Best Practices Recommendations in the Application of Immunohistochemistry in the Kidney Tumors

Report From the International Society of Urologic Pathology Consensus Conference

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Abstract: Primary renal neoplasms comprise multiple distinct entities, some of which are well understood and others that are not. It is not uncommon for some of these entities to have overlapping morphologic features. Their clinical behavior is varied, ranging from highly malignant to benign, and metastatic renal cell carcinoma oftentimes enters into the differential diagnosis of tumors of unknown primary. In this age of personalized medicine, identifying biomarkers that can better predict clinical outcome and response to therapy is a pressing need. In 2013 the International Society of Urological Pathology held a meeting in which best practices recommendations on the use of immunohistochemical markers in urologic malignancies were discussed. In this review we make recommendations regarding immunohistochemical markers that are best suited to aid in establishing a diagnosis of renal primary, panels of antibodies that are most useful in classifying renal tumors, and the current status of prognostic and predictive biomarkers. Although no prognostic or predictive marker and set of markers have yet to be validated, ongoing research suggests that this fact is likely to change in the near future.

Key Words: ISUP recommendations, immunohistochemistry, best practices, kidney tumors, renal neoplasm, renal cell carcinoma

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Renal cell neoplasms comprise a heterogenous group of tumors that vary greatly in genetic composition as well as clinical behavior and therapeutic response. Although most have characteristic histologic features, some may share morphologic similarities, at least focally. In addition, high-grade and poorly differentiated tumors may be difficult to classify on the basis of morphology alone. This fact is true not only within the kidney but also at metastatic sites. In these settings one must consider a differential diagnosis that includes not only several renal tumors but also tumors arising at other sites.

The purpose of the conference was to evaluate and put forth best practice recommendations pertaining to 3 areas in which immunohistochemistry (IHC) can help in the evaluation of renal neoplasms.

(1) Markers to establish the diagnosis of renal neoplasm (site of origin).
(2) Markers to aid in the classification of primary renal neoplasms.
(3) Prognostic and predictive markers in renal neoplasms.

In 2012, the International Society of Urological Pathology (ISUP) met in Vancouver, BC, and the member participants reviewed all aspects of the pathology of adult renal tumors, including diagnostic and prognostic biomarkers. What followed was a series of papers published in the *American Journal of Surgical Pathology* in October, 2013. The statements made therein represent the consensus opinions of the participants. The present document is not a duplication of that effort but rather a contemporary review of the pertinent literature with specific recommendations. Ultimately, the goal is to avoid overutilization of biomarkers and to recommend panels of analytes that focus on resolving specific diagnostic problems.

MARKERS TO ESTABLISH THE DIAGNOSIS OF RENAL NEOPLASM (SITE OF ORIGIN)

IHC is often utilized to establish the diagnosis of metastatic renal cell carcinoma (RCC). However, the sensitivity and specificity of a given marker for RCC is

variable and depends upon multiple factors. These factors include

(1) *The subtype of RCC*. Along these lines, a marker that consistently detects the major subtypes of RCC that metastasize (such as clear cell and papillary RCC [PRCC]) is generally more useful than a marker that preferentially detects less common subtypes that infrequently metastasize (such as chromophobe RCC and mucinous tubular and spindle cell carcinoma [MTSC]).
(2) *The grade of the RCC*. Many markers, including PAX8, show greater labeling in more differentiated, low-grade RCC than in poorly differentiated, high-grade RCC.
(3) *The size of the specimen*. The sensitivity of a marker that labels only focally can be affected by specimen size, particularly when one is dealing with a small core biopsy or fine needle aspiration specimen.
(4) *The specific antibody clone and the method of detection used*.

Although no single IHC marker is perfect, PAX8 (reviewed by Ordoñez[1]) is the most useful antibody for establishing a diagnosis of metastatic RCC. PAX8 is a 415 amino acid, 48 kDa transcription factor, which mediates development/maintenance of the kidney, thyroid, and Müllerian systems. It is normally expressed diffusely in the renal tubules, with preferential labeling of the distal tubules, and shows patchy labeling of the renal pelvic urothelium. Most of the published experience with PAX8 IHC is with the polyclonal antibody from Proteintech (#10336-1-Ap) (Chicago, IL, which binds the 212 N-terminal amino acids of PAX8. However, this region includes 128 amino acids of a DNA-binding domain that is common for all PAX transcription factors, which explains the cross-reaction with other PAX proteins, which diminishes the specificity of the polyclonal PAX8 preparation. A newer monoclonal antibody to PAX8 (PAX8R1) binds to the C-terminal amino acids 318-426 of PAX8, which is highly divergent among PAX proteins and thus should not cross-react with other PAX proteins. Consistent with its pattern of expression in development, PAX8 consistently labels RCC, Müllerian neoplasms, and thyroid neoplasms. A subset of urothelial carcinomas of the renal pelvis (20%) labels for PAX8, as do Wolffian duct lesions and thymic neoplasms. Endocrine neoplasms such as pancreatic islet cell tumor and gastrointestinal tract carcinoids often label for PAX8 using the polyclonal preparation; however, this reflects cross-reaction with PAX6.[2] B-cell lymphomas also label with the polyclonal PAX8 preparation; however, this represents a cross-reaction with PAX5.[3]

PAX8 is expressed in all RCC subtypes, with a sensitivity of approximately 95%.[4] This includes labeling of sarcomatoid RCC, MTSC, and MiT-family translocation RCC. PAX2 labels similarly to PAX8[5–7]; however, PAX8 is generally a more sensitive marker,[8] and PAX2 is reportedly negative in thyroid neoplasms in a small series.[9]

The utility of RCC marker antigen (RCCm), CD10, and Ksp-cadherin is limited. RCCm detects a proximal tubular antigen and demonstrates focal labeling in approximately 80% of RCC.[10,11] However, RCCm has notoriously poor specificity, in that it labels many other carcinomas such as those of the breast, lung, colon, and adrenal origin. There are specific situations in which it may be useful, for example in differentiating clear cell RCC (ccRCC) from clear cell carcinoma of the ovary. PAX8 would be positive in both tumors, whereas RCCm would be positive only in the renal neoplasm.[12] PAX8 is negative in adrenal cortical neoplasms, which preferentially stain for steroid factor-1.[12] CD10 is another proximal tubular marker, which is highly sensitive but again not at all specific for RCC, as lung, bladder, colon, and ovarian carcinomas all label for CD10.[13] However, as CD10 fairly consistently labels ccRCC, the absence of CD10 labeling in a metastatic lesion argues against this diagnosis. Ksp-cadherin is a distal tubular marker which is very sensitive for chromophobe RCC. However, the latter rarely presents as a metastatic lesion, limiting the utility of Ksp-cadherin.[14] At least focal staining can also be seen in other renal tumors, including high-grade ccRCC.

It should be noted that several commonly used but highly effective IHC markers are almost always negative in RCC. This includes the pulmonary marker TTF-1, the intestinal marker CDX2, p63, prostate-specific antigen, and estrogen receptor. Hence, labeling with any of these markers is a strong argument against the diagnosis of metastatic RCC.

ISUP Recommendations

- PAX8 is the most useful IHC marker for establishing the diagnosis of metastatic RCC. One can use IHC for other markers such as estrogen receptor, CDX2, prostate-specific antigen, TTF-1, GATA3, and p63 to help exclude other carcinomas, including those that also may label for PAX8. The other IHC markers currently in common practice (CD10, RCCm, Ksp-cadherin) are supportive of metastatic RCC but usually not indicated or useful.

