Role of Immunohistochemistry in Diagnosing Renal Neoplasms
When Is It Really Useful?

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Context.—With the refinement of molecular and histologic classifications of renal neoplasms and the availability of more-effective molecular targeted therapy for specific renal neoplasms, immunohistochemical techniques will play an increasingly important role in the diagnosis of renal neoplasm. During the past few decades, many markers have been evaluated for their role in the diagnosis, prognosis, and prediction of treatment for renal neoplasms. The number of useful markers in our routine practice continues to increase. The challenge will be to choose among them and to decide in which situations immunohistochemistry will be truly useful.

Objectives.—To review the diagnostic utility of molecular markers for renal neoplasms and common diagnostic scenarios that call for immunohistochemistry in routine practice.

Data Sources.—This review is based on published literature and personal experience.

Conclusions.—Some of the most important and useful markers for the diagnosis of renal neoplasm include cytokeratins, vimentin, PAX2, PAX8, RCC marker, CD10, E-cadherin, kidney-specific cadherin, parvalbumin, claudin-7, claudin-8, α-methylacyl coenzyme A racemase, CD117, TFE3, thrombomodulin, uroplakin III, p63, CD57, and carbonic anhydrase IX. Each marker has its diagnostic role in a specific diagnostic setting. The common diagnostic situations that call for immunohistochemical staining are differential diagnoses of renal versus nonrenal neoplasms, histologic subtyping of renal cell carcinoma, diagnosis of rare primary renal neoplasms, diagnosis of renal neoplasms in small core-biopsy specimens, diagnosis of possible metastatic renal carcinomas, and less frequently, molecular prognostication.

Renal cell carcinoma (RCC) is the third most common cancer of the genitourinary tract and the most lethal urologic cancer, accounting for approximately 2% of all cancer deaths.1 Approximately one-third of the patients with RCC will present with metastases, and many patients will develop metastasis after surgical resection.2 Traditionally, RCC is known to be resistant to chemotherapy. However, there has been tremendous development in effective molecular targeted therapies in the past few years for specific types of RCC with well-defined histology and molecular abnormalities.3-6 Therefore, accurate histologic diagnosis and classification is increasingly important. Almost all malignant renal tumors arise from the renal tubules, collecting duct, or renal pelvic urothelium. In a normal kidney, each segment of renal tubules has a distinct and specific immunohistochemical expression profile. Each type of renal neoplasm is thought to be derived from or displays differentiation toward a specific segment of renal tubules. For example, the RCC marker antigen and CD10 are preferentially expressed in proximal tubules and the corresponding clear cell RCC; S100A, claudins, and kidney-specific cadherin are preferentially expressed in distal convoluted tubules and corresponding chromophobe RCC and oncocytoma. Similarly, high–molecular-weight cytokeratin is expressed in the collecting duct and urothelium; therefore, it is expected to be expressed in collecting duct carcinoma and urothelial carcinoma.

IMMUNOHISTOCHEMICAL TECHNIQUES IN RENAL NEOPLASMS

Immunohistochemical techniques with a variety of markers have been applied more frequently in diagnostic pathology of renal neoplasms, and in some situations, those techniques become indispensable.7-10 In this article, we will review the immunohistochemical markers most commonly used for diagnosis of renal neoplasms and discuss situations in which application of immunohistochemistry is truly valuable. Among the numerous markers that have been studied in renal neoplasms, some of the most commonly used markers are described below.
Cytokeratin

Differential expression of broad-spectrum and specific cytokeratin (CK) markers are useful for the diagnosis of RCC. Almost all renal cell neoplasms are positive for broad-spectrum CK (pancytokeratin); however, some commonly used broad-spectrum CK antibodies, such as AE1/AE3, lack the specificity for CK18, a low–molecular-weight CK expressed in all simple epithelia and commonly paired with CK8. In this setting, the widely used CAM 5.2 antibody is specific for CK8 and, to a lesser extent, for the closely related CK7, but shows no reactivity with CK18, CK7 is positive in most papillary RCC, collecting duct RCC, and urothelial carcinoma but is negative for clear cell RCC. High–molecular-weight CK (34βE12) and CK5/6 are positive in most of the urothelial carcinomas and in collecting duct RCCs. All RCCs are negative for CK20, which is important in differential diagnoses from the many CK20+ carcinomas.

