hyalinized stromal background. Necrosis and mitoses are not usually observed in oncocytoma. Nuclear degenerative atypia can be present as well as infiltration into the surrounding adipose tissue. Oncocytomas can be CD117⁺ and show positivity in rare cells or small clusters of cells for CK7. The differential diagnosis of oncocytoma includes hybrid tumors, which are relatively rare tumors composed of foci of chromophobe RCC in the midst of a tumor that looks like an oncocytoma. Another relatively more-common entity in the differential diagnosis is the eosinophilic/oncocytic variant of chromophobe RCC, which usually has subtle foci of cells with classic nuclear features of chromophobe RCC (raisinoïd nuclei, binucleation, perinuclear halos) scattered throughout. It is often challenging to make the distinction among these 3 entities even on resection specimens; therefore, in our opinion, a definitive diagnosis of an oncocytoma should not be rendered on needle core biopsies. We prefer instead the diagnosis of renal oncocytic neoplasm, favor oncocytoma (Figure 5, E through G). When nuclear atypia is present and chromophobe RCC or a hybrid tumor cannot be excluded with certainty, a diagnosis of renal oncocytic neoplasm with a comment explaining the differential diagnosis is preferred. When nuclear features of chromophobe RCC are seen and/or are accompanied by the characteristic vasculature, plant-cell wall-like cytoplasmic membranes, and diffuse CK7 positivity, a diagnosis of chromophobe RCC is made. Chromophobe RCC represents less than 10% of RCCs and, in general, has a better prognosis than does clear cell RCC or papillary RCC. Chromophobe RCC is composed of sheets of eosinophilic cells with raisinoïd nuclei, binucleation, and perinuclear halos, and they are usually diffusely CK7⁺ and CD117⁺.

The most common RCC with eosinophilic cytoplasm after the above-discussed tumors is clear cell RCC with eosinophilic cytoplasm. It is not uncommon for clear cell RCC to have an eosinophilic cytoplasm and the likelihood increases the higher the grade of the carcinoma. The characteristic nuclear features of chromophobe RCC are absent, and the characteristic vascular pattern of clear cell RCC is usually maintained. Clear cell RCC with eosinophilic cytoplasm is also positive for epithelial membrane antigen, CAIX, vimentin, and CD10.

A much rarer confounder is “epithelioid” angio(myolipo-)ma (AML), which often has eosinophilic cytoplasm. Immunohistochemical stains are positive for HMB-45 and Melan-A and are negative for pancytokeratin (see discussion below regarding classic AML).

**Tumors With Spindle Cell Morphology**

The most-common spindle cell tumor of the kidney is AML. In general, AML with a considerable fatty component can be readily diagnosed radiologically, but tumors that are “fat poor” are more diagnostically challenging by imaging studies. AMLs are mesenchymal tumors that usually display 3 components: a vascular component (angio-), a spindle cell or myoid component (myo-), and a fatty component (lipo-). They usually have bland
cytology. These tumors are positive for HMB-45 and Melan-A (Figure 5, H through J) and are negative for pancytokeratin. Smooth muscle actin may also be positive in the spindle or myoid component. The most important differential diagnosis is between a spindle cell–predominant AML and sarcomatoid RCC. The differential diagnosis is usually resolved based on histologic grounds, in which sarcomatoid carcinoma displays malignant cytologic features, including nuclear pleomorphism and atypia, mitotic activity, and necrosis. In addition, a panel of IHC stains can be helpful; sarcomatoid RCC usually maintains pancytokeratin positivity (CK8/18, if available, also appears to be a useful marker in this setting).

In conclusion, needle core biopsies of renal masses are diagnostic in most cases. An approach that combines the histologic features with a panel of targeted IHC stains is recommended for a definitive diagnosis, especially in challenging cases. Although a few prognostic markers have been proposed in the literature, there is still no gold standard for prognostication in kidney tumors.