MARKERS TO AID IN THE CLASSIFICATION OF PRIMARY RENAL NEOPLASMS

The morphologies of most renal neoplasms are well defined, and proper classification rarely requires ancillary tests. However, the fact of the matter is that dozens of entities exist, some of which have a spectrum of morphologic findings that are either poorly defined or remain controversial. Even within common tumors, high-grade or poorly preserved areas may exhibit nonspecific features that can be seen in other tumors. These features include solid, cystic, tubular, sarcomatoid, rhabdoid, or papillary growth, as well as cytoplasmic clearing, eosinophilia, or basophilia, to name a few (Table 1). Dozens of antibodies have been tested and published in peer-reviewed journals and review articles.[15–21] Unfortunately, the information provided is no better than level II or III evidence, which lacks validation studies. In addition, reports vary in the type of antibody (monoclonal or polyclonal, antibody clone) and methodology used, making comparison among studies difficult. Having said this, experience has taught us

TABLE 1. When to Use IHC in Evaluating Renal Tumors

Tumors with complex morphology
Papillary, solid, tubular, etc.
Suggestive of distal nephron origin
Solid, tubular, papillary, desmoplasia
Differential diagnosis of oncocytic tumors
When PEComa enters in the differential diagnosis
When MiTF/TFE tumors enter in the differential diagnosis
Tumors with clear cell features but unusual growth pattern characteristics
Tumors with clear cell features and papillary growth
Carcinomas arising in young patients

when and how best to use these antibodies. These markers should be used as a panel rather than singly, and the panel utilized will depend on the differential diagnosis being considered (Tables 2–6). Quantitative and qualitative assessment of the staining results is equally important. The recommendations we put forth are based on original contributions published in peer-reviewed journals as well as review articles and book chapters, which also take into account the professional experience of the authors. We include commercially available antibodies only. It is important to note that there are limited data for the application of these analytes on needle biopsies, cell blocks, and fine needle aspirates.[22,23]

In neoplasms that are difficult to classify, we recommend paying close attention to areas of transition between low-grade, well-differentiated areas of the tumor and more pleomorphic areas; it is the former that will provide the best clues on how to best classify the tumor and where the IHC findings will more likely be informative. Along these lines, additional sampling of the gross specimen to uncover such areas may be more useful (and more cost-effective) than a large battery of IHC markers. Finally, new IHC markers and new clones of established markers are continually entering the market, and certainly some will complement or even supplant some of the antibodies recommended in this publication. As such, we recommend evaluating and refining diagnostic panels on a continuous basis, taking into account additional information as it becomes available.

ISUP Recommendations

- When evaluating the published reports of any new analyte, play close attention to the specific information regarding the type of antibody and methodology used.
- Use antibodies in panels rather than singly.
- The panel should include only those that are relevant for the differential diagnosis being considered.
- Quantitative and qualitative features of the stain should be taken into account.
- If the IHC results are conflicting or inconclusive, a diagnosis of unclassified RCC is appropriate.
- Panels will need to be adjusted as novel markers of proven value enter the market.

TUMORS PREDOMINANTLY COMPOSED OF CLEAR CELLS

Although ccRCC is the most common tumor in this category, many others can contain clear cells, at least focally but often diffusely.[20] It is important to keep in mind that ccRCC is likely not to have entirely clear cytoplasm in high-grade areas (Fig. 1A). Table 2 highlights other tumors that may contain abundant clear cells. It is noteworthy that this list includes both epithelial-derived and nonepithelial-derived tumors.

Carbonic anhydrase IX (CAIX) in a transmembrane member of the carbonic anhydrase family of genes and has a role in CO_2 transport and in regulation of pH. It is under the regulation of the hypoxia-inducible factor, which is invariably dysregulated in ccRCC. For this reason, CAIX is characteristically overexpressed in these tumors diffusely and in a membranous pattern (Fig. 1B). Staining is diffuse in 75% to 100% and focal in up to 25% of cases.[24–29] For the stain to be considered positive, only membranous and not cytoplasmic staining should be taken into consideration.[20,24] Decreased expression may be seen in high-grade, poorly differentiated tumors, although many will retain the characteristic pattern of immunoreactivity.[25,27] Immunoreactivity for this marker should not be evaluated adjacent to areas of necrosis, as positive staining can be seen in any tumor because of hypoxia. ccRCC will commonly express epithelial markers such as AE1/AE3, CAM 5.2, and epithelial membrane antigen (EMA). CK7 is rare and then limited to isolated cells or cluster of cells in high-grade tumors. CD10, a proximal tubule marker, is routinely positive in ccRCC in a membranous distribution. However, at least focal immunoreactivity may be seen in other tumors, decreasing its utility. Given other options, it is rarely required. Vimentin is positive in ccRCC, more intensely in high-grade areas, as well as in some high-grade PRCCs. The fact that other high-grade renal tumors can express at least focal vimentin positivity limits its utility in

TABLE 2. Tumors Composed Predominantly of "Clear" Cells

Tumor Type	CA IX	CK7	CD117	Cathepsin-K	HMB-45
Clear cell RCC	Positive, diffuse membranous	Negative	Negative	Negative	Negative
Clear cell PRCC	Positive, cup-like	Positive	Negative	Negative	Negative
Chromophobe RCC, classic	Negative	Positive, cytoplasmic	Positive, membranous	Negative	Negative
Epithelioid-AML	Negative	Negative	Negative	Positive, cytoplasmic	Positive, cytoplasmic
MiTF-TFE tumors					
Xp11 family	Variable but focal	Negative	Variable	Positive (50%), cytoplasmic	Negative
t(6;11)	Variable but focal	Negative	Negative	Positive, cytoplasmic	Positive (always focal)

TABLE 3. Tumors With a Significant Papillary Component

	CAIX	CK7	AMACR	Cathepsin-K	34βE12	TFE3/TFEB
ccRCC with papillary growth	Positive, membranous	Negative	Negative	Negative	Negative	Negative
PRCC "type I"	Negative	Positive	Positive	Negative	Negative	Negative
PRCC "type II"	Negative	± Positive	Positive	Negative	Negative	Negative
Clear cell PRCC	Positive, cup-like	Positive, diffuse	Negative	Negative	Negative	Negative
MiTF-TFE trans-assoc	Variable but focal	Negative	Positive	Positive (50%)	Negative	Positive*

*Antibodies are difficult to standardize on automated platforms. FISH assays are more reliable.

difficult-to-classify cases.[20] RCCm is a monoclonal antibody directed against a 200 kDa glycoprotein in the brush border of the proximal renal tubules. Although it exhibits cytoplasmic and membranous immunoreactivity in the majority of ccRCCs and PRCCs, expression decreases with increasing grade, and immunoreactivity can be seen in other renal tumor types, limiting its discriminating ability.[15,17,30,31]

CAIX staining in clear cell papillary carcinoma is very distinctive.[21,32–34] Although virtually all tumor cells are positive, staining is limited to the cytoplasmic membrane along the basal and lateral aspects of the cell, sparing the luminal border ("cup-like" staining) (Fig. 2A). These tumors exhibit diffuse immunoreactivity for CK7 but, unlike the usual PRCC, lack staining for racemase (AMACR) (Fig. 2B). Cytokeratin 34βE12 will be positive but is not required in the panel when the differential diagnosis includes ccRCC and PRCC.[21]

Chromophobe RCC does not express CAIX but stains for CK7 and CD117 (c-kit).[20] Although other markers such as kidney-specific cadherin (Ksp-cadherin) and parvalbumin are positive in chromophobe RCC and rarely in other tumors that harbor clear cells, they serve little added utility in resolving this differential diagnosis.[35]

Epithelioid angiomyolipoma (E-AML) is characterized by immunoreactivity for HMB-45 and MART-1 as well as cathepsin-K, whereas epithelial markers and CAIX are negative[36,37] (Figs. 3A–C). Cathepsin-K is a cysteine protease expressed in osteoclasts. Microphthalmia transcription factor (MiTF) activates genes associated with melanin production and binds to selective elements in the cathepsin-K promoter. Studies have shown that this marker is expressed in all variants of PEComas of the kidney, and the percentage of cells positive is greater than that seen with HMB-45 and MART-1, which can be quite variable.[37] Unfortunately, cathepsin-K is not available in most diagnostic laboratories; however, we strongly recommend its implementation.