Vimentin

As a broad mesenchymal marker, vimentin, interestingly, is expressed in most types of RCC, which can be useful in the differential diagnosis of carcinoma because very few other types of carcinoma (endometrial carcinoma, thyroid and adrenal cortical carcinoma) coexpress vimentin and CK. Among the common RCCs, 87% to 100% of the clear cell RCCs and papillary RCCs are positive for vimentin, whereas chromophobe RCCs and oncocytomas are typically negative.

PAX2 and PAX8

Both PAX2 and PAX8 are transcription factors that are essential for the development of kidney, Müllerian, and other organs. They are expressed in normal kidney as well as in most of the renal neoplasms. PAX2 and PAX8 have very similar expression profiles in RCC and in ovarian and endometrial carcinoma. However, PAX8 is also expressed in thyroid follicular cells and thyroid carcinoma, but PAX2 is typically negative in thyroid tumors. Their strong nuclear immunoreactivity, as well as their higher sensitivity than other renal tissue markers, makes them the front runners during the workup for metastatic RCC.

RCC Marker

The RCC marker is a monoclonal antibody directed against a glycoprotein on the brush border of proximal renal tubular cells. It is positive in almost all low-grade clear cell RCCs and papillary RCCs but is usually negative in chromophobe RCCs, oncocytomas, and collecting duct RCCs. Early studies have shown that RCC marker is relatively specific for carcinoma of renal origin; however, weak expression has been reported in a few other neoplasms, including breast ductal carcinoma and testicular embryonal carcinoma, among others.

CD10

CD10 is a cell-surface glycoprotein expressed in a variety of tissues and malignancies. For renal neoplasms, the CD10 expression profile is similar to that of RCC marker and almost all clear cell RCCs and papillary RCCs stain positive for CD10, whereas other types of RCCs stain negative. Although still useful in selective situations, the broad expression of CD10 in many other malignant neoplasms limits its use as a confirmatory marker for metastatic RCC.

E-Cadherin and Kidney-Specific Cadherin

Both E-cadherin and kidney-specific cadherin are cell-adhesion molecules and are involved in cell-cell interaction. Almost all chromophobe RCCs and oncocytomas are positive for E-cadherin and kidney-specific cadherin, but clear cell RCCs and papillary RCCs are typically negative. In contrast to E-cadherin, which is expressed in many other neoplasms, kidney-specific cadherin expression in nonrenal neoplasms has not been reported.

Parvalbumin, Claudin 7 and 8, and CD117

This group of markers (parvalbumin, claudin 7 and 8, and CD117) has been shown to be positive in a high percentage of chromophobe RCCs and oncocytomas but is typically negative in other types of RCC. Claudins and CD117 are expressed in other malignant neoplasms, and the expression of parvalbumin in other neoplasms has not, to our knowledge, been well studied.

α-Methylacyl Coenzyme A Racemase

α-Methylacyl coenzyme a racemase (AMACR) is a mitochondrial enzyme mediating the oxidation of fatty acids and is commonly expressed in normal hepatocytes, the epithelium of the proximal renal tubules, and the bronchus. It is a well-known, positive tumor marker for prostatic adenocarcinoma. Almost all papillary RCCs are positive for AMACR, but other types of RCCs are rarely positive.

TFE3, TFEB, and Cathepsin-K

TFE3 is a transcription factor that is overexpressed in a group of RCCs with translocation involving Xp11.2. Positive nuclear labeling for TFE3 by immunohistochemistry is a sensitive and specific marker for Xp11.2 translocation RCC, which occurs primarily in children and young adults. Similarly, TFEB is also a transcription factor that is overexpressed in pediatric RCCs with t(6;11)(p21;q12). Nuclear staining with the TFE3 protein is a highly sensitive and specific marker for these renal neoplasms. Cathepsin-K is overexpressed in all TFE3 translocation carcinomas and most TFE3 translocation carcinomas and, therefore, appears to be useful with differentiating translocation RCCs from other adult RCCs.

