RENAL EPITHELIAL NEOPLASMS: IS THERE A ROLE OF IMMUNOSTAINS IN DIAGNOSIS?

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The majority of renal epithelial neoplasms are diagnosed on morphologic grounds. In renal cell carcinoma (RCC), there are unique histologic features that help to distinguish subtypes, and separate them from benign tumors such as oncocytoma and metanephric adenoma. These unique morphologic features are associated with specific genetic and chromosomal abnormalities as well as different tumor behaviors. However, renal epithelial tumors can exhibit overlapping features. In this setting, immunostains may play a role in the differential diagnosis (Table 1).

Table 1. Differential Diagnosis in Select Renal Epithelial Neoplasms

1. Renal epithelial neoplasms with eosinophilic granular cytoplasm
   - Clear cell renal cell carcinoma
   - Papillary renal cell carcinoma (type II)
   - Chromophobe renal cell carcinoma (eosinophilic variant)
   - Oncocytoma

2. Renal epithelial neoplasms with “small blue cells”
   - Papillary renal cell carcinoma (type I)
   - Metanephric adenoma
   - Wilms tumor

3. Renal epithelial neoplasms with tubular, tubulopapillary or papillary architecture
   - Papillary renal cell carcinoma
   - Metanephric adenoma
   - Translocation-associated renal cell carcinomas (TFE3 gene fusions)
   - Oncocytoma
   - Urothelial carcinoma of the collecting system
   - Collecting duct carcinoma

4. Renal epithelial neoplasms with spindle cells
Renal cell carcinoma (all subtypes) with sarcomatoid differentiation
Primary renal sarcomas
Retroperitoneal sarcomas with secondary involvement of the kidney
Solitary fibrous tumor
Angiomyolipoma

5. Metastatic renal epithelial neoplasms

Adrenal gland
- Primary adrenocortical adenoma/carcinoma
- Metastatic clear cell renal cell carcinoma

Identification of primary site for metastases
- Clear cell carcinomas from other sites

1. Renal Epithelial Neoplasms with Eosinophilic Granular Cytoplasm

This differential diagnosis includes clear cell, chromophobe, and type II papillary RCC and oncocytoma, and number of published studies have shown the utility of immunostains in separating these various renal tumors (Table 2). The immunostains of note are CD10, parvalbumin, RCC, KIT, alpha-methylacyl-CoA racemase or AMACR (P504S), PAX-2, antimitochondrial antibody (112-1), vimentin, and cytokeratins (CK) including CK7.

CD10

CD10, the common acute lymphoblastic leukemia antigen, is a cell surface metalloproteinase, and expressed by lymphoid precursor cells, germinal center B cells, some myeloid cells, and a number of non-hematolymphoid tissues including the normal proximal nephron of the kidney. A number of studies have shown that CD10 is expressed in the majority of clear cell and papillary RCC, and a minority of chromophobe RCC and oncocytoma, and therefore may be useful in the diagnostic distinction between these tumor types. It is of note that in clear cell RCC, increasing tumor grade is associated with decreased expression of CD10. In one study, low-grade clear cell RCC (grade 1 and 2) was positive in 89% of cases compared to 69% of higher-grade RCC. Also, CD10 appears to be more frequently expressed in type II papillary RCC (88% of tumors positive) compared to type I papillary RCC (45%). CD10 is expressed in a number of other tumors including urothelial carcinoma, prostatic adenocarcinoma, melanoma, pancreatic adenocarcinoma, and others. Therefore, in the immunostains workup of a tumor of unknown primary, CD10 may have limited utility.

Parvalbumin
Parvalbumin is a cytosolic calcium-binding protein that regulates intracellular calcium and this protein is expressed in the collecting ducts of the adult kidney. A limited number of studies have shown that the immunostain to this protein is positive in chromophobe RCC and oncocytoma, and negative in papillary and clear cell RCC. This finding has potential diagnostic utility and supports the concept that chromophobe RCC is derived from the intercalated cell of the collecting duct and has a close relationship with oncocytoma.