A small percentage of renal tumors are associated with translocations, and these commonly exhibit cytoplasmic clearing with solid, alveolar, or papillary growth (Fig. 4A). Although more commonly seen in young patients, they may develop at any age. As a group they are referred as MiTF-TFE translocation–associated carcinomas.[38–40] These tumors may express CAIX and epithelial markers, although most are negative for these markers or show focal immunoreactivity. Two thirds will be negative for EMA and cytokeratin AE1/AE3, with the remainder showing only focal and weak staining.[41] These tumors fall into 2 categories, those that harbor a translocation involving Xp11 with one of several possible fusion partners and those with a translocation between chromosomes 6 and 11 (t[6;11]). The former express TFE3 protein, whereas the latter stain for TFEB (Table 4 and Fig. 4B). However, these antibodies, although commercially available, have shown to be unreliable when used on automated platforms. The consensus among most academic pathologists is that it is much more reliable to use fluorescence in situ hybridization (FISH) with specific break-apart probes to establish the diagnosis with certainty. Unfortunately, highly validated commercially available probes for TFE3 and TFEB are lacking, with most laboratories designing and validating probes internally. Melanoma-associated markers may be present in 50% of TFEB and up to 15% of TFE3 tumors (Fig. 4C). Cathepsin-K has been shown to be a valuable assay in these tumors, with virtually all t(6;11) tumors having diffuse cytoplasmic positivity and a subset of Xp11 tumors, with the exception of t(X;17), having a similar staining pattern.[42]

ISUP RECOMMENDATIONS

- ccRCC can be distinguished from chromophobe RCC using a panel that includes CAIX, CD117, and CK7
- ccRCC can be distinguished from clear cell PRCC using a panel that includes CAIX, CK7, and racemase (AMACR). Qualitative differences in CAIX staining between the 2 entities are important to evaluate.
- ccRCC can be distinguished from E-AML using a panel that includes CAIX and cathepsin-K. We suggest

TABLE 4. Solid PRCC Versus Metanephric Adenoma Versus Wilms Tumor

	CK7	AMACR	WT-1	CD57
Solid papillary	Positive	Positive	Negative	Negative
Metanephric adenoma	Negative or isolated cells	Negative	Positive, nuclear	Positive
Wilms	Negative or isolated cells	Negative	Positive, nuclear	Negative

TABLE 5. Tumors With Oncocytic Features*

	CD117	CK7	Ksp-cadherin	HMB-45	Cathepsin-K
Oncocytoma	Positive, membranous	Negative	Positive	Negative	Negative
Chromophobe RCC, eosinophilic	Positive, membranous	Positive but variable	+/−Positive	Negative	Negative
Oncocytic PRCC	Negative	Positive but focal	Not known	Negative	Unknown
Oncocytic AML	Negative	Negative	Negative	Positive, focal	Negative

Other Abs said to be differentially expressed on oncocytomas and chromophobe RCC.
Positive in oncocytoma, negative in chromophobe: S100A1.
*Hale colloidal iron: Although a histochemical rather than an IHC stain, it can be useful in differentiating chromophobe carcinoma (cytoplasmic granular staining) from oncocytoma (negative or luminal staining). However, this is a technically demanding stain and reliability is laboratory-dependent.

to include a wide-spectrum cytokeratin (AE1/AE3) or EMA to document the presence or absence of epithelial lineage. If the laboratory does not have cathepsin-K, the melanoma-associated markers HMB-45 and MART-1 can be used.

- ccRCC can be distinguished from MiTF-TFE translocation–associated carcinomas using a panel of antibodies that include cathepsin-K, TFE3, and TFEB. If TFE3 and TFEB are inconclusive (only weak or patchy nuclear staining or high background), FISH assays should be performed.
- E-AML can be distinguished from MiTF-TFE translocation–associated carcinoma by nuclear expression of PAX8 in the latter and total absence of CAIX and epithelial differentiation (AE1/AE3, EMA) in the former.

TUMORS WITH A SIGNIFICANT PAPILLARY COMPONENT

PRCCs have been subdivided into type 1 and type 2 tumors. It is fair to assume that type 1 tumors comprise the majority of these neoplasms, and their morphology is relatively well understood. Type 2 tumors, characterized by multilayering of nuclei and cytoplasmic eosinophilia, are poorly understood and as currently defined are likely to encompass more than one entity. Although we include this category in this review, some prefer to consider type 2 PRCC as a descriptive term. However, at the recent consensus conference of the ISUP, a majority consensus of participants advocated retaining this classification for PRCC.[43] It is possible that foci within a type 1 tumor can closely mimic a type 2 PRCC.

Occasional ccRCC may have a papillary component, but this is either a very focal finding or a high-grade tumor with a pseudopapillary pattern of growth due to cell drop-off. CAIX will be diffusely positive (membranous) in most cases, whereas racemase and CK7 are negative (Table 3).[20] PRCC type 1 is classically diffusely immunoreactive for CK7 and for racemase (AMACR) in a cytoplasmic distribution (Figs. 5A, B). Cathepsin-K, p63 (clone 4A4), and TFE3/TFEB are negative.[21,42] CAIX is usually negative, but focal staining can be seen at the tips of the papillae. The staining pattern for so-called type 2 PRCC can be more variable. Whereas racemase (AMACR) is usually positive, CK7 immunoreactivity is variable and is usually negative (Figs. 6A, B). CAIX is also usually negative as is p63 (clone 4A4) and TFE3/TFEB.[21,39] Collecting duct carcinoma (CDC) may occasionally contain a papillary component, although rarely it does comprise the majority of the tumor. Tumor cells exhibit high-grade nuclei arranged in nests and tubules embedded in a fibrotic or desmoplastic stroma. These tumors classically stain for CK7 and p63 (clone 4A4) but not CAIX, TFE3, and TFEB. Racemase expression can be variable.

Some type 1 PRCCs are characterized mostly by a solid pattern of growth, mimicking metanephric adenoma and even highly differentiated epithelial-predominant Wilms tumor (Table 4). Although close attention to nuclear detail can help us solve this differential diagnosis, a panel that includes CK7, racemase (AMACR), WT-1, and CD57 is also useful.[44] Solid PRCC is immunoreactive for CK7 and racemase but negative for WT-1 and CD57. Alternatively, metanephric adenoma is only positive for

TABLE 6. Tumors With a Predominant Sarcomatoid Pattern of Growth*

	Vimentin†	CAIX‡	PAX 8	CK7	34βE12	GATA3	P63
ccRCC	Positive	Positive, membranous	Positive	Negative	Negative	Negative	Negative
PRCC	Positive	Negative	Positive	Focal or negative	Negative	Negative	Negative
Chromophobe RCC	Positive	Negative	Positive	Positive	Negative	Negative	Negative
MTSC	Positive	Negative	Positive	Positive	Variable	Negative	Negative
Urothelial CA	Positive	+/−Negative	Negative§	Positive	Positive	Positive	Positive
Sarcoma	Positive	Negative	Negative	Negative	Negative	Negative	Negative

*Stains should be performed in the better differentiated or most epithelioid areas.
†In sarcomatoid component.
‡Positive adjacent to necrosis or focal cytoplasmic in high-grade areas of various tumors.
§Positive in up to 20% of upper tract UC.

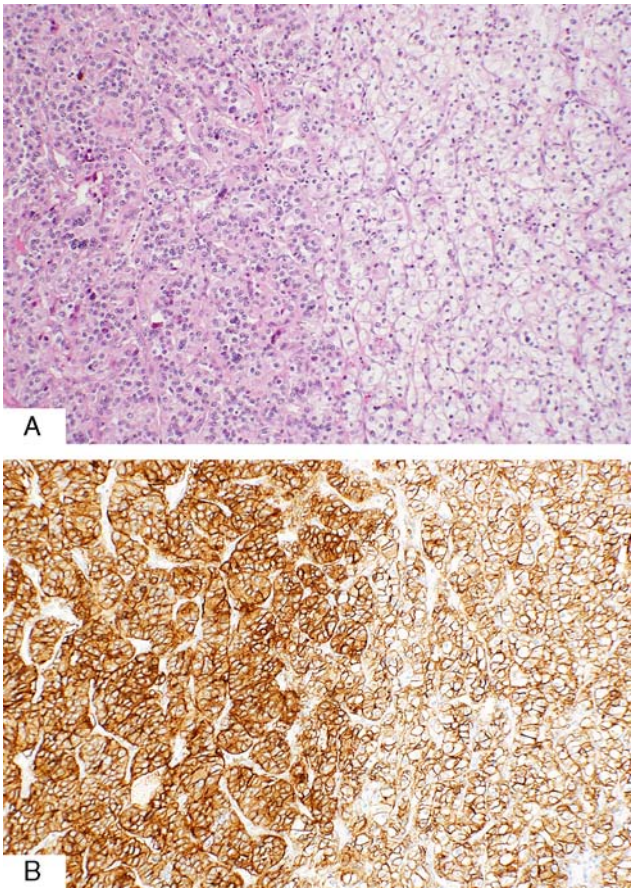

FIGURE 1. Clear cell carcinoma. A, Transition between classic low-grade and higher-grade areas, the latter exhibiting more cytoplasmic eosinophilia and less prominent vascularity. B, CAIX staining is diffuse and membranous staining is maintained throughout the lesion.

