

Immunohistochemical Analysis of Chromophobe Renal Cell Carcinoma, Renal Oncocytoma, and Clear Cell Carcinoma

An Optimal and Practical Panel for Differential Diagnosis

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• **Context.**—The separation of chromophobe renal cell carcinoma, oncocytoma, and clear cell renal cell carcinoma using light microscopy remains problematic in some cases.

Objective.—To determine a practical immunohistochemical panel for the differential diagnosis of chromophobe carcinoma.

Design.—Vimentin, glutathione S-transferase α (GST- α), CD10, CD117, cytokeratin (CK) 7, and epithelial cell adhesion molecule (EpCAM) were investigated in 22 cases of chromophobe carcinoma, 17 cases of oncocytoma, and 45 cases of clear cell carcinoma.

Results.—Vimentin and GST- α expression were exclusively observed in clear cell carcinoma. CD10 staining was more frequently detected in clear cell carcinoma (91%) than in chromophobe carcinoma (45%) and oncocytoma (29%). CD117 was strongly expressed in chromophobe carcinoma (82%) and oncocytoma (100%), whereas none of the cases of clear cell carcinomas were immunoreactive.

Cytokeratin 7 was positive in 18 (86%) of 22 cases of chromophobe carcinoma, whereas all oncocytomas were negative for CK7. EpCAM protein was expressed in all 22 cases of chromophobe carcinoma in more than 90% of cells, whereas all EpCAM-positive oncocytomas (5/17; 29%) displayed positivity in single cells or small cell clusters.

Conclusions.—Using the combination of 3 markers (vimentin, GST- α , and EpCAM), we achieved 100% sensitivity and 100% specificity for the differential diagnosis of chromophobe carcinoma, oncocytoma, and clear cell carcinoma. The pattern of “vimentin⁻/GST- α ⁻” effectively excluded clear cell carcinoma, and homogeneous EpCAM expression confirmed the diagnosis of chromophobe carcinoma rather than oncocytoma. CD117 and CK7 were also useful markers and could be used as second-line markers for the differential diagnosis, with high specificity (100%) and high sensitivity (90% and 86%, respectively).

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Chromophobe renal cell carcinoma (RCC) is an uncommon variant of RCC, accounting for approximately 5% of renal cancer. In many cases, it is possible to distinguish chromophobe RCC from other renal tumors on the basis of hematoxylin-eosin (H&E)-stained tissue sections and Hale colloidal iron staining alone. However, overlapping morphologic characteristics pose some difficulties in making a proper diagnosis in a small but significant number of kidney tumors even in the hands of experienced pathologists. The eosinophilic variant of chromophobe RCC is particularly difficult to distinguish from renal oncocytoma and the eosinophilic variant of clear cell RCC, whereas the typical variant can resemble clear cell RCC.

To render an accurate diagnosis of chromophobe RCC, additional methods have been recommended. Electron mi-

croscopy is a useful means for diagnosis when one identifies the characteristic cytoplasmic microvesicles. Genetic abnormalities such as deletion of chromosomes 1, 2, 6, 10, 13, 17, and 21 detected in chromophobe RCC are sometimes used for differential diagnosis.¹ However, both methods are time-consuming, expensive, and not available in most facilities. Hale colloidal iron stain is also a useful adjunct, but it is technically demanding and often difficult to interpret. Therefore, increasing interest has focused on identification of a fast, reliable set of immunohistochemical markers that is applicable in most pathology laboratories. To date, a small but significant number of immunohistochemical stains have been reported individually to be useful for distinguishing chromophobe RCC from oncocytoma and clear cell RCC. Vimentin, CD10, and cytokeratin (CK) 7 are useful according to most investigators, but conflicting results have been reported.^{2–9} In recent years, other studies have suggested that glutathione S-transferase α (GST- α), CD117, and epithelial cell adhesion molecule (EpCAM) were also valuable for differentiation.^{10–12} However, no single marker appears to be sufficiently accurate by itself. Moreover, reliance on a single marker in differential diagnosis of tumors with overlapping morphology may be insufficient or even misleading, especially when the interpretation of the stain is not straightforward or the

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Table 1. Summary of Correlation of Staining of All 6 Markers Between Tissue Microarrays and Large Sections in 10 Cases*

	Cases Without Discrepancy, No. (%)	Cases With Discrepancy, No. (%)		
		Difference Range†		
		≤10%	11%–20%	21%–30%
Vimentin	10 (100)	0 (0)	0 (0)	0 (0)
GST-α	9 (90)	1 (10)‡	0 (0)	0 (0)
CD10	7 (70)	1 (10)‡	0 (0)	2 (20)§
CD117	7 (70)	1 (10)‡	1 (20)‡	1 (10)§
CK7	8 (80)	0 (0)	1 (10)‡	1 (10)‡
EpCAM	10 (100)	0 (0)	0 (0)	0 (0)

* GST-α indicates glutathione S-transferase α; CK, cytokeratin; and EpCAM, epithelial cell adhesion molecule.

† The difference in the percentage of positive cells.

‡ The differences did not change the category of immunoreactivity.

§ The differences changed the category of immunoreactivity from diffuse to moderate positivity.

tissue sample is small. This prompted us to explore a set of immunohistochemical markers that would improve the accuracy for the diagnosis of chromophobe RCC with an eye toward optimization and, as needed, redundancy to provide confirmatory evidence of identity of a neoplasm. Accordingly, we examined 22 chromophobe carcinomas, 17 oncocytomas, and 45 conventional RCCs using immunohistochemistry on tissue microarrays (TMAs) as well as on routinely collected tumor blocks. The objectives of this study were (1) to identify the specific staining patterns of multiple markers in 3 different types of renal tumor, (2) to compare the sensitivity and specificity of these markers for differential diagnosis, and (3) to determine an optimal diagnostic strategy for chromophobe RCC.