Uroplakin III, p63, Thrombomodulin, and GATA3

These markers (uroplakin III, p63, thrombomodulin, and GATA3) are expressed in a high percentage of urothelial carcinomas but are not usually expressed in RCCs; therefore, these markers are useful in the differential diagnosis of a high-grade carcinoma involving the kidney, when a definitive diagnosis cannot be made solely on morphologic findings.

Other Useful Markers

CD57 is a useful marker for the diagnosis of metanephric adenoma. HMB-45 and Melan-A immunohistochemical stains are important for making a diagnosis of angiomylipoma. Many other markers that are useful in the diagnosis of nonrenal malignant neoplasms are available and may be applied in workup of renal tumors. Immunohistochemical methods have become increasingly important in diagnostic pathology because of
Immunohistochemical Profile of Common Renal Neoplasms

<table>
<thead>
<tr>
<th>Renal Neoplasm</th>
<th>Immunohistochemical stain(s)</th>
</tr>
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<tbody>
<tr>
<td>Clear cell RCC</td>
<td>Positive for vimentin, AE1/AE3, CD10, RCCM, PAX2, PAX8, and CAIX</td>
</tr>
<tr>
<td></td>
<td>Negative for HMWCK, CK7, CK20, CD117, kidney-specific cadherin, and parvalbumin</td>
</tr>
<tr>
<td>Papillary RCC and mucinous tubular and spindle cell carcinoma</td>
<td>Positive for vimentin, AE1/AE3, CK7, AMACR, RCCM, PAX2, and PAX8</td>
</tr>
<tr>
<td></td>
<td>Negative for CD117, kidney-specific cadherin, and parvalbumin</td>
</tr>
<tr>
<td>Chromophobe RCC/oncocytoma</td>
<td>Positive for E-cadherin, kidney-specific cadherin, parvalbumin, CD117, AE1/AE3, CK7 strong/diffuse for chromophobe RCC</td>
</tr>
<tr>
<td></td>
<td>Negative for vimentin, CK7 (or weak for oncocytoma), CAIX, and AMACR</td>
</tr>
<tr>
<td>Collecting duct carcinoma</td>
<td>Positive for p63 and HMWCK; some are positive for PAX2 and PAX8</td>
</tr>
<tr>
<td></td>
<td>Negative for RCCM, CD10, CD117, kidney-specific cadherin, and parvalbumin</td>
</tr>
<tr>
<td>Xp11.2 translocation carcinoma</td>
<td>Positive for CD10, RCCM, TFE3, PAX2, PAX8, and cathepsin-K</td>
</tr>
<tr>
<td></td>
<td>Usually negative or focally positive for AE1/AE3</td>
</tr>
<tr>
<td>Clear cell papillary renal cell carcinoma</td>
<td>Positive for CK7, PAX2, and PAX8</td>
</tr>
<tr>
<td></td>
<td>Negative for AMACR, RCCM, and CD10</td>
</tr>
<tr>
<td>Urothelial carcinoma</td>
<td>Positive for HMWCK, CK7, p63, CK5/6, CK20, uroplakin III, thrombomodulin, and GATA-3</td>
</tr>
<tr>
<td></td>
<td>Negative for RCCM, CD10, PAX2, and PAX8</td>
</tr>
<tr>
<td>Myoid-rich or epithelioid angiomyolipoma</td>
<td>Positive for HMB-45, Melan-A, MSA, or SMA</td>
</tr>
<tr>
<td></td>
<td>Negative for AE1/AE3, CD10, RCCM, PAX2, and PAX8</td>
</tr>
</tbody>
</table>

Abbreviations: AMACR, α-methylacyl-coenzyme A racemase; CAIX, carbonic anhydrase IX; CK, cytokeratin; HMWCK, high–molecular-weight cytokeratin; MSA, muscle-specific actin; RCCM, renal cell carcinoma marker; SMA, smooth muscle actin.

improved detection techniques and the availability of monoclonal antibodies against specific cellular proteins. The approach to pathologic diagnoses of renal neoplasms is similar to that of any other tissue or organ system pathology, starting with the gathering of information from gross findings and microscopic examination of the tumor. Often, immunohistochemistry has either a confirmatory role or is indispensable in making a definite diagnosis. However, immunohistochemistry can only be helpful when logical differential diagnoses are generated after an appropriate sampling of the lesion, a careful microscopic examination, and clinical and radiologic correlation.