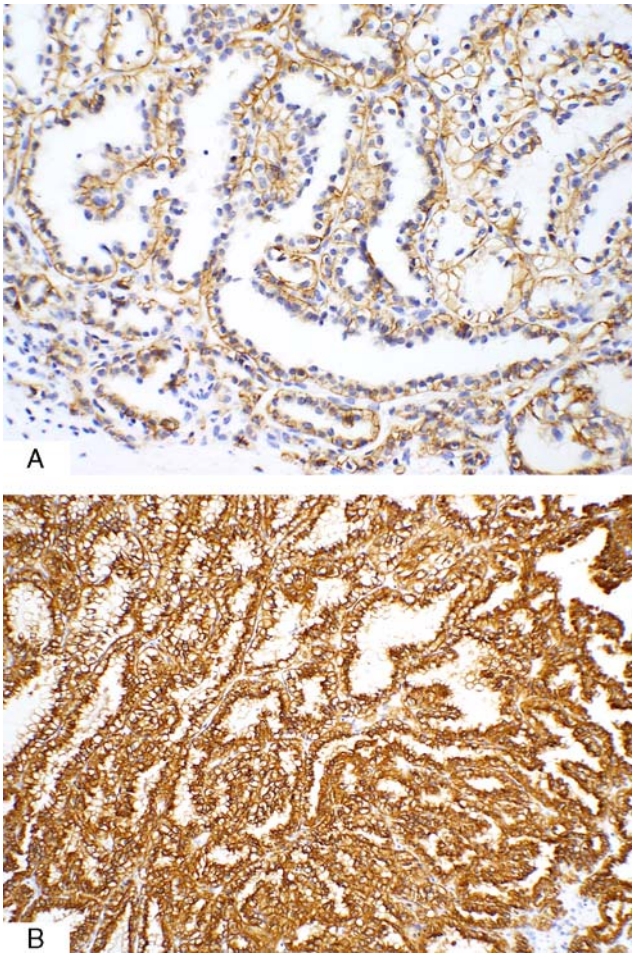

FIGURE 2. Clear cell PRCC. A, CAIX staining spares the luminal border of the tumor cells (cup-like). B, Tumors cells exhibit strong and diffuse CK7 cytoplasmic immunoreactivity.

WT-1 and CD57, whereas Wilms tumor expresses only nuclear immunoreactivity for WT-1 and rarely isolated cells with CK7 cytoplasmic positivity. Rarely will you need to apply IHC to solve this differential diagnosis. A more difficult problem is distinguishing type 1 PRCC from MTSC, as some rare PRCCs are associated with a cytologically bland spindle cell component, mimicking MTSC.[45] No IHC panel can reliably solve this differential diagnosis. At least one publication has suggested that centromeric FISH probes for chromosomes 7 and 17 can reliably distinguish these tumors, but this fact remains to be validated by others.[46] It is undeniable that most type 1 PRCCs exhibit polysomy 7/17, but whether some MTSCs can exhibit similar chromosomal gains is unsettled.

ISUP Recommendations

- Special attention should be paid to areas of the tumor that transition from low grade to high grade. Proper prosecting can help select key diagnostic areas.
- A panel that includes CAIX, CK7, and racemase (AMACR) can discriminate between ccRCC with papillary areas and PRCC. This same panel can be used to distinguish ccRCC from true PRCC and clear cell PRCC.
- Type 2 PRCC is likely a heterogenous group of tumors, which usually stain for racemase but may exhibit variable results with CK7, including total lack of staining. Before this diagnosis is rendered, other specific entities should be considered in the differential diagnosis.
- MiTF/TFE translocation–associated carcinomas may have a prominent papillary component. Cathepsin-K shows diffuse immunoreactivity in most variants except t(X;17), whereas epithelial markers are usually negative. Antibodies directed toward the TFE3 and TFEB proteins are highly specific but somewhat unreliable, so break-apart FISH probes directed against these gene fusions are better. A subset of these tumors will exhibit immunoreactivity with melanocytic markers (HMB-45 and MART-1).

TUMORS WITH EXTENSIVE CYTOPLASMIC EOSINOPHILIA

Although many renal tumors may exhibit some degree of cytoplasmic eosinophilia, here we are referring

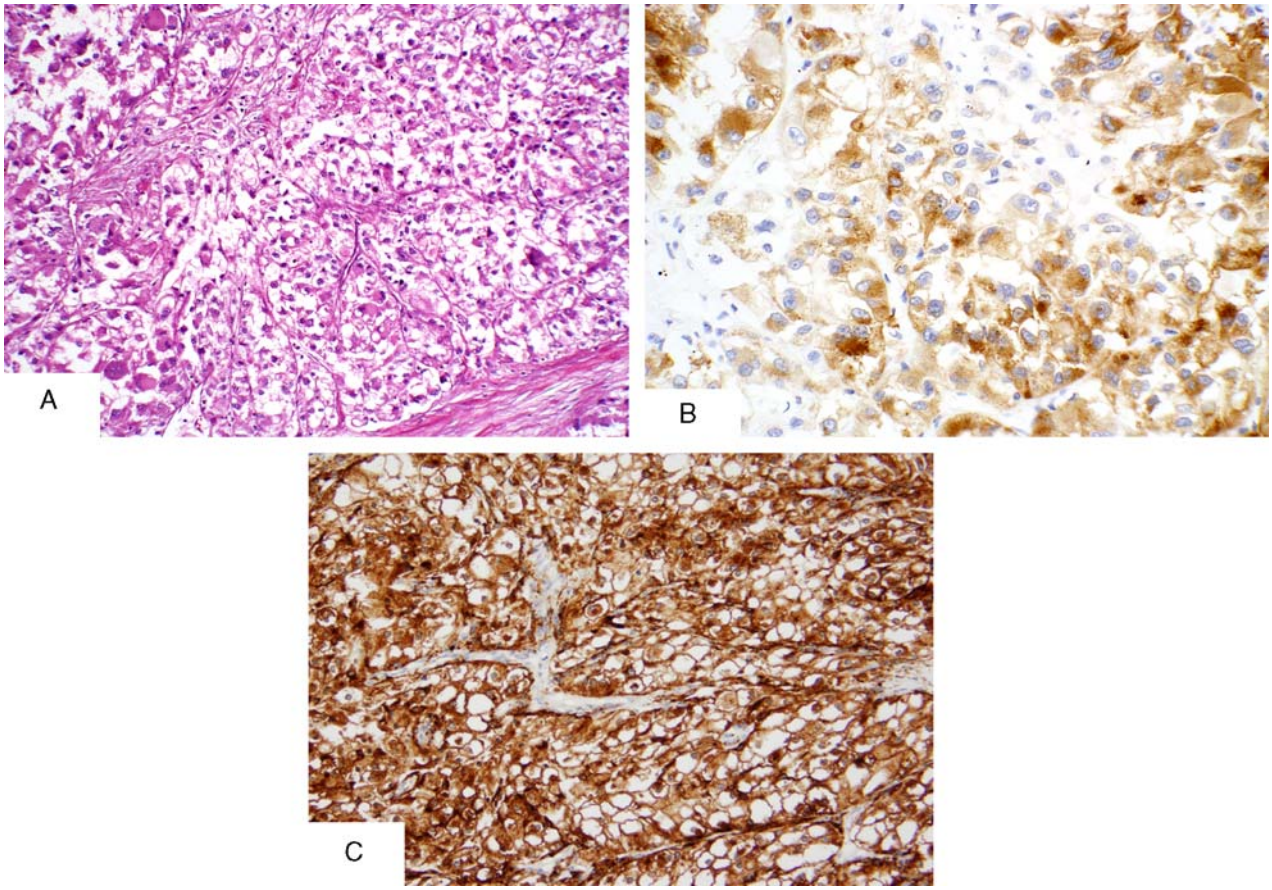

FIGURE 3. E-AML. A, A hematoxylin and eosin stain reveals that the tumor has an alveolar pattern of growth, and the tumor cells have abundant clear to granular cytoplasm. B, Strong cytoplasmic immunoreactivity for HMB-45. C, Strong and diffuse cytoplasmic staining for cathepsin-K.

to those cases in which renal oncocytoma enter in the differential diagnosis (Table 5). A very common problem confronted by pathologists is distinguishing between a renal oncocytoma and an eosinophilic variant of chromophobe RCC (Figs. 7A, B). Admittedly, most of these cases can be resolved by careful examination of the growth pattern characteristics, as well as nuclear and cytoplasmic features.[47] The cells of renal oncocytoma will exhibit membranous CD117 immunoreactivity, whereas CK7 is negative or labels only single tumor cells, clusters of cells in a patchy distribution, or entrapped native renal tubules.[15–17,48] Chromophobe RCC will have similar immunoreactivity for CD117 but also expresses CK7 diffusely in a membranous distribution. Unfortunately, the eosinophilic variant of chromophobe RCC can have either no or much fewer cells expressing this cytokeratin (Figs. 8A, B). Ksp-cadherin is a cell adhesion glycoprotein expressed in distal tubules of the nephron. Although it is expressed in the majority of both oncocytoma and chromophobe RCCs, it is predominantly cytoplasmic in the former but membranous/cytoplasmic in the latter.[14,35] Whether this marker is useful in distinguishing tumors that are truly difficult to classify remains to be proven; it is rarely needed in classifying classic cases.