MATERIALS AND METHODS

Case Selection

The study group consisted of 22 cases of chromophobe RCC, 17 cases of oncocytoma, and 45 cases of clear cell RCC; all specimens were obtained by radical or partial nephrectomy. Cases with needle biopsies were excluded. All cases were retrieved from the files of Bostwick Laboratories, Richmond, Va, or the Department of Pathology at Beijing Friendship Hospital, Beijing, China. The World Health Organization classification of renal tumors was used for diagnosis.¹³ Three pathologists independently reviewed the H&E slides with accompanying Hale colloidal iron stain without knowledge of the previous diagnosis, and complete agreement was reached in all cases for chromophobe RCC, oncocytoma, and clear cell RCC. Four of the 22 chromophobe RCCs were of the eosinophilic variant (all were positive for Hale colloidal iron stain); none of the chromophobe RCCs showed sarcomatoid change. Chromophobe RCCs were graded as 2 (13 cases), 3 (8 cases), and 4 (1 case), and clear cell RCCs were graded as 1 (16 cases), 2 (16 cases), 3 (7 cases), and 4 (6 cases) using the Fuhrman grading system.¹⁴ Two cases of chromophobe RCC and 2 cases of clear cell RCC were initially signed out elsewhere as oncocytoma but reclassified in our consultation service.

Tissue Microarrays

Representative areas were identified on H&E slides and marked for sampling with TMAs. Using the Beecher Instruments TMA processor (4508-DM, Sun Prairie, Wis), 1 to 3 cores 2.0 mm in diameter were extracted from 1 to 2 paraffin-embedded tissue blocks in each case and incorporated into 4 tissue array blocks—TMA1, TMA2, TMA3, and TMA4, which included 45, 35, 35, and 23 cores, respectively. Normal renal parenchyma cores were also included in each TMA block to serve as positive and negative controls. After the TMAs were constructed, we added 2 more cases of chromophobe RCC, 4 more cases of clear cell RCC, and 9 more cases of oncocytomas to this study, and immunohisto-

chemistry was performed on conventional tissue blocks in these cases. To deal with the heterogeneity of each tumor, multiple 2-mm-diameter tissue cylinders were used to offer more tissue surface instead of the 0.6-mm-diameter cylinders commonly produced by the typical tissue array instrument. To further evaluate whether TMA expression was representative of each tumor, we additionally performed the battery of immunohistochemical tests on routine tissue sections from 10 tumors that were used to construct the TMAs (1–6 tumors for each kind).

Immunohistochemistry

The following antibodies were included in this study: vimentin (V9 monoclonal, 1:500; Dako, Carpinteria, Calif), CD10 (56C monoclonal, 1:100; Biocare, Concord, Calif), GST-α (rabbit polyclonal, 1:50; Neomarker, Fremont, Calif), CD117 (rabbit polyclonal, 1:400; Biocare), CK7 (K72.7 monoclonal, 1:50; Biocare), and EpCAM (C10 monoclonal, 1:100; Santa Cruz Biotechnology, Santa Cruz, Calif). Sections (3–4 μm) were cut and mounted on silane-coated slides, dried, deparaffinized in xylene, and rehydrated in ethanol. Antigenic retrieval used the Biocare pressure cooker, heating slides to 125°C for 2.5 min (1mM EDTA except for CK7 and CD117, which were treated with 10mM citrate buffer [pH 6.0]) and cooling the slides to 90°C. Endogenous peroxidase was quenched by 3% hydrogen peroxide for 10 minutes. Slides were incubated for 30 minutes with primary antibody. The rabbit polyclonal antibodies were detected by MACH2 rabbit horseradish peroxidase (Biocare) and mouse monoclonal antibodies detected by the EnVision+ system (DAKO) with 30 minutes of incubation. Both secondary detection systems were biotin free. The antigen-antibody immunoreaction was visualized using 3,3'-diaminobenzidine. All immunoreactions were carried out at room temperature.

Tumor cells were considered positive only when the appropriate staining pattern was noted (CD117 and EpCAM give membranous staining, CD10 gives cell surface staining, CK7 gives cytoplasmic and membranous staining, vimentin gives cytoplasmic staining, and GST-α gives cytoplasmic and nuclear or cytoplasmic staining). The extent of immunoreactivity was categorized as negative (0), less than 5%; focal (1+), 5% to 10%; moderate (2+), 11% to 50%; and diffuse (3+), greater than 50% positivity of tumor cells. The sensitivity and specificity were calculated for each marker.

RESULTS

Agreement of Results Between Tissue Arrays and Conventional Large Tissue Sections

The expression patterns of the markers on the TMAs were compared with those on routine tissue sections from 10 of the tumors that were constructed into TMAs. There was a good correlation between the staining pattern in the TMA and large sections (Table 1).

Immunostaining in Normal Kidney Tissue

In the normal renal parenchyma, renal tubules did not express vimentin. GST- α and CD10 selectively labeled the proximal tubules, with CD10 additionally staining the glomerular epithelium and Bowman capsule. Interestingly, CD117 selectively labeled some of the lining cells of distal tubules and collecting ducts in an intermittent fashion, in which the positive cells in the collecting ducts probably represent intercalated cells, and the expression was cytoplasmic with accentuation in the basal portion of the cell membrane (Figure 1). Cytokeratin 7 and EpCAM preferentially labeled distal tubules and collecting ducts: CK7 expression was cytoplasmic with cell membrane accentuation, whereas EpCAM expression was in the basolateral cell membranes; weak cytoplasmic staining was also seen.