RENAAL CELL VERSUS NON–RENAAL CELL NEOPLASMS

Different types of renal neoplasms may simulate non–renal cell neoplasms, including both benign and malignant entities, such as angiomylipoma, malignant lymphoma, urothelial carcinoma, adrenal malignant neoplasms (cortical carcinoma, pheochromocytoma, or neuroblastoma), and metastatic carcinoma. In rare situations, nonneoplastic processes, such as xanthogranulomatous pyelonephritis or malakoplakia, may present as a tumoral mass clinically, grossly, or microscopically. When RCCs present with a typical morphology, the diagnosis is straightforward, and immunohistochemical stains are not necessary. When renal tumors present with unusual morphologies or features that do not readily conform to any known histologic types of renal tumor, the possibility of nonrenal neoplasms has to be ruled out with the help of immunohistochemical stains. The most common situations include an undifferentiated high-grade malignant neoplasm, a small blue cell tumor, or a spindle cell neoplasm. The key immunohistochemical markers for making a diagnosis of primary RCC are currently PAX2, PAX8, RCC marker, CD10, and a combination of vimentin and CK. Consider tissue-specific markers for metastasis from other sites if there is a possible clinical history of a nonrenal tumor, such as TTF-1 from the lung, prostate-specific antigen from the prostate, CDX2 from the colon-rectum, hepar-1 from the liver, inhibin from an adrenal cortical carcinoma, and S100 and HMB-45 from melanoma.

HISTOLOGIC SUBTYPING OF RENAL CELL NEOPLASMS

Different histologic subtypes of RCC are known to have distinct clinical presentations and prognoses. Additionally, histologic subtype is a consideration for the choice among increasingly popular neoadjuvant or adjuvant therapy regimens for patients with advanced RCC. During the past few decades, the histologic classification of the renal neoplasm has been continuously revised. Several relatively new and specific morphologic entities of RCC have been recognized. Even though the immunohistochemical profiles of most RCCs are fairly well established, the results of any particular study may be quite varied because of several factors, most importantly, the diagnostic criteria used for the histologic RCC subtyping, the immunohistochemical result interpretation, and the antibody used. A general immunohistochemical profile of the most common and well-classified renal neoplasms is shown in the Table. These results are generated from typical histologic subtypes of RCC or the renal tumor. It is costly, unnecessary, and impractical to use a large panel of markers for the differential diagnosis of different RCC subtypes. For a renal cell neoplasm with clear and eosinophilic cells, the differential diagnosis of clear cell and chromophobe RCC can be achieved with vimentin, RCC marker, carbonic anhydrase IX (for clear cell) and kidney-specific cadherin, and CD117 or parval-
The utility of immunohistochemical stains in the differential diagnosis of an oncocytic renal neoplasm is shown in Figure 1, A through D. For a renal tumor with a papillary growth pattern, the useful antibody panel may include CK7, AMACR, CD10 or RCC marker, TFE3, and CD57. For a diagnosis of unclassified RCC, immunohistochemical stains with PAX2 and PAX8 will be helpful in establishing the diagnosis of carcinoma of renal cell origin. Many studies have attempted to identify markers that are useful for distinguishing oncocytomas and chromophobe RCCs. Histogenetically, these 2 neoplasms are closely related and share overlapping morphologic and immunohistochemical features. Strong and diffuse CK7 staining favors a diagnosis of chromophobe RCC. Some studies have shown the value of S100A1 and CD82 in distinguishing chromophobe RCC from oncocytoma, but those findings need to be further verified. So far, it appears no markers can reliably distinguish between an oncocytoma and an eosinophilic variant chromophobe RCC. In general, papillary RCC and mucinous tubular spindle cell carcinoma share very similar immunohistochemical profiles. In addition to RCC, renal pelvic urothelial carcinoma accounts for approximately 7% to 8% of all malignant renal carcinomas. For an invasive high-grade urothelial carcinoma involving the kidney, a differential diagnosis from a high-grade RCC, such as collecting duct RCC or a papillary or clear cell RCC, can be difficult. A panel of markers, including RCC marker, PAX2 or PAX8, uroplakin III, thrombomodulin, and p63, can be helpful.