GLOBAL TRANSCRIPTOME ANALYSIS AND RNA BIOMARKERS OF PROSTATE CANCER

Prostate cancer remains the most-common noncutaneous malignancy diagnosed in men, with more than 200,000 new cases and almost 28,000 deaths per year in the United States. Urologic oncologists, genitourinary radiation oncologists, genitourinary medical oncologists, and patients are faced with difficult management decisions when a diagnosis of prostate cancer is made because it is often challenging to accurately predict whether a patient is likely to progress to aggressive and metastatic disease. Most patients with prostate cancer are clinically asymptomatic with early stage and organ-confined disease. In fact, more than 50% of men who are 80 years and older develop clinically insignificant prostate cancer. However, a subset of patients, initially diagnosed with early stage and organ-confined prostate cancer, may progress to highly aggressive, androgen-independent/castration-resistant, metastatic disease.

Although there are already well-established clinical/pathologic/prognostic parameters for patients with prostate cancer, including age, prostate specific antigen, Gleason score (grade group), stage, margin status, among others, it is well known that these parameters alone are insufficient for accurate prediction of tumor recurrence in a subset of patients. One of the greatest challenges in the management of prostate cancer is identifying patients with clinically significant tumors that require aggressive treatment in contrast to patients who have indolent tumors that are unlikely to progress and may be successfully managed with active surveillance. Biomarkers that predict the likely clinical outcome/prognosis and tumor-recurrence status in patients with prostate cancer are urgently needed to aid clinicians involved in the management of these patients for the best course of treatment (aggressive versus active surveillance) and accurate prognostication. At our institution, we recently published a panel of 10 protein-coding genes and 2 microRNA (miRNA) genes that could be used to stratify patients with and without biochemical recurrence after radical prostatectomy. This panel of 12 biomarkers could significantly predict clinical recurrence for patients with a Gleason score of 7 (grade groups 2 and 3), but this analysis was limited to a selected set of approximately 522 prostate cancer–relevant genes and 1146 human miRNAs. In a more recent study to take a more-global approach to the identification of biomarkers of recurrence, we collected formalin-fixed, paraffin-embedded radical prostatectomy specimens from 3 independent sites and prepared libraries for RNA sequencing. We performed RNA sequencing on prostatectomy samples from 100 patients and identified a new set of biomarkers of biochemical recurrence composed of a 24-gene panel. This panel showed significant improvement of prediction of biochemical recurrence over clinical parameters alone and over previously described biomarker panels based on cellular proliferation. The findings were especially significant in demonstrating that RNAseq analysis of formalin-fixed, paraffin-embedded prostate cancer specimens is feasible and can provide critical insights into the mechanisms of prostate cancer progression that may be translated into clinically useful biomarkers, which can then be used in institutions and settings that may not have access to frozen, banked specimens.

ROBOTIC-ASSISTED UROLOGIC SURGERY

There is no doubt that there has been a paradigm shift over the years from open radical urologic surgery to minimally invasive robotic surgery. One of the first procedures in urologic oncology to undergo this transformation was radical prostatectomy. The first major article on robotic-assisted radical prostatectomy was published in 2001. One of the first centers in the United States to pioneer this procedure was the Vattikuti Urology Institute, Henry Ford Hospital (Detroit, Michigan). Since then, there has been an exponential increase in the utility of this procedure, and currently, about 60% to 80% of all radical prostatectomies (both academic and nonacademic institutions) in the United States are done robotically. More than 100 robotic-assisted urologic surgeries are performed at our institution annually (Figure 6, A through C). Many landmark articles have been published by...
The advantages of this procedure include minimally invasive surgery, with minimal perioperative/postoperative complications; superior ergonomics; better visibility; improved lymph node dissection; less positive margins (in experienced hands); and shorter postoperative stays in the hospital. This is indeed a testament to the reduction in the positive margin rate at our institution over the years. One of the most challenging aspects of both open and robotic-assisted radical prostatectomy is the dissection of the apex of the prostate. There has to be a delicate balance between cancer control (with negative margins) and maintenance of urinary continence and potency. At our institution, some of the urologists have used intraoperative frozen sections to assess the margin status at the apex and other aspects of the prostate gland in challenging cases. Recent studies from other institutions have also demonstrated the feasibility and adoption of the neurovascular structure–adjacent frozen section examination (NeuroSAFE) procedure during robot-assisted radical prostatectomy. There was no significant increase in blood loss and/or operating time with this procedure. In addition, this technique could maximize nerve-sparing frequency and reduce positive surgical margins even in non–organ-confined tumors. We have also noticed an increase in the number of lymph nodes excised by urologists during robotic-assisted radical prostatectomy at our institution, which has led to more-accurate and reliable staging of tumors. Robotic-assisted surgery has been used in several other oncologic procedures at our institution, including radical cystoprostatectomy, radical nephrectomy, partial nephrectomy, and adrenalectomy, among others.