Immunostaining in Chromophobe RCC, Oncocytoma, and Clear Cell RCC

The results of vimentin, GST- α , CD10, CD117, CK7, and EpCAM immunohistochemical staining in chromophobe RCC, oncocytoma, and clear cell RCC are detailed in Table 2. Representative H&E and immunohistochemistry staining is illustrated in Figure 2, A through C, for chromophobe RCC and in Figure 3, A and B, for clear cell RCC.

Vimentin staining was absent in all chromophobe RCCs (0/22) and oncocytomas (0/17), whereas diffuse cytoplasmic staining was present in all clear cell RCCs (45/45), with 90% to 100% tumor cells positive.

None of the chromophobe RCCs and oncocytomas showed GST- α staining. Positive cytoplasmic and nuclear GST- α staining was present in 41 (91%) of 45 clear cell RCCs.

CD10 was expressed in most (41/45; 91%) clear cell RCC cases. CD10 expression was also observed in 45% (10/22) of chromophobe RCCs (Figure 4) and 29% (5/17) of oncocytomas.

Immunoreactivity for CD117 was present in 18 (82%) of 22 chromophobe RCCs and all 17 oncocytomas with moderate to diffuse staining in all positive cases. The staining was complete-membranous (Figure 5) rather than the basal staining of cell membranes as seen in the normal distal tubules. None of the 45 clear cell RCCs were positive for CD117.

Nineteen (86%) of 22 chromophobe RCCs showed cytoplasmic positivity with membrane accentuation for CK7, whereas the remaining 3 cases (13%) were considered to be negative with staining detected in 1% of tumor cells in all 3 cases. All 17 oncocytomas were negative for CK7, of which 11 cases (65%) showed only single scattered immunoreactivity in less than 5% of tumor cells (Figure 6). Five (11%) of the 45 clear cell RCCs demonstrated positivity for CK7.

EpCAM protein was strongly expressed in all chromophobe RCCs (100%; 22/22) with positivity in 100% of tumor cells in 21 cases and 90% of tumor cells in 1 case (Figure 7, A), and the staining was complete-membranous or basolateral in tumor cells arranged in tubules, similar to normal renal tubules. Five (29%) of 17 oncocytomas were positive for EpCAM, of which 2 tumors showed focal positivity in 10% of tumor cells and the remaining 3 tumors showed moderate positivity in 30%, 40%, and 50% of tumor cells, respectively. The staining pattern in positive cases was invariably single scattered or in small cell clusters (Figure 7, B), in contrast to the homogeneous

staining pattern seen in chromophobe RCC. EpCAM positivity was also found in 15 (33%) of 45 clear cell RCCs.

Sensitivity and Specificity

This study aimed to separate the tumors initially into 2 groups, that is, chromophobe RCC/oncocytoma and clear cell RCC, by vimentin, GST- α , CD10, and CD117, and to further discriminate between chromophobe RCC and oncocytoma by CK7 and EpCAM. To separate clear cell RCC from chromophobe RCC and oncocytoma, vimentin, GST- α , and CD117 each showed 100% specificity, whereas the sensitivity was 100%, 91%, and 90%, respectively; CD10 had high sensitivity (91%) but low specificity (62%). To separate oncocytoma from chromophobe RCC, CK7 expression yielded 100% specificity and 86% sensitivity, whereas EpCAM expression with homogeneous staining pattern gave 100% specificity and 100% sensitivity.

COMMENT

Owing to the overlapping morphologic characteristics, separation of chromophobe RCC from oncocytoma and conventional clear cell carcinoma based on conventional H&E staining is often challenging, even in the hands of experienced pathologists.^{2,4,7} Immunohistochemistry is available in most pathology laboratories as an adjunct and is technically easier to perform and interpret than Hale colloidal iron and electron microscopy. Our results show that an optimal diagnostic strategy for separation of chromophobe RCC, clear cell RCC, and oncocytoma can be achieved by using a set of immunohistochemical markers, which includes vimentin, GST- α , CD117, CK7, and EpCAM (Figure 8; Table 3). To exclude clear cell RCC, the combination of vimentin⁻/GST- α ⁻/CD117⁺ can be used. Then, to exclude oncocytoma, the combination of Hale colloidal iron⁺/CK7⁺/EpCAM⁺ can be used.

Coexpression of keratin and vimentin is a widely used profile for clear cell RCC^{4,6} in contrast to chromophobe RCC and oncocytoma, which are negative for vimentin. Bazille et al¹⁵ found that vimentin was only positive in clear cell RCC, whereas all of their 50 chromophobe RCCs and 96 oncocytomas were negative. Likewise, our study showed vimentin was the most sensitive and specific marker for conventional RCC. However, other reports showed vimentin positivity varied from 54.5% to 85% in clear cell RCC.^{4,9,15} In a large study by Pan et al⁷ of 256 clear cell RCCs, 164 (64.1%) expressed vimentin, similar to the finding of Mazal et al¹⁶ (65.7%; 67/102). Few studies have documented vimentin positivity in chromophobe RCC (21.4%; 6/28) and oncocytoma (9.7%; 3/31).^{7,16} These differences could be caused by the variance in pathologic diagnosis, use of different antibodies and reagents for the studies, and different laboratory staining procedures. Despite these variations, the diagnosis of chromophobe RCC or oncocytoma should be rendered with caution if diffuse vimentin staining is detected.