S100A1 is a calcium-binding protein of the S100 gene family, which has been shown to be differentially expressed in renal oncocytomas and chromophobe RCC.[49–51] Whereas the majority of oncocytomas express the antigen in a nuclear and cytoplasmic distribution, most chromophobe RCCs are negative. This marker is also expressed in a large subset of ccRCCs and PRCCs. The issue of S100A1 staining in eosinophilic variants of chromophobe RCC has been poorly described in the literature but deserves further study. Claudin 7 and claudin 8 code for tight junction proteins located in epithelial cells of the distal nephron. Several studies have shown that they are differentially expressed in renal oncocytoma and chromophobe RCC, both qualitatively and quantitatively.[52,53] Once again, very little is known regarding their discriminatory ability in truly difficult-to-classify tumors, particularly the eosinophilic variant of chromophobe RCC.

Although not an IHC assay, much has been written about the utility of Hale colloidal iron in distinguishing oncocytomas from chromophobe RCCs.[54,55] Indeed, many experienced pathologists regard this histochemical stain as the best marker in this setting. However, this assay has not been easy to standardize in many laboratories, and its staining characteristics in chromophobe

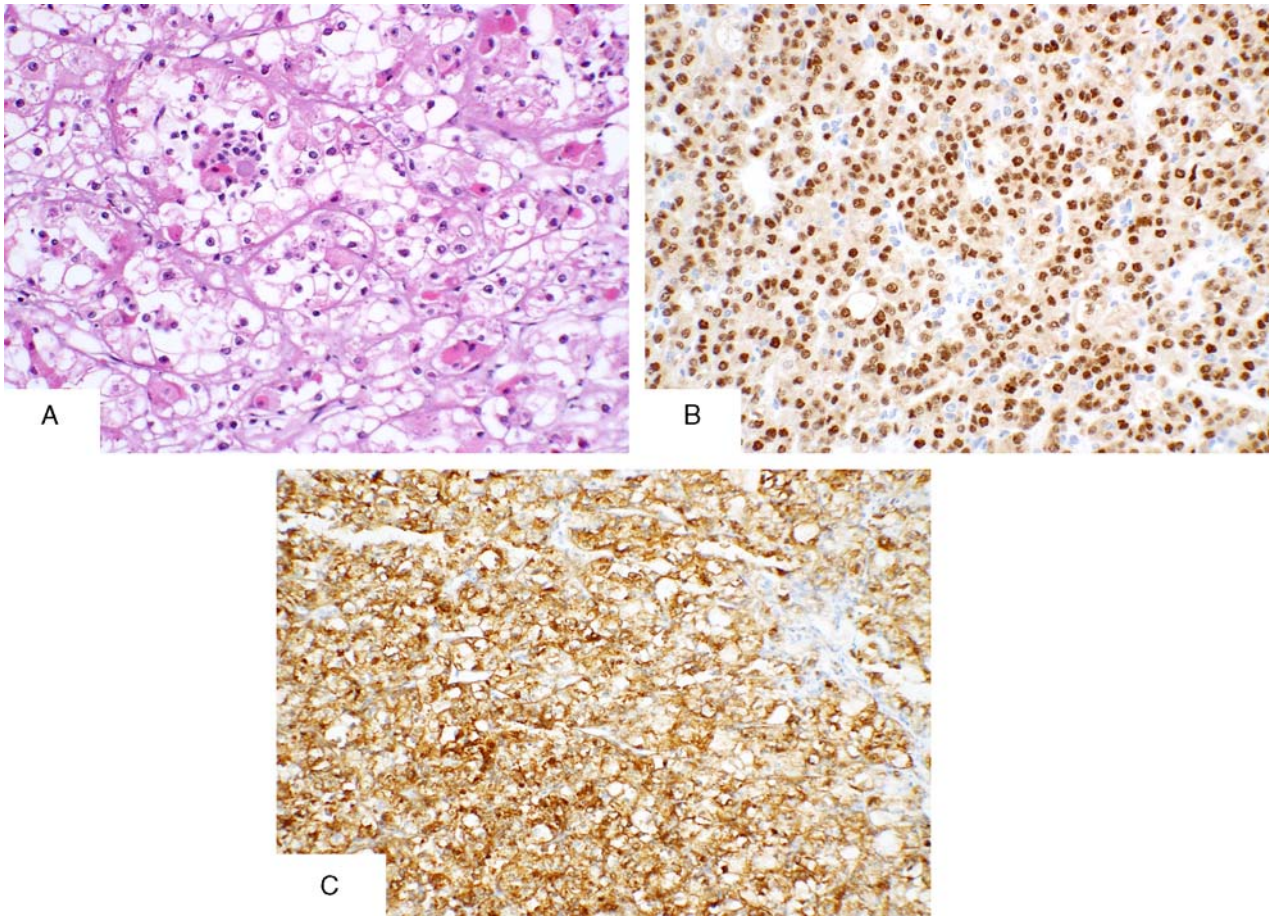

FIGURE 4. TFE-B translocation–associated renal carcinoma. A, A hematoxylin and eosin stain reveals an acinar pattern of growth and clear cytoplasm, easily mistaken from ccRCC. B, Strong and diffuse nuclear immunoreactivity for TFEB protein. When the stain is strong and diffuse, correlation with FISH results is high. C, Cytoplasmic staining for the melanoma-associated marker MART-1. Staining is usually focal or patchy rather than diffuse.

RCC with extensive cytoplasmic eosinophilia remains in doubt. For those pathologists lucky enough to have access to a dependable assay in their laboratory, Hale colloidal iron remains a useful diagnostic tool.

Oncocytic PRCC is a rare proposed variant of renal carcinoma that has cytoplasmic eosinophilia but has a very characteristic growth pattern with a luminal orientation to the tumor nuclei.[15–17] The expression pattern seen in these tumors is similar to what is seen in type 1 PRCC, but applying IHC stains to this tumor is rarely required, if ever.

Rarely renal AML may be composed entirely of oncocytic epithelioid cells. A clue to the proper diagnosis is the presence of occasional adipocytes within the renal parenchyma and in close proximity to the eosinophilic epithelioid cells.[37,43] Focal melanocytic marker (HMB-45, MART-1) expression will be present as will diffuse cathepsin-K labeling but not CD117 or CK7.

ISUP Recommendations

- Distinguishing oncocytoma from the eosinophilic variant of chromophobe RCC is the most common diagnostic challenge for which close attention to nuclear cytology and cytoplasmic features can be supplemented by performing IHC stains for CK7. Laboratories that have Ksp-cadherin may find this marker useful, although qualitative rather than quantitative differences in staining must be taken into account. S100A1 is potentially a very useful marker, which needs further validation.
- Oncocytic PRCC is a rare diagnosis that does not require IHC for proper classification.
- Oncocytic AML will express melanocytic markers and cathepsin-K.