MINIMALLY INVASIVE VIDEOSCOPIC INGUINAL LYMPHADENECTOMY

Another important procedure, introduced relatively recently at our institution, is minimally invasive videoscopic inguinal lymphadenectomy. Although open inguinal lymphadenectomy is the typical procedure performed for the analysis of inguinal lymph nodes in patients with malignancies including penile carcinoma, the minimally invasive approach is gaining traction at our institution. This approach was initially pioneered at our institution for the evaluation of inguinal lymph nodes in patients with malignant melanoma. However, it was subsequently used in patients with penile cancer with great success. The historic aspect of the introduction of laparoscopic surgery is interesting. Because of the publication of a major randomized trial comparing oncologic outcomes of patients who had laparoscopic versus open colectomy for colorectal carcinoma, the utility of minimally invasive approaches in the management of this and other malignancies has been accepted. When a 3-port approach is used for inguinal lymphadenectomy in the management of patients with metastatic penile carcinoma (or malignant melanoma), the groin scar/incision is eliminated. The typical complications (including wound dehiscence) associated with “conventional” open inguinal lymphadenectomy are less frequent with the minimally invasive videoscopic inguinal lymphadenectomy, and the lymph node yield is also improved. This is important from a pathologic, clinical, and prognostic standpoint. Even relatively large inguinal lymph nodes can be removed via
this laparoscopic approach. In a series published at our institution, lymph nodes as large as 4.0 cm were excised using a specimen bag, without having to increase the size of the 1.2-cm apical port. In addition, larger nodes up to 5.6 cm were successfully excised by increasing the size of the apical port to 1.5 mm.

TARGETED MRI-GUIDED PROSTATE NEEDLE CORE BIOPSIES

The prostate gland is one of the few organs that is typically sampled by a standardized, nontumor-targeted technique (transrectal ultrasound-guided biopsy). In most other solid organs, suspicious areas are identified with the aid of direct visualization or imaging studies before biopsy. Because prostate cancer is frequently multifocal and the volume of prostate sampled is relatively small, sampling error is relatively common in transrectal ultrasound-guided biopsy. The rate of prostate cancer detection decreases from 22% on first transrectal ultrasound-guided biopsy to 4% by the fourth. Targeted MRI-guided biopsy has recently emerged as a technique that could improve cancer-detection rates and potentially limit the number of prostate biopsies performed in patients with a high clinical suspicion for prostate cancer. At our institution, our urologic pathology team works closely with an experienced interventional radiologist who has expertise in targeted MRI-guided biopsies (Figure 8, A through D), and we have noted an upward trend in the detection of prostate cancer in patients with previously negative transrectal ultrasound-guided biopsy. We believe that more cores were retrieved from malignant lesions because of radiologic findings that raised the suspicions of the interventional radiologist. These particular characteristics have not yet been fully elucidated, but we intend to characterize them in future studies. Because we also document benign histologic findings in our pathology

Figure 8. Targeted magnetic resonance imaging–guided prostate needle core biopsies. (Images courtesy of Sherif G. Nour, MD, FRCR.) A, An interventional radiologist about to obtain magnetic resonance imaging–guided biopsies. B, High-resolution T2-weighted axial magnetic resonance imaging section. C, High-resolution T2-weighted coronal magnetic resonance imaging section. D, High-resolution T2-weighted sagittal magnetic resonance imaging section. Arrows in each figure denote malignant tumors.
reports, we may be able to characterize findings that are more likely associated with benign prostatic hyperplasia, high-grade prostatic intraepithelial neoplasia, or atypical small acinar proliferation and are less likely associated with cancer.