In recent years, GST- α , which functions to protect cells by catalyzing the detoxification of xenobiotics and carcinogens, was found to be of diagnostic value in renal tumors.^{11,17} GST- α overexpression is present in clear cell RCC at the transcript level by complementary DNA microarray analysis and at the protein level by immunohistochemistry. Our result extended these observations by demonstrating GST- α immunoreactivity in most cases of clear cell RCC (91%; 41/45) but in 0 of 22 chromophobe RCCs and 0 of 17 oncocytomas. A similar positive rate (82.2%; 166/

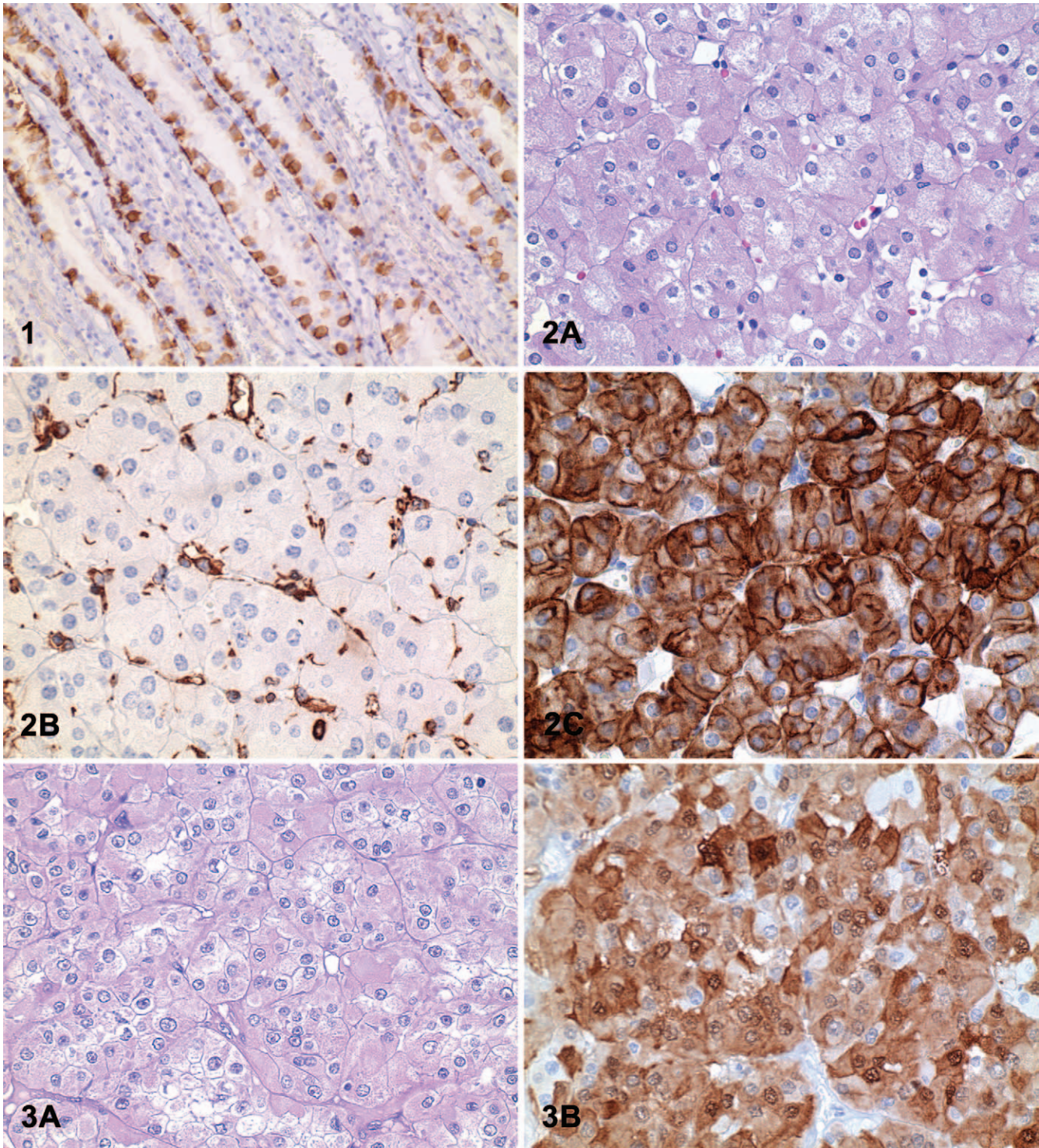


Figure 1. Normal adult renal medulla. CD117 exhibiting cytoplasmic staining and basal staining of cell membranes in an intermittent fashion in collecting ducts (immunoperoxidase, original magnification $\times 200$).

Figure 2. A, Eosinophilic variant of chromophobe renal cell carcinoma (RCC) (hematoxylin-eosin, original magnification $\times 400$). B, Eosinophilic variant of chromophobe RCC. Negative expression of vimentin in tumor cells in contrast with positive staining in vascular network (immunoperoxidase, original magnification $\times 400$). C, Eosinophilic variant of chromophobe RCC. Diffuse positive expression of cytokeratin 7 (immunoperoxidase, original magnification $\times 400$).

Figure 3. A, Eosinophilic variant of clear cell renal cell carcinoma (hematoxylin-eosin, original magnification $\times 400$). B, Eosinophilic variant of clear cell renal cell carcinoma. Positive expression of glutathione S-transferase α (immunoperoxidase, original magnification $\times 400$).