RARE, PRIMARY RENAL NEOPLASMS

A small subset of renal neoplasms composed of small blue cells, such as lymphoma, synovial sarcoma, neuroblastoma, small cell carcinoma, Wilms tumor, and Ewing sarcoma/primitive neuroepithelial tumor, can occur in the kidney and distinguishing them can be difficult on morphologic examination alone. Malignant lymphoma, small cell carcinoma, and neuroendocrine carcinoma can occur either as primary kidney tumors or as a systemic spread from other locations. Correlation

Figure 1. Application of immunohistochemical stains in the diagnosis of an oncocytic renal neoplasm. A, Hematoxylin-eosin stain showing a tumor composed of a solid growth of oncocytic/eosinophilic cells. Differential diagnosis may include clear cell renal cell carcinoma (RCC) with granular cells, chromophobe RCC or oncocytoma, and type-2 papillary RCC. B, Vimentin immunostaining showing strong and diffuse positive stain. C, The RCC marker stain showing unique apical membrane cellular pattern. D, α-Methylacyl coenzyme A racemase stain showing diffuse and strong cytoplasmic staining. The overall features support a diagnosis of papillary RCC (original magnifications ×200 [A through D]).
with clinical history and imaging studies is mandatory. Primary synovial sarcoma, adult Wilms tumor, and Ewing sarcoma/peripheral neuroectodermal tumor have rarely been reported in the kidney. A panel of antibodies, such as leukocyte common antigen, epithelial membrane antigen (EMA), AE1/AE3, neuroendocrine markers, WT1, CD99, and TLE1, is helpful in establishing the diagnosis.

Figure 2. A through C, Immunohistochemical staining of metastatic renal cell carcinoma (RCC). A, Metastatic, high-grade clear cell RCC to the lung (hematoxylin-eosin staining). B, PAX8 immunostain showing strong nuclear positivity. C, The RCC marker showing focal, faint apical membrane staining. D through F, Metastatic collecting duct carcinoma to the liver. D, Hematoxylin-eosin staining. E, PAX8 immunostain showing strong nuclear positivity. F, The RCC marker is completely negative (original magnifications ×200 [A through F]).
SMALL BIOPSY SPECIMENS

Fine-needle aspiration biopsy or core needle biopsy has recently become more frequently used for preoperative diagnosis, not only for traditional indications, such as inoperable tumor or tumors where surgical resection is considered to be contraindicated or ineffective, such as malignant lymphoma or metastatic tumors, but also in response to new therapies where preoperative diagnosis will help make decisions about the choice of treatment (partial versus total nephrectomy, radiofrequency, or cryoablation).72–74 Several studies have demonstrated the relatively high diagnostic accuracy of needle biopsies based on the hematoxylin-eosin section alone.74,75 Recently, Al-Ahmadie et al76 showed that standard morphologic evaluation, in combination with the judicious use of 5 markers (CAIX, CD117, AMACR, CK7, and CD10), can produce an accurate diagnoses in greater than 90% of cases in an ex vivo core needle biopsy of renal tumors after nephrectomy. The use of immunohistochemical staining could conceivably continue to increase in this setting. The availability of renal tissue–specific markers and differential markers as outlined previously will allow confirmatory diagnoses, particularly when diagnostic tissue samples are too small or not well preserved.

METASTATIC RCC IN DISTANT SITES

Historic data have shown that approximately 30% of RCCs have distant metastases at the time of presentation. Furthermore, many patients will present with recurrence after surgical resection. Renal cell carcinoma is also known to metastasize to virtually any body site, including odd locations.77,78 Patients can present with either a second malignancy or potential metastatic RCC.79 Fine-needle aspiration or core biopsy is often performed in this context. In these situations, the amount of tumor tissue available is often quite limited, and ancillary studies, including immunohistochemistry, are needed for initial or confirmatory diagnosis.