Because cancer detection rates improve with targeted MRI-guided biopsy (and as specific radiologic characteristics of suspicious lesions become better elucidated), we may be able to decrease the number of unnecessary biopsies performed on patients with a high clinical suspicion for prostate cancer and focus more on targeted lesions within the prostate gland. We predict that targeted biopsies will ultimately decrease the number of biopsy sessions and the cores sampled per biopsy that are required to diagnose prostate cancer, thereby decreasing cost. It is also conceivable that targeted MRI-guided biopsy may have a critical role as a tool for active surveillance, tumor mapping, and primary detection of prostate cancer in the future.

**POSITRON EMISSION TOMOGRAPHY–COMPUTED TOMOGRAPHY FOR DETECTION OF PRIMARY, RECURRENT, AND METASTATIC PROSTATE CANCER**

One of the main diagnostic pitfalls of ultrasound-guided needle core biopsies is the inability to directly and reliably visualize prostate cancer on imaging via this technique. Therefore, there has been a push to identify potential molecular/radiologic markers that might highlight the focus of prostate cancer on imaging.

Anti-1-amino-3-fluorine 18-fluorocyclobutane-1-carboxylic acid (anti-3-[18F] FACBC) is a synthetic amino acid analog positron emission tomography (PET) radiotracer that has demonstrated great utility for the staging and restaging of prostate carcinoma. The uptake of FACBC, likely mediated via sodium-dependent (ASC system) and sodium-independent (type) amino acid transporters. This marker has been used at our institution in combined PET and computed tomography (CT), and we have found that anti-3-[18F] FACBC PET CT had significantly greater accuracy than did routine CT or MRI, detecting more prostatic and extraprostatic disease and effectively up-staging 25.7% of cases. Our recent experience with anti-3-[18F] FACBC PET CT in the evaluation of primary prostate carcinoma has shown a sensitivity of 81.3%, a specificity of 50.0%, with positive predictive and negative predictive values of 76.5% and 57.1%, respectively. There was also a statistically significant correlation between Gleason scores (grade groups) and anti-3-[18F] FACBC maximum standardized uptake (Figure 9, A and B). Even though a statistically significant difference between mean maximum standardized uptake and total activity between nonmalignant and malignant regions of the prostate has been demonstrated, overlap of radiotracer activity between nonmalignant and malignant regions may limit practical utility for radiotherapy planning. However, the correlation between both higher Gleason scores and anti-3-[18F] FACBC maximum standardized uptake could be useful to direct biopsy to the areas of the most aggressive disease, especially in patients undergoing active surveillance or tumor mapping. This technology has also had a critical role in the detection of occult metastatic prostate cancer at our institution.

**CRYOTHERAPY FOR PROSTATE CANCER**

Although there are several emerging approaches for the treatment of prostate cancer, radical prostatectomy still remains a mainstay of treatment. However, focal therapy for prostate cancer is also being encouraged in a subset of patients, especially those considering a nonsurgical therapeutic option. In addition, recent advances in cryotherapy, such as the use of smaller-gauge probes, argon-based probes (as opposed to traditional nitrogen-based probes), and improved imaging techniques have all lead to increased efficacy with a reduction in severe side effects. This approach to the treatment of prostate cancer is also seen as a safe alternative for patients who are not good surgical candidates for radical prostatectomy.
Several studies have demonstrated the advantages and disadvantages of focal therapy. Those that support focal therapy demonstrate that only a few men would have been undertreated when radical prostatectomy would have been curative. Those against focal therapy argue that, even using stringent selection criteria, 20% of the men who would have undergone focal cryotherapy would have had potentially significant prostate cancer that was not adequately treated. In a study looking at 100 completely embedded radical prostatectomy specimens in which the previous needle biopsies predicted limited disease (fewer than 3 positive cores, 50% or less involvement of any positive core, Gleason score 6/grade group 1, and all unilateral positive cores) in 65% of cases, there was some tumor contralateral to the positive biopsy side in the radical prostatectomy specimens. Keeping these studies in mind, urologists at our institution always inform patients about the potential risk of residual cancer following focal therapy.