TUMORS WITH A PREDOMINANT SARCOMATOID PATTERN OF GROWTH

A small but significant number of high-grade renal epithelial neoplasms can exhibit a sarcomatoid pattern of growth (Table 6). When this component is focal, proper classification of the lesion is usually straightforward. However, if the sarcomatoid component comprises all or the overwhelming majority of the tumor or if no clear cut transitions to lower-grade areas are present, then classification will be challenging. Vimentin, a marker that has been

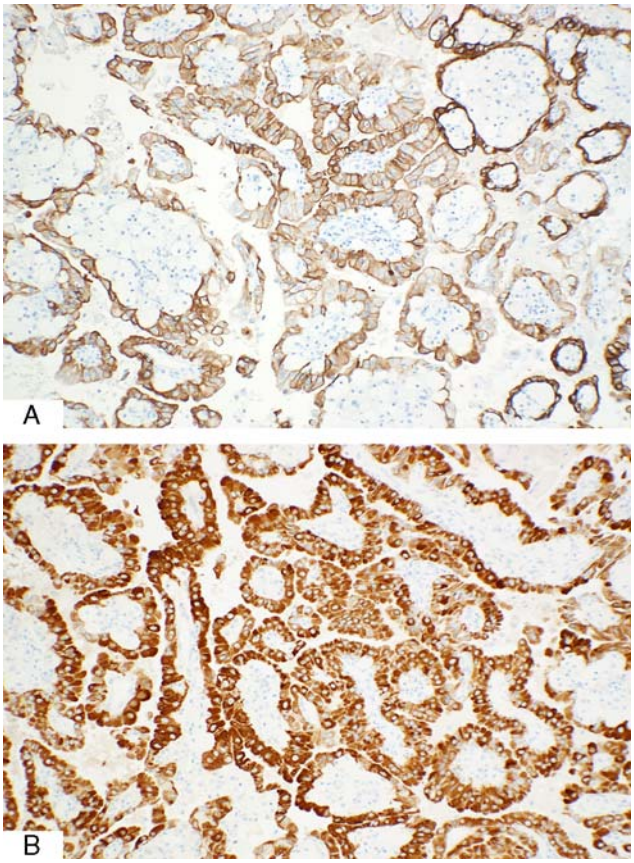

FIGURE 5. PRCC, type 1. A, Strong and disuse staining for CK7. B, Strong and diffuse granular cytoplasmic staining racemase (AMACR).

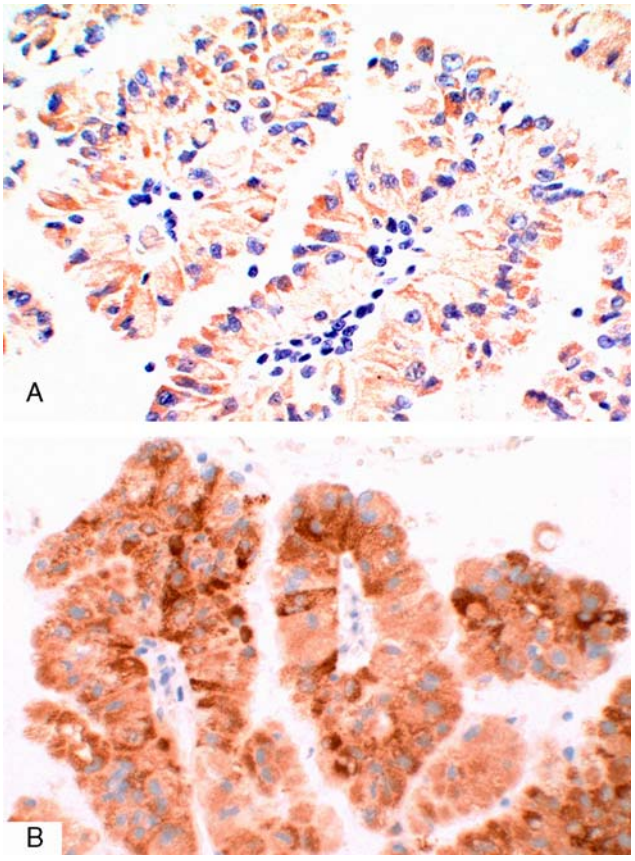

FIGURE 6. PRCC, type 2. A, Weak and variable staining for CK7. B, Strong and diffuse granular cytoplasmic staining for racemase (AMACR).

associated with ccRCC, is of limited utility in this setting, as many high-grade spindle cell epithelial neoplasms will express this antigen, as will mesenchymal tumors. CAIX has been reported to be positive in the majority of sarcomatoid ccRCCs in a diffuse membranous pattern but not in other RCC variants.[56] It is important to keep in mind that for this marker to be considered positive the pattern of expression must be membranous, that immunoreactivity cannot be evaluated adjacent to areas of necrosis, and that a significant percentage of urothelial carcinomas, some of which may exhibit sarcomatoid features, will also express CAIX. In this scenario, it is likely that urothelial carcinoma will also express urothelial markers such as GATA3, p63 (clone 4A4), cytokeratin 34βE12, and thrombomodulin.[43] PAX8 is universally expressed in all epithelial tumors of renal origin but also in up to 20% of urothelial carcinomas originating in the renal pelvis. The epithelial cells lining the cystic component of renal monophasic synovial sarcomas will express PAX2 and PAX8 as well.[57]

Leiomyosarcoma is the most common primary renal sarcoma, but even this tumor is extremely rare. It is more common for sarcomas to secondarily involve the kidney, either by metastasis or by direct extension. As expected, sarcomas that secondarily involve the kidney are those that arise in the retroperitoneum, mostly liposarcoma or leiomyosarcoma. Both tumors, including the dedifferentiated form of liposarcoma, may mimic sarcomatoid carcinoma. MDM2 and CDK4 should be positive in a nuclear distribution in liposarcoma, whereas actin and desmin should de positive in leiomyosarcoma. Both tumors should be negative for epithelial markers PAX8 and CAIX.

ISUP Recommendations

- Careful prosecting and attention to areas of morphologic transition can aid in establishing a proper diagnosis and choosing the most appropriate section(s) to perform ancillary studies.
- Vimentin and PAX8 are unlikely to contribute significantly to the diagnosis, although PAX8 can narrow down the diagnosis to either renal or urothelial origin, ruling out metastatic disease.
- In combination with other markers, CAIX may help in classifying a tumor as a ccRCC, but interpretation must be performed carefully.
- Traditional markers of urothelial lineage but not PAX8 can help identify a sarcomatoid urothelial carcinoma.
- If sarcoma enters in the differential diagnosis, the possibility of leiomyosarcoma and dedifferentiated liposarcoma can be ruled out by performing appropriate stains.