**RCC**

RCC is a glycoprotein found in the brush border of proximal tubule of the nephron. The immunostain for RCC is found in 47 to 85% of clear cell RCC and 63 to 91% of papillary RCC in contrast to a minority (0 to 4%) of chromophobe RCC and (0 to 14%) of oncocytoma. The staining pattern is either surface membrane or a combination of surface membrane and cytoplasmic.

**KIT**

KIT is a transmembrane tyrosine kinase receptor protein encoded by the proto-oncogene c-kit. Mutations in certain exons of the gene are found in a number of tumors, most notably gastrointestinal stromal tumors. Malignant GISTs that exhibit these mutations are responsive to imatinib mesylate therapy. Recently, gene expression studies have identified that c-kit is upregulated in chromophobe RCC, and immunostains have confirmed the presence of KIT protein in both chromophobe RCC and oncocytoma and its absence in clear cell RCC and papillary RCC. However, a recent study reported that a significant percentage of high-grade clear cell RCC including those with sarcomatoid differentiation expressed KIT, and these patients could potentially benefit from imatinib mesylate therapy. We recently reported that KIT protein staining is very infrequent in high-grade RCC and its presence is not associated with the specific mutations indicative of response to imatinib mesylate. KIT protein immunostaining is not specific to GIST, oncocytoma or chromophobe RCC, and it is seen in a number of different tumor types.

**Alpha-methylacyl-CoA Racemase (AMACR or P504S)**

Alpha-methylacyl-CoA racemase was identified in gene expression analyses of prostate cancer where the mRNA (followed by protein studies) was found to be increased in prostate cancer and absent in benign prostatic tissue. Gene expression analyses followed by immunohistochemical staining indicated that AMACR was also increased in papillary RCC but not the other RCC subtypes or oncocytoma. In addition, AMACR protein expression was identified in metastatic papillary RCC. Like other tumor markers, AMACR is not specific to papillary RCC and may be seen in other tumor types so its utility in determining a specific tumor lineage is limited.
Pax-2

Pax-2 is a homeogene that is expressed in the metanephric mesenchyme of the developing human kidney, and it functions in the mesenchymal-epithelial transformation that occurs in fetal kidney development. Although the number of studies and cases examined are low, the preliminary findings suggest that Pax-2 protein is more frequently expressed in clear cell RCC than papillary and chromophobe RCC and oncocytoma. In addition, Pax-2 expression appears to be higher in higher grade tumors. Additional studies are needed to determine the utility of this diagnostic marker in the evaluation of primary and metastatic renal epithelial tumors.

Vimentin

Immunohistochemical studies examining vimentin expression in renal tumors indicate that vimentin is more frequently expressed in clear cell and papillary RCC compared to oncocytoma and chromophobe RCC. However, as in most of the immunostains discussed here, there is overlap in expression of this protein between tumor types. In addition it is thought by some pathologists that cytokeratin and vimentin co-expression is supportive of a diagnosis of metastatic RCC. However, this co-expression profile is not specific to either papillary or clear cell RCC, and can be seen in other tumor types particularly spindle cell carcinomas from a number of sites.

Cytokeratins

The cytokeratins compromise a family of 20 distinct intermediate filaments, and their expression is indicative of epithelial differentiation. Differential CK protein expression has become common in diagnostic pathology with the use of CK7, CK20, CK5/6, and HMWCK. Recently, differential cytokeratin expression has been identified in the human nephron with the CKs of simple epithelia (CK7, CK8, CK18, and CK19) predominating particularly CK8 and CK18. Using this differential CK expression, Skinnider et al examined a panel of various CK subtypes and vimentin to differentiate between various renal cortical neoplasms consisting of clear cell, papillary, chromophobe, collecting duct, renal medullary, tubulocystic, mucinous tubular and spindle cell RCC, oncocytoma and metanephric adenoma and they identified the following profiles:

Clear cell RCC (15 cases):  CK7/-;CK8/-;CK18+;vimentin+
Papillary RCC (15 cases):  CK7+;CK8+;CK19+;vimentin+
Chromophobe RCC (15 cases):  CK7+;CK8+;CK18+;vimentin+
Oncocytoma (10 cases):  CK7-;CK8+;CK18+;vimentin-;CK14-
Urothelial Ca (12 cases):  CK5/6+/-;CK17+/-;vimentin-/+;HMWCK+-
Collecting duct RCC (6 cases):  CK5/6/-;CK17+/+;vimentin+;HMWCK+
Renal medullary RCC (3 cases):
CK7+;CK8+;CK18+;CK19+;vimentin+

Tubulocystic RCC (3 cases): 7-/+;CK8+CK18+;CK19+;vimentin+

Skinnider et al concluded that immunohistochemical determination of CK subtypes can be helpful in the diagnostic workup of some renal tumors. Cytokeratin 20 has also been examined in RCCs and oncocytoma, and is negative in clear cell RCC, positive in papillary RCC (type II), negative in chromophobe RCC and variably positive in oncocytoma (53-80% of cases positive).

Other Markers

Other potentially useful diagnostic markers of renal cell carcinoma include Ron protein. The protein is derived from a RON proto-oncogene that encodes for a tyrosine kinase receptor. In immunohistochemical analyses, Patton et al identified Ron protein expression in 99% (69 of 70 cases) of oncocytomas, 96% (55 of 57 cases) of chromophobe RCC, and in only 17% of other renal cell carcinoma subtypes. Antimitochondrial antibody (113-1) is a monoclonal antibody that recognizes a protein portion of the human mitochondria. In the assessment of granular renal cell tumors, distinctive staining profiles for this antibody were detected in oncocytoma (diffuse and fine granularity), chromophobe RCC (peripheral accentuation with coarse granularity), and granular variant of clear cell RCC (irregular distribution of coarse cytoplasmic granules). These distinctive staining patterns were reported as useful in the diagnostic workup of renal tumors with granular eosinophilic cytoplasm. E-cadherin is positive in chromophobe RCC and oncocytoma, and negative in clear cell RCC. In papillary RCC, e-cadherin is positive in approximately 40% of type II tumors, and 0% of type I tumors.
Table 2. Select Renal Epithelial Neoplasms and Immunostain Results by Study (% Cases Positive)

<table>
<thead>
<tr>
<th>Immunostain</th>
<th>Study</th>
<th>Clear Cell RCC</th>
<th>Papillary RCC</th>
<th>Chromophobe RCC</th>
<th>Oncocytoma</th>
<th>Collecting Duct RCC</th>
<th>Urothelial Carcinoma</th>
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<td>CD10</td>
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<td>93% (45 cases)</td>
<td>100% (20 cases)</td>
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<td></td>
<td>Mazal et al</td>
<td>85% (102)</td>
<td>23% (44)</td>
<td>4% (24)</td>
<td>0% (24)</td>
<td>0% (3)</td>
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<td></td>
<td>Pan et al</td>
<td>82% (256)</td>
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<td>32% (28)</td>
<td>0% (7)</td>
<td>40% (5)</td>
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<td></td>
<td>Martignoni et al</td>
<td>100% (75)</td>
<td>63% (51)</td>
<td>26% (42)</td>
<td>33% (9)</td>
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<td>0% (19)</td>
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<td>86% (22)</td>
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2. **Renal Epithelial Neoplasms with “Small Blue Cells”**

The differential diagnosis in this setting includes type I (basophilic) papillary renal cell carcinoma (immunophenotype already discussed), metanephric adenoma and Wilms tumor. The following immunostains may be helpful with the following typical immunophenotype:

- Metanephric adenoma: EMA-;CK7-/++;WT-1+
- Papillary RCC, type I: EMA++;CK7++;WT-1-
- PNET: EMA-;CD99+
- Wilms: EMA-;CK7-/++;WT-1++;CD99-/+

In the differential diagnosis of metanephric adenoma and Wilms tumor, immunostains play a minimal role as the tumors exhibit identical immunoreactivities including positivity for WT-1. In addition, it must be remembered that WT-1 is not specific to Wilms tumor and can be seen in a number of other tumors including carcinomas of the ovary, breast, lung, pancreas, endometrium, and colon as well as mesotheliomas.