Table 2. Immunohistochemical Results of Renal Cell Tumors*

	Staining Extent	Oncocytoma, No. (%)	Chromophobe RCC, No. (%)	Clear Cell RCC, No. (%)
Vim	0	17 (100)	22 (100)	0 (0)
	1+	0 (0)	0 (0)	0 (0)
	2+	0 (0)	0 (0)	0 (0)
	3+	0 (0)	0 (0)	45 (100)
GST-α	0	17 (100)	22 (100)	4 (9)
	1+	0 (0)	0 (0)	2 (4)
	2+	0 (0)	0 (0)	7 (16)
	3+	0 (0)	0 (0)	32 (71)
CD10	0	12 (71)	12 (55)	4 (9)
	1+	1 (6)	2 (9)	3 (7)
	2+	0 (0)	3 (13)	4 (9)
	3+	4 (23)	5 (23)	34 (75)
CD117	0	0 (0)	4 (18)	45 (100)
	1+	0 (0)	0 (0)	0 (0)
	2+	2 (12)	1 (5)	0 (0)
	3+	15 (88)	17 (77)	0 (0)
CK7	0	17 (100)	3 (13)	40 (89)
	1+	0 (0)	1 (5)	1 (2)
	2+	0 (0)	1 (5)	0 (0)
	3+	0 (0)	17 (77)	4 (9)
EpCAM	0	12 (71)	0 (0)	30 (67)
	1+	2 (12)	0 (0)	5 (11)
	2+	3 (17)	0 (0)	4 (9)
	3+	0 (0)	22 (100)	6 (13)
Total		17	22	45

* RCC indicates renal cell carcinoma; Vim, vimentin; GST-α, glutathione S-transferase α; CK, cytokeratin; and EpCAM, epithelial cell adhesion molecule.

202) in clear cell RCC was observed by Chuang et al.¹⁷ However, 1 study documented immunoreactivity in 1 of 10 chromophobe RCCs.¹¹

CD117 recently has been reported as a useful diagnostic marker for renal cancer. This transmembrane growth factor receptor, encoded by the proto-oncogene *c-kit*, was widely expressed in various normal tissues and many tumors.^{18,19} Pan et al¹⁰ found that 83% (24/29) of chromophobe RCCs and 71% (5/7) of oncocytomas had membranous immunoreactivity for CD117, whereas all 256 clear cell RCCs were negative, similar to another study with 88% (22/25) positivity in chromophobe RCC, 71% (10/14) in oncocytoma, and 0% (0/29) in clear cell RCC.²⁰ Wang and Mills²¹ observed 100% immunoreactivity with CD117 in both chromophobe RCC (11/11) and oncocytoma (12/12). Our study confirmed the accuracy of CD117 and its expression in chromophobe RCC and oncocytoma, in contrast with negative staining in clear cell RCC. Of note, in tumors of other organs, the expression pattern of CD117 was primarily cytoplasmic except for a few tumors such as germ cell tumor with typical membranous staining.^{18,19} In general, only membranous reactivity was accepted as positive staining for renal cell tumors,¹⁰ although cytoplasmic staining was documented elsewhere to be positive in 2 of 13 clear cell RCCs.²² We found CD10 immunoreactivity in most clear cell RCCs, but it was of little benefit in the separation of clear cell RCC from chromophobe RCC and oncocytoma, unlike the results of Avery et al.³ Other investigators observed CD10 expression in 26% (11/42) to 32% (9/28) of chromophobe RCCs and in 25% (3/12) of oncocytomas,^{4,7,23} which is comparable to our data that shows positivity in 45% of chromophobe RCCs and in 29% of oncocytomas.

The distinction between oncocytoma and chromophobe

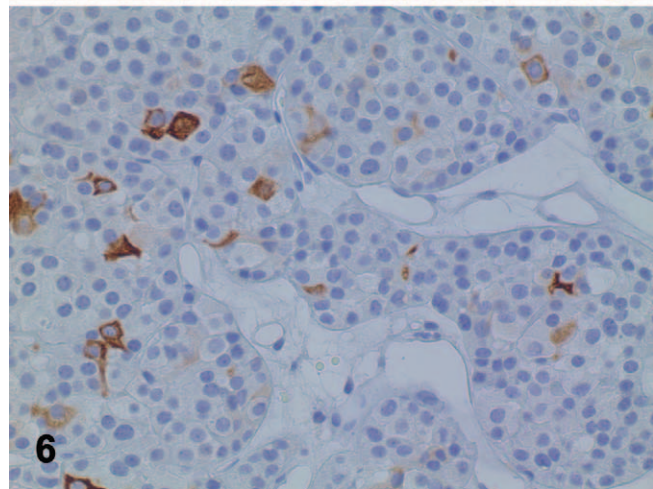
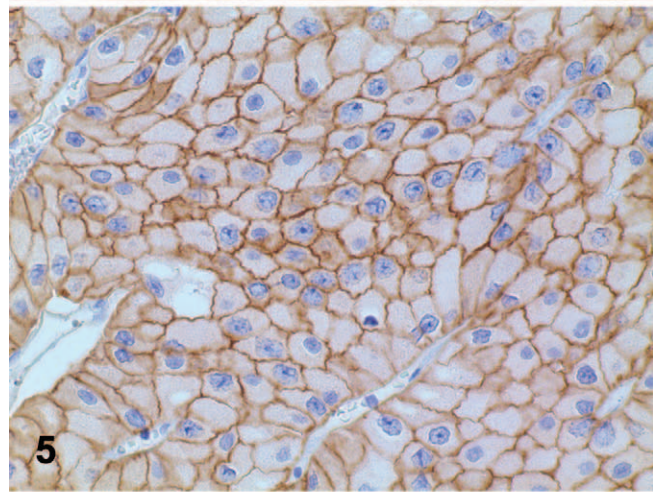
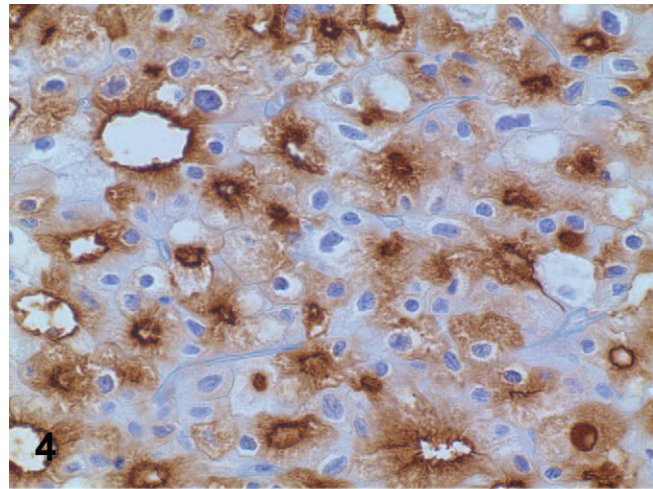