In spite of the availability of several “renal” markers, the diagnosis of metastatic RCC is often not straightforward for the following reasons: (1) metastatic RCC may be less well differentiated and thus appear morphologically different from its primary tumor, (2) the metastasis may not conform to the known morphologic spectrum of primary RCCs, (3) morphologic changes can occur after adjuvant therapy, and (4) although several markers have been described for RCC, many of them are also noted in other types of neoplasms.

Fortunately, among markers for RCC, the tumor type-specific profiles for primary RCCs are largely retained in their metastases, but significant attenuation of both staining frequency and extent may supervene.23,30 PAX-2 and PAX-8 have emerged as the most useful markers for metastatic RCC, with a frequency and extent of staining similar to those for the primary tumors, including metastatic collecting duct RCC.80 The RCC marker and CD10 have been useful markers for the diagnosis of metastatic RCC; however, comparative studies have shown that their sensitivity is lower than that of PAX-2 and PAX-8. Figure 2, A through F, shows 2 examples of metastatic RCC with comparative staining of PAX-8 and RCC marker. Parvalbumin and kidney-specific cadherin are useful markers for detecting metastatic chromophobe RCC, and AMACR is expressed in a high percentage of primary papillary RCCs and was detected in 23 of 28 metastatic papillary RCCs (82%) in one study39 and 6 of 6 metastatic papillary RCCs (100%) in another study.40 For metastatic sarcomatoid RCC, immunohistochemical identification remains problematic. The tumor cells are either completely negative or focally and weakly positive for the “renal markers,” such as RCC marker, CD10, PAX-2, or PAX-8.23,30

Each marker that is used for a renal tumor also expresses in nonrenal tumors and, therefore, is not specific for metastatic RCC. For example, CD10 is helpful for the diagnosis of metastatic RCC, but it is expressed in many other primary or metastatic carcinomas.8,29,41–44 Although AMACR is expressed in almost all metastatic papillary RCCs, it is expressed by other carcinomas, most notably, prostate adenocarcinoma; PAX8 detects most metastatic RCCs but is seen in most müllerian tumors and thyroid malignancies.21

The available data suggest that the panel for evaluating potential metastatic RCC should include PAX2 or PAX8, and RCC marker or CD10, supplemented by other markers dictated by the affected organ and/or the type of nonrenal tumors that may coexist.

PROGNOSTIC MARKERS

Metastatic RCC has traditionally been considered resistant to chemotherapy. However, recent advances in molecular targeted therapy have revolutionized the landscape for treatment of patients with advanced RCC. There is great interest in identifying markers that can help predict tumor progression and response to various therapies.85,86 Currently, although several promising prognostic markers have been shown to be useful in specific situations, none of them have been validated or proven for clinical application. The most extensively studied markers include the von Hippel Lindau pathway-related markers (hypoxia-inducible factor-α, vascular endothelial growth factor, CAIX).67 Among them, CAIX is one of the most important molecular markers for RCC. Several studies have shown that high tumoral expression of CAIX is associated with better prognosis and a greater likelihood of response to immunotherapy based on interleukin-2.88 Other general tumor markers that have been studied extensively include p53,39 Ki-67,89 CXCXR3 and CXCXR4,91 matrix metalloproteinase 2 or 9,92 insulin-like growth factor II mRNA-binding protein (IMP3),93 B7-H1, B7-H3, and B7-H4,94 and survivin,95 among others.

In summary, with the availability of numerous traditional and emerging new markers, immunohistochemistry is increasingly important in the accurate diagnosis of primary or metastatic renal neoplasms. Familiarity with the sensitivity and specificity of each marker for each type of tumor and understanding each of their uses in different diagnostic situations are critical. Many studies have shown the potential utility of molecular markers as prognostic and predictive factors for RCC; however, vigorous clinical validation is necessary before they can be used routinely in the clinical setting.

References
12. Han CP, Hsu JD, Koo CC, Yang SF. Antibody to cytokeratin (CK8/CK18) is not derived from CAM5.2 clone, and anticytokeratin CAM5.2 (Becton Dickinson) is not synonymous with the antibody (CK8/CK18). Hum Pathol. 2010;41(4):616–617; author reply. Hum Pathol. 2010;41(4):617.
carcinoma of the kidney: significant immunophenotypic overlap warrants diagnostic caution.