3. **Renal Epithelial Neoplasms with Tubular, Tubulopapillary or Papillary Architecture**

The immunophenotype of the tumors in this diagnostic category have been covered previously (Table 2). One very important exception is renal cell carcinomas that arise from TFE3 gene fusions, the so-called “translocation carcinomas of the kidney”. These tumors most commonly affect young individuals and have a characteristic chromosomal translocation that involves chromosome Xp11.2 that results in gene fusions that involve the TFE3 transcription factor gene. Although renal cell carcinomas comprise less than 5% of renal tumors in young patients, it is likely that these translocation carcinomas of the kidney compromise a significant proportion of these childhood RCCs. The gene fusion combinations include ASPL-TFE3, PRCC-TFE3, PSF-TFE3, CLTC-TFE3 and nonO-TFE3. A polyclonal antibody to the C-terminal portion of TFE3 has been developed, and is a sensitive and specific marker for these tumors. Finally, another translocation carcinoma involves t(6;11)9p21;q12) that results in a Alpha-TFEB gene fusion whose product can be recognized by an antibody to TFEB. These translocation carcinomas of the kidney have malignant potential and will require further study to determine their frequency in the childhood and adult populations as well as their biologic behavior. Utilization of immunostains to identify these tumors will help greatly in this regard. In rare instances, urothelial carcinoma can have an unusual architecture. In addition to some stains listed in Table 2, p63 may be helpful as p63 is reported as positive in urothelial carcinoma and negative in RCC.
4. Renal Epithelial Neoplasms with Spindle Cells

The primary differential diagnosis in this setting rests between RCC with sarcomatoid differentiation, spindle cell sarcoma, and some variants of angiomyolipoma. The presence of an epithelial component (of any RCC subtype) associated with a malignant spindle cells is diagnostic of sarcomatoid RCC, and no immunostains are warranted. In most instances, adequate sampling will reveal an underlying RCC. In cases where the tumor is composed entirely of spindle cells, cytokeratin positivity is indicative of epithelial differentiation and sarcomatoid RCC. Immunostains are helpful for the other spindle cells tumors as well including smooth muscle markers for leiomyosarcoma and leiomyoma, CD34 for solitary fibrous tumor, and smooth muscle actin, muscle-specific actin, melan A and HMB45 for angiomylipoma (particularly useful for the epithelioid variant). In cases where the kidney is involved secondarily from a retroperitoneal sarcoma (which can be difficult to determine), dedifferentiated liposarcoma must always be kept in mind, and the tumor sampled well to evaluate for a diagnostic low grade component.

5. Metastatic Renal Epithelial Neoplasms

The identification of the kidney as a primary site in the immunostains workup of metastatic tumors of unknown primary is problematic. When the adrenal gland is involved by a tumor with clear cells and the differential includes adrenocortical tumors and metastatic clear cell RCC in a patient with known RCC, immunostains are potentially helpful (Table 3). At other sites, however, the lack of specificity of the immunostains (except in the unusual cases ) presented here prevents an unequivocal identification of kidney as the primary site. In addition, a thorough clinical examination including radiologic studies will determine if the kidney contains the likely primary tumor. In patients with known RCC who develop tumors at other sites, histologic comparisons of the metastasis with the renal tumor can be extremely helpful particularly knowledge of the primary tumor subtype, nuclear grade and stage. Hopefully, in the future additional specific immunostains will become available through gene expression profiles of RCC subtypes that will help in this regard.

Table 3. Renal Versus Adrenocortical Neoplasia.

<table>
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<th>Immunostain</th>
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<th>Clear Cell Renal Cell Carcinoma</th>
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<tr>
<td>Melan A</td>
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<tr>
<td>Inhibin</td>
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References