Figure 4. Chromophobe renal cell carcinoma. Cell surface immunoreactivity for CD10 (immunoperoxidase, original magnification $\times 400$).

Figure 5. Chromophobe renal cell carcinoma. Complete-membranous expression of CD117 (immunoperoxidase, original magnification $\times 400$).

Figure 6. Oncocytoma. Single scattered cytoplasmic expression of cytokeratin 7 (immunoperoxidase, original magnification $\times 400$).

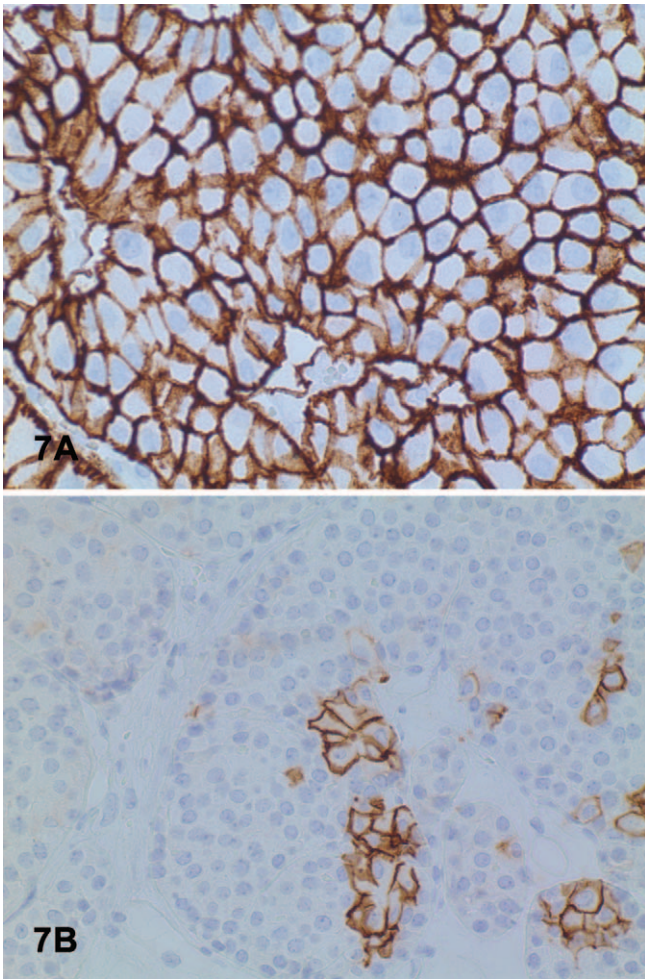


Figure 7. A, Chromophobe renal cell carcinoma. Diffuse membrane-bound staining of epithelial cell adhesion molecule (EpCAM) (immunoperoxidase, original magnification $\times 400$). B, Oncocytoma. EpCAM expression in single cells or in small cell clusters (immunoperoxidase, original magnification $\times 400$).

RCC, especially the eosinophilic variant of chromophobe RCC, is most challenging because both tumors share morphologic and immunophenotypic features. Previous studies have reported conflicting results with CK7 in making this distinction.^{5,8,24} In our study, CK7 positivity with membrane accentuation was found in 19 (86%) of 22 chromophobe RCCs, whereas all oncocytomas were negative with only scattered staining in less than 5% of tumor cells observed. These results confirm the discriminant power of CK7 for chromophobe RCC and oncocytoma, similar to the results of Leroy et al⁵ and Mathers et al.²⁴ Conversely, results from other studies argue against the diagnostic value of CK7 by demonstrating 19% (4/21) to 100% (3/3) positivity in oncocytoma,^{8,25,26} but these reports found either only focal staining without giving the percentage of positive tumor cells or only cytoplasmic staining without distinct membrane accentuation as in chromophobe RCC. In our study, 3 cases (13%) of chromophobe RCC were negative for CK7 with only single scattered staining as seen in oncocytoma, indicating the need for a more sensitive antibody for differential diagnosis between these 2 entities.