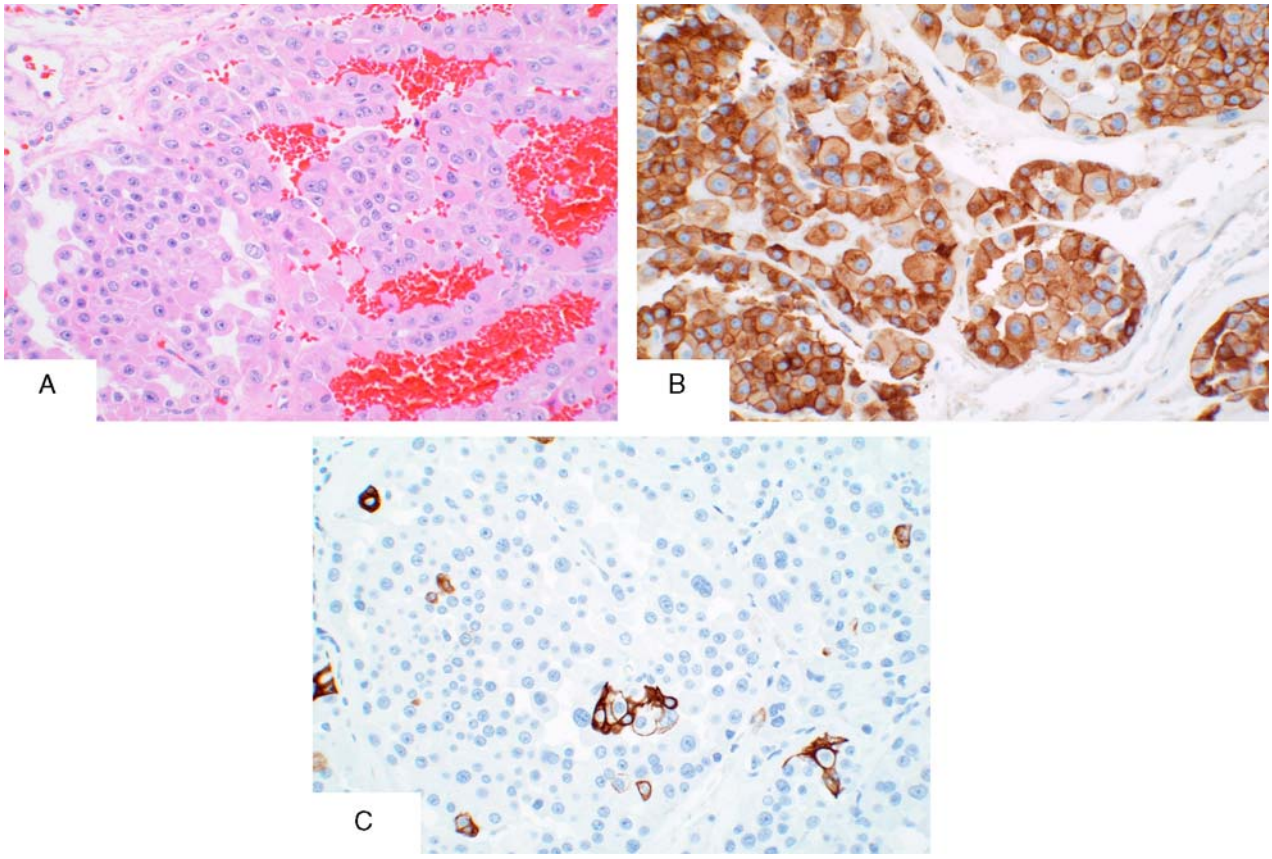

FIGURE 7. Renal tumor with cytoplasmic eosinophilia. A, Hematoxylin and eosin stain reveals an oncocytic tumor in which the nuclear irregularity and solid growth do not support oncocytoma, whereas the cytoplasm has no evidence of perinuclear clearing. B, CD117 is diffusely positive, which can be seen in either tumor. C, CK7 is limited to isolated cells, which supports neither diagnosis. Cases such as this are usually classified descriptively as "renal oncocytic neoplasm, type unclassified."

TUMORS WITH MORPHOLOGY SUGGESTIVE OF THE DISTAL NEPHRON ORIGIN (COLLECTING DUCT AND MEDULLARY CARCINOMA)

"Distal nephron-like carcinomas" is a descriptive term, which includes tumors with overlapping histology (Table 7). Classic examples include CDC and medullary carcinoma, wherein tumors are invariably high grade, have a solid, tubular or papillary growth, and are associated with some level of stromal reaction and inflammatory infiltrate. Other tumors that fit in this category include those with a predominant papillary growth, high nuclear grade, highly infiltrative growth, and stromal fibrosis. In our experience, when high-grade PRCC, CDC, and medullary carcinoma enter into the differential diagnosis, most cases end up being placed in the "unclassified" category. The possibility of urothelial carcinoma arising in the renal pelvis should always enter in the differential diagnosis of tumors with this morphologic spectrum. Sickle cell trait or some other type of hemoglobinopathy (such as hemoglobin SC) is present in patients with medullary carcinoma but is not a feature of other renal or urothelial tumors. The *SMARCB1/INI-1* gene is a member of the SWI/SNF chromatin remodeling complex. BAF47 is an antibody directed at the product of this gene, the loss of expression of which is characteristic of medullary carcinoma.[58–60] Whereas INI-1 loss is seen in 100% of medullary carcinoma, it has also been reported in 15% of CDC (Figs. 9A, B). Given the difficulty in establishing a diagnosis of CDC, this result remains to be validated. Alternatively, whether all medullary carcinomas must be associated with sickle cell trait or some other hemoglobinopathy remains controversial. Recent unpublished data suggest that the stem cell transcription factor OCT4 is selectively overexpressed in medullary carcinomas. Once again, this result requires validation, as overexpression has been described in a host of high-grade primitive tumors.

PAX8 cannot be used in resolving this differential diagnosis, as it will be expressed in up to 20% of upper tract urothelial carcinomas and all tumors arising from renal tubular epithelium.[43] GATA3 belongs to the GATA family of transcription factors and has a role in cell differentiation and proliferation in many tissues and cell types. It is expressed in most urothelial but not renal epithelial tumors[61,62] (Figs. 10A, B). Cytokeratins are differentially expressed in these tumors, but we find them, for the most part, unreliable in any given case. All tumors discussed in the section can express immunoreactivity for

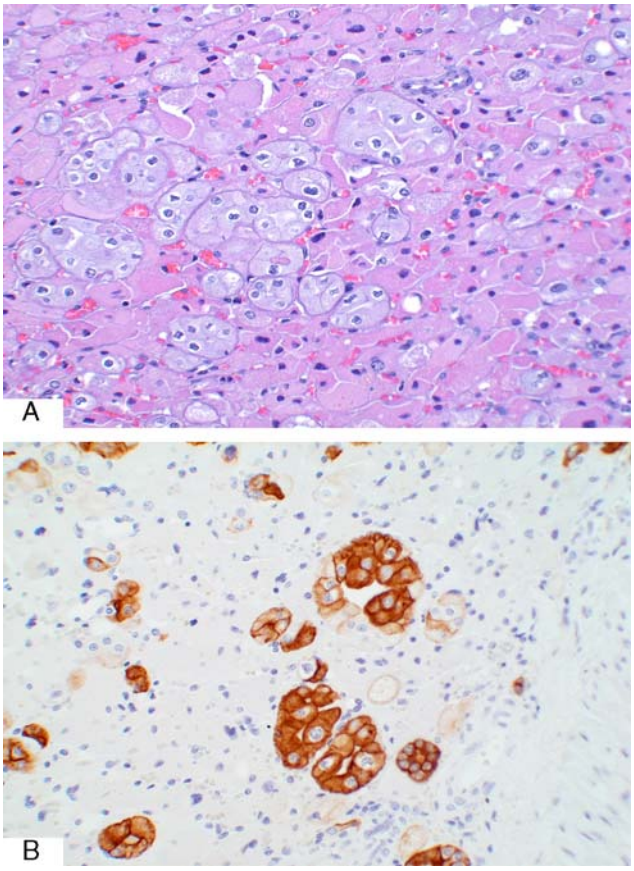

FIGURE 8. Eosinophilic variant of chromophobe RCC. A, Clusters of tumor cells with classic nuclear and cytoplasmic features are surrounded by more densely eosinophilic cells with degenerating nuclei. B, CK7 is preferentially positive in the classic chromophobe RCC areas.

CK7. Cytokeratin 34βE12 is positive in most urothelial tumors and also in a subset of CDC but not in medullary carcinoma. P63 (clone 4A4) immunoreactivity is a feature of urothelial tumors but not CDC or medullary carcinoma. Of course, any high-grade carcinoma can have a small percentage of cells with immunoreactivity to any given intermediate filament.

ISUP Recommendations

- CDC, on the basis of limited data, can be distinguished from medullary carcinoma using a panel that includes INI-1 and OCT4, taking into consideration the clinical history of sickle cell trait. Cytokeratin 34βE12 may be expressed in CDC but not in medullary carcinoma.
- Urothelial carcinoma can be distinguished from both CDC and medullary carcinoma using a panel that includes GATA3 and p63 (clone 4A4).

TABLE 7. "Distal Nephron-like" Carcinomas

	Collecting Duct Ca	Medullary Ca	Urothelial Ca
IN-1/BAF47	Retained*	Lost	Retained
OCT4	Negative	Positive†	Negative
GATA3	Negative	Negative	Positive
PAX8	Positive	Positive	Negative‡

*One study reports 15% of CDC with *INI-1* loss.
†Unpublished data.
‡20% positive.