The pathologic changes following radiation therapy and hormone/androgen deprivation therapy are well known to most pathologists. However, there are few publications that describe the histologic changes in the prostate following cryotherapy, especially in the monotherapy setting. Unlike radiation therapy and hormone/androgen-deprivation therapy, the changes seen after cryotherapy predominantly involve the prostatic stroma. The acinar changes typically associated with radiation therapy or hormone/androgen-deprivation therapy, such as acinar atrophy, cytoplasmic vacuolization, nuclear pyknosis, and basal cell hyperplasia, are not usually seen in the postcryotherapy setting. Based on these observations, it is conceivable that the component of the prostatic glands and stroma (benign or malignant) that are in the center of the ice ball (which has lethal temperatures below \(-20^\circ\text{C}\)) are completely destroyed and that those in the periphery of the ice ball are left unaltered. Therefore, the typical histologic findings following cryotherapy include necrosis, calcifications, hemosiderin deposition, stromal fibrosis with loss of glands, and vessel-wall thickening (Figure 10, A through C).

**BLUE-LIGHT/FLUORESCENCE CYSTOSCOPY FOR BLADDER LESIONS**

There is no doubt that complete transurethral resection of bladder tumors is critical in maximizing oncologic outcomes and minimizing costs, especially in patients who are poor surgical candidates. Nonmuscle-invasive bladder cancer is associated with increased risk of progression and high rates of recurrence, especially in patients with high-grade urothelial carcinoma or urothelial carcinoma in situ. It is, therefore, critical to diagnose urothelial carcinoma as early as possible.

Routine/white light cystoscopy misses up to 20% of tumors. Because 50% to 70% of superficial urothelial carcinomas recur, there has been a high demand for better detection techniques. Fluorescence/blue light cystoscopy is a relatively new diagnostic and therapeutic technique that is performed at our institution. In contrast to regular white light cystoscopy where some tumors may be missed because of their small size, blue light cystoscopy, which is an enhanced imaging procedure, helps urologists identify bladder lesions/tumors of any size more easily by using Cysview (hexaminolevulinate hydrochloride, Photocure...
ASA, Oslo, Norway), which turns malignant cells in the bladder bright pink or red under a blue light (Figure 11, A through F). This allows for more complete excision of the lesion/tumor and a decreased likelihood of tumor recurrence. The procedure is performed by preinstilling 5-aminolevulinic acid or hexaminolevulinate in the bladder 30 to 60 minutes before using ultraviolet/blue light cystoscopy. The chemicals are concentrated in the malignant urothelial cells, which are then easily identified with blue light cystoscopy.

Figures 11. Contrast between regular white light cystoscopy (A through C) and corresponding blue light/fluorescence cystoscopy (D through F). Images courtesy of Mehrdad Alemozaffar, MD, FACS.
A meta-analysis using raw data from prospective studies on 1345 patients with known or suspected nonmuscle-invasive bladder cancer compared blue light cystoscopy with white light cystoscopy.\cite{123,127} Blue light cystoscopy detected significantly more pTa tumors (14.7%; \(P < .001\); odds ratio, 4.89; 95% CI, 1.94–12.39) and carcinoma in situ lesions (40.8%; \(P < .001\); odds ratio, 12.37; 95% CI, 6.34–24.13) than did white light cystoscopy.\cite{23,27} A study showed that, with fluorescence cystoscopy, residual tumor rate was 4.5%, compared with 25.2% for white light cystoscopy, and recurrence-free survival rates for fluorescence cystoscopy were 78% and 45%, respectively.\cite{150}

There appears to be considerable evidence that blue light cystoscopy improves detection of additional lesions at the time of transurethral resection of bladder tumors, especially carcinoma in situ, and this contributes to reduced recurrence rates. The increased detection of lesions should also improve staging of patients, which may enhance overall outcomes but still needs to be validated in prospective studies.\cite{150} Whether blue light cystoscopy will affect long-term, recurrence-free survival requires further investigation.

**CONCLUSIONS**

In summary, there have been many advances in the field of urologic pathology, oncology, and imaging, and we are very fortunate at Emory University Hospital (Atlanta, Georgia) to have access to these advances for both patient care and research. This synopsis demonstrates that the multidisciplinary approach to patient care and research ultimately leads to the best outcomes from the diagnostic, therapeutic, and prognostic standpoints.

**References**


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**Current Practice: Urologic Pathology—Ellis et al**