EpCAM is another marker that is potentially useful in differentiating chromophobe RCC and oncocytoma.^{12,27} EpCAM, also known as KSA, KS1/4, and 17-1 antigen, is a transmembrane cell surface epithelial protein encoded on chromosome 2p21.²⁸ EpCAM has gained interest as a potential therapeutic target because it is widely expressed on the surface of many carcinomas.²⁹ We found that EpCAM was an accurate diagnostic marker to differentiate chromophobe RCC from oncocytoma; 21 of 22 chromophobe RCCs demonstrated membranous expression in 100% of tumor cells while 1 demonstrated staining in 90% of tumor cells, whereas immunoreactive cells in oncocytoma were invariably distributed singly or in small cell clusters. Although 5 oncocytomas in our study displayed expression in 10% to 50% of tumor cells, similar to 35% and 60% of tumor cells in 2 oncocytomas reported by Went et al,¹² the distribution pattern of positive cells was restricted to small cell clusters, and the discrimination

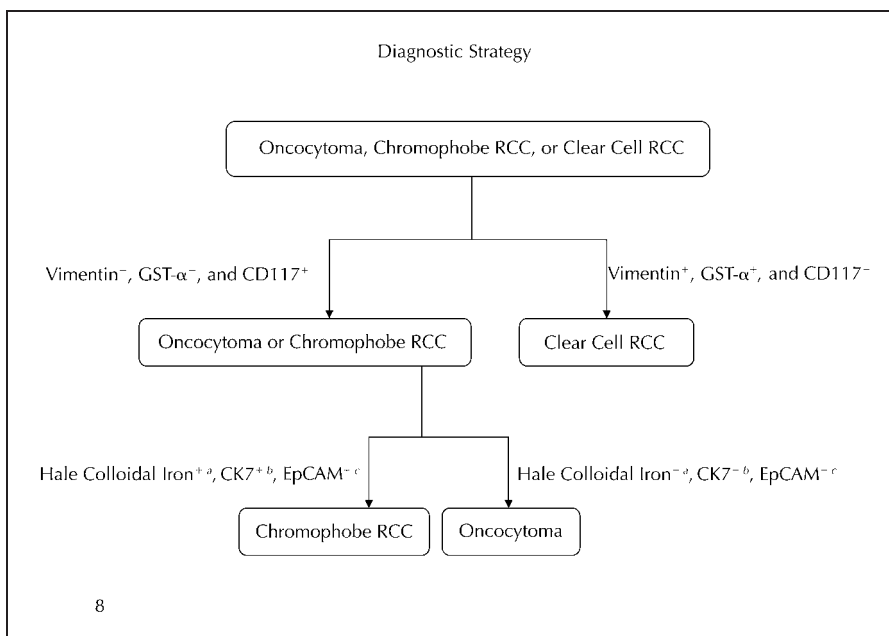


Figure 8. Diagnostic strategy for the differential diagnosis of chromophobe renal cell carcinoma (RCC). To exclude clear cell RCC, the combination of vimentin⁻/GST-α⁻/CD117⁺ can be used. To exclude oncocytoma, the combination of Hale colloidal iron⁺/CK7⁺/EpCAM⁺ can be used. ^aHale colloidal iron stain is positive in the cytoplasm of cells of chromophobe RCC but may show moderate staining of the cell membranes in oncocytoma. ^bCK7 staining is diffuse in most cases of chromophobe RCC, but focal staining is often observed in oncocytoma. ^cEpCAM staining is invariably diffuse in chromophobe RCC and focal in oncocytoma. GST-α indicates glutathione S transferase α; CK, cytokeratin; and EpCAM, epithelial cell adhesion molecule.

Table 3. Distinguishing Features of Oncocytoma, Chromophobe Renal Cell Carcinoma (RCC), and Clear Cell RCC*

Neoplasm	Oncocytoma	Chromophobe RCC	Clear Cell RCC
Distinguishing light microscopic features	Nests, tubules; edematous stroma; eosinophilic and granular cytoplasm	Sheets; large polygonal cells; prominent cell membranes; perinuclear halo and reticular cytoplasm	Compact alveoli, tubules; prominent delicate vascular network; clear cytoplasm
Distinguishing histochemical features	Hale colloidal iron stain ⁻	Hale colloidal iron stain ⁺	Hale colloidal iron stain ⁻ ; oil red O ⁺
Distinguishing ultrastructural features	Numerous mitochondria	Numerous vesicles, 150–300 nm in diameter	Lipid and glycogen
Distinguishing immunohistochemical features	Vimentin ⁻ ; GST- α ⁻ ; CD117 ⁺ ; CK7 ⁺ (focal); EpCAM ⁺ (focal)	Vimentin ⁻ ; GST- α ⁻ ; CD117 ⁺ ; CK7 ⁺ (diffuse); EpCAM ⁺ (diffuse)	Vimentin ⁺ ; GST- α ⁺ ; CD117 ⁻

* – indicates negative; +, positive; GST- α , glutathione S-transferase α ; CK, cytokeratin; and EpCAM, epithelial cell adhesion molecule.

from chromophobe RCC was invariably straightforward. EpCAM antibody yielded 100% sensitivity for chromophobe RCC in this study, although Went et al observed an absence of EpCAM in 1 chromophobe RCC (1/21; 5%) and focal positivity in another (1/21; 5%). In addition, those authors described complete absence of EpCAM expression in sarcomatoid areas of chromophobe RCC; however, we believe that, given the presence of sarcomatoid RCC, the separation of chromophobe RCC from other subtypes of RCC is not clinically important because sarcomatoid RCC of any subtype implies a poor prognosis and is treated similarly.

A novel finding in our study involved CD117 expression in normal adult renal parenchyma. We observed staining in both the cytoplasmic and basal portions of cell membranes in collecting ducts but not in the proximal tubules, and only some of the lining cells stained positively for CD117 in an intermittent fashion, which probably represent intercalated cells of the collecting ducts. Nonetheless, membrane staining was not seen in previous studies^{10,22}; furthermore, Miliaras et al²² observed cytoplasmic positivity only in proximal and distal tubules not in collecting tubules. Our findings of membranous expression for CD117 in normal collecting ducts as well as in chromophobe RCC and oncocytoma, but not in clear cell RCC, provided further evidence supporting the hypothesis that chromophobe RCC and oncocytoma are related tumors that may originate from the intercalated cells of renal collecting tubules, whereas clear cell RCC probably arises from proximal tubular epithelium.

Our study is limited by a relatively modest number of cases and the use of routine microscopic evaluation of slides. The use of machine vision may provide more precise quantitation of results. Further, the diagnosis of most of our cases was not independently confirmed by genetic studies or ultrastructural investigation. It was also noted that the number of eosinophilic variants of chromophobe carcinoma in this study was higher than observed in routine practice, probably reflecting referral bias of our consultation practice.

Treatment and prognostic implications make it imperative for pathologists to correctly diagnose chromophobe RCC. However, no single immunomarker is sufficient to definitively identify chromophobe RCC; moreover, reliance on a single antibody can be misleading. Our study provides an optimal and practical solution to this dilemma. The combination of vimentin, GST- α , and EpCAM can be used as the first-line choice, whereas a combination of CD117 and CK7 (with Hale colloidal iron) can be used as the second-line choice for the differential diagnosis of

chromophobe RCC. Because either the first-line or second-line combination yields 100% specificity, the most appropriate combination can be selected based on availability and on which combination yields the best staining results in a given laboratory.

References

1. Speicher MR, Schoell B, du Manoir S, et al. Specific loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 in chromophobe renal cell carcinomas revealed by comparative genomic hybridization. *Am J Pathol.* 1994;145:356–364.
2. Abrahams NA, MacLennan GT, Khoury JD, et al. Chromophobe renal cell carcinoma: a comparative study of histological, immunohistochemical and ultrastructural features using high throughput tissue microarray. *Histopathology.* 2004;45:593–602.
3. Avery AK, Beckstead J, Renshaw AA, Corless CL. Use of antibodies to RCC and CD10 in the differential diagnosis of renal neoplasms. *Am J Surg Pathol.* 2000;24:203–210.
4. Kim MK, Kim S. Immunohistochemical profile of common epithelial neoplasms arising in the kidney. *Appl Immunohistochem Mol Morphol.* 2002;10:332–338.
5. Leroy X, Moukassa D, Copin MC, Saint F, Mazeman E, Gosselin B. Utility of cytokeratin 7 for distinguishing chromophobe renal cell carcinoma from renal oncocytoma. *Eur Urol.* 2000;37:484–487.
6. Pitz S, Moll R, Storkel S, Thoenes W. Expression of intermediate filament proteins in subtypes of renal cell carcinomas and in renal oncocytomas: distinction of two classes of renal cell tumors. *Lab Invest.* 1987;56:642–653.
7. Pan CC, Chen PC, Ho DM. The diagnostic utility of MOC31, BerEP4, RCC marker and CD10 in the classification of renal cell carcinoma and renal oncocytoma: an immunohistochemical analysis of 328 cases. *Histopathology.* 2004;45:452–459.
8. Wu SL, Kothari P, Wheeler TM, Reese T, Connelly JH. Cytokeratins 7 and 20 immunoreactivity in chromophobe renal cell carcinomas and renal oncocytomas. *Mod Pathol.* 2002;15:712–717.
9. Young AN, Amin MB, Moreno CS, et al. Expression profiling of renal epithelial neoplasms: a method for tumor classification and discovery of diagnostic molecular markers. *Am J Pathol.* 2001;158:1639–1651.
10. Pan CC, Chen PC, Chiang H. Overexpression of KIT (CD117) in chromophobe renal cell carcinoma and renal oncocytoma. *Am J Clin Pathol.* 2004;121:878–883.
11. Takahashi M, Yang XJ, Sugimura J, et al. Molecular subclassification of kidney tumors and the discovery of new diagnostic markers. *Oncogene.* 2003;22:6810–6818.
12. Went P, Dimhofer S, Salvisberg T, et al. Expression of epithelial cell adhesion molecule (EpCAM) in renal epithelial tumors. *Am J Surg Pathol.* 2005;29:83–88.
13. Eble JN, Epstein JI, Sesterhenn IA. *Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs.* Lyon, France: IARC Press; 2004. *World Health Organization Classification of Tumours*; vol 6.
14. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol.* 1982;6:655–663.
15. Bazille C, Allory Y, Molinie V, et al. Immunohistochemical characterisation of the main histologic subtypes of epithelial renal tumours on tissue-microarrays: study of 310 cases [in French]. *Ann Pathol.* 2004;24:395–406.
16. Mazal PR, Exner M, Haitel A, et al. Expression of kidney-specific cadherin distinguishes chromophobe renal cell carcinoma from renal oncocytoma. *Hum Pathol.* 2005;36:22–28.
17. Chuang ST, Chu P, Sugimura J, et al. Overexpression of glutathione s-transferase alpha in clear cell renal cell carcinoma. *Am J Clin Pathol.* 2005;123:421–429.
18. Arber DA, Tamayo R, Weiss LM. Paraffin section detection of the c-Kit gene product (CD117) in human tissues: value in the diagnosis of mast cell disorders. *Hum Pathol.* 1998;29:498