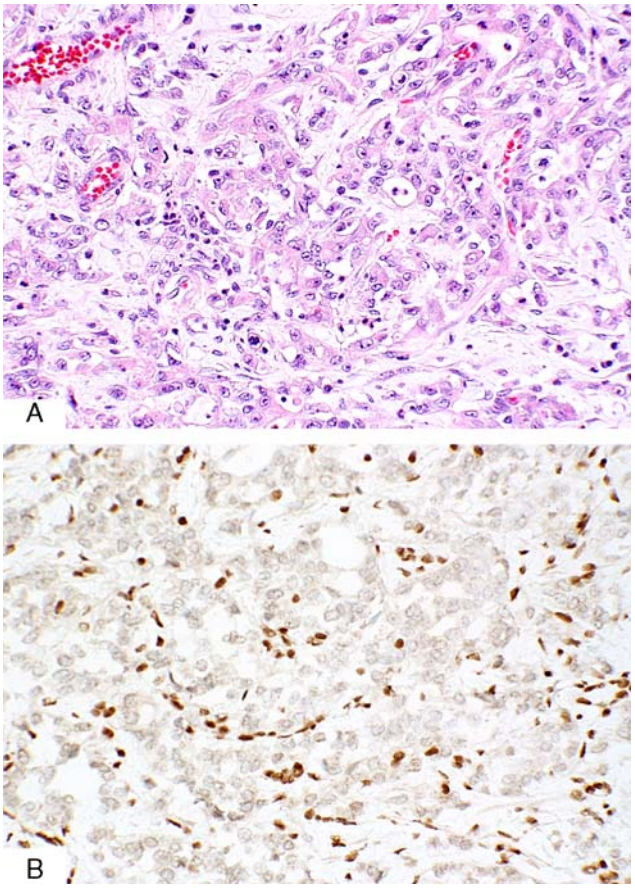

FIGURE 9. Medullary carcinoma of the kidney. A, Hematoxylin and eosin stain demonstrating high-grade tumor cells associated with stromal fibrosis and an inflammatory infiltrate. This pattern can be seen in collecting duct and urothelial carcinomas as well. B, INI-1 loss as demonstrated by lack of nuclear staining in the tumor cells, with surrounding endothelial and stromal cells retaining expression.

RCC, UNCLASSIFIED TYPE

By definition this category of renal tumors is not an entity but rather a diagnosis of doubt. It was added in the 2004 World Health Organization classification of renal neoplasms as a placeholder for tumors that do not fit into any of the other categories, despite our best efforts.[15–17,43] As such, there is no specific panel of IHC markers that characterize this group. In fact, it is expected that these cases have been submitted to a large battery of stains in an attempt to arrive at a more specific diagnosis. We cannot recommend a specific panel of antibodies to be used; the stains used will depend on which entities are being considered in the differential diagnosis. For

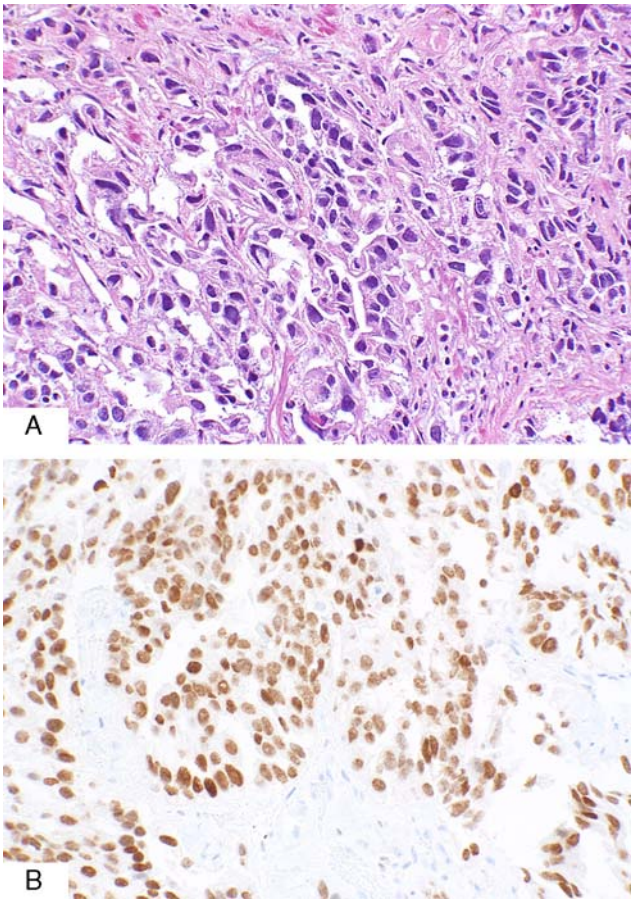

FIGURE 10. Urothelial carcinoma of the renal pelvis involving renal parenchyma. A, Hematoxylin and eosin stain showing a high-grade carcinoma with stromal fibrosis. B, Diffuse nuclear immunoreactivity for GATA3.

example, if the lesion is an oncocytic renal neoplasm in which the morphology is neither classic oncocytoma nor an eosinophilic variant of chromophobe RCC, the appropriate panel may include CD117, CK7, S100A1, and Hale colloidal iron (Table 5). If the tumor has high-grade nuclear features, voluminous reticulated cytoplasm, and a solid/alveolar growth, the panel should include markers to rule out ccRCC, MiTF-TFE translocation–associated carcinoma, and E-AML (Table 2). The category of unclassified RCC should not be used until all efforts to classify the tumor definitively have been exhausted. At the very least, renal epithelial origin should be established (PAX8) in cases of poorly differentiated tumors, and some attempt should be made to predict the level of aggressiveness of the tumors, on the basis of standard morphologic parameters.

ISUP Recommendations

- This category should only been used when all efforts to place the tumor into a distinct entity have been exhausted or when the immunophenotype is entirely at odds with the morphologic impression.
- The antibody panel performed will depend on the entities being considered in the differential diagnosis.

PROGNOSTIC AND PREDICTIVE MARKERS IN RCC

Over the last 3 decades investigators have evaluated and in some cases proposed multiple markers as having prognostic or predictive value in renal cancer.[63–67] However, most of these studies are retrospective, contain small number of cases with limited annotated clinical data, and lack subsequent validation studies. As such, no marker or sets of markers have yet to emerge as reproducible and of clinical utility in predicting disease progression or response to therapy. Given the pace of genomic discovery and molecular pathway–driven clinical trials that are being performed, this is likely to change in the near future, as it has in adenocarcinomas of the lung, breast, melanoma, and hematopoietic neoplasms.

In recent years, we have gained a significant amount of insight into the molecular pathways involved in renal carcinoma, particularly ccRCC. This infusion of data has grown exponentially in the last few years, because of the work of International Genomics Consortium, The Cancer Genome Atlas, and multiple other research collaborations.[68,69] It is expected that follow-up studies focusing on molecular-clinical correlations will yield new tools to aid in predicting outcome and response to therapy.[70–74] These studies have revealed additional recurrent mutations involving several chromatin remodeling and histone modifying genes, all of which reside on chromosome 3p, similar to the *vhl* gene.[75–80] Tumors that harbor *PBRM1* mutations are the most common, and its presence predicts for extrarenal extension (pT3a) in small renal masses, although it does not appear to predict an adverse clinical outcome. Mutations to *SETD2* and *BAP-1* have been associated with high Fuhrman grade and worse clinical outcome.[78–80] Unfortunately, validated antibodies used on an automated platform in a CLIA laboratory have yet to be described, although the clinical utility of loss of BAP-1 expression appears to be very promising in predicting an adverse outcome, even in apparent low-risk disease. It must be stated that virtually all of the studies mentioned here apply to ccRCC, with virtually no strong data available for other types of renal tumors.

It is known that the vHL pathway is inactivated in virtually all ccRCCs, either by chromosomal loss, mutation, or epigenetic mechanisms. The end result is inactivation of the vHL complex resulting in activation of the hypoxia-inducible factor pathway and upregulation of downstream molecules, such as CAIX, and vascular endothelial growth factor (VEGF), among others.[65,68,69] Many of these molecules have a role in tumor initiation and growth. In addition, data from the Cancer Genome Atlas demonstrate that up to 28% of ccRCCs have alterations of the PI(3)K pathway, including 6% with activating mutations of mTOR. Given these findings, it is understandable that inhibitors of VEGF and mTOR have shown therapeutic efficacy in patients with advanced ccRCC,

prolonging disease-free survival but not overall survival.[70,71,74] There are no IHC markers targeting this pathway that can predict response, with one possible exception. As previously mentioned, CAIX is constitutively expressed in ccRCC.[80–85] Some authors have suggested that decreased expression in the primary tumor predicts poor survival, whereas tumors that express the marker in >85% of cells are more likely to respond to IL-2 therapy and to mTOR inhibitors. These data remain controversial and have yet to be validated in prospective trials.

Markers that look at cell cycle progression (p53, Ki-67) have been shown to correlate with adverse outcome.[86,87] However, it is unclear how much they add to grade, stage, and clinical performance status and are not used clinically to determine therapy.

ISUP Recommendations

- There are no markers or sets of markers ready for routine clinical use.
- Novel markers have been recently described that are associated with high-risk pathologic features and disease progression. However, these should not be used routinely until further clinical and technical validation is performed.

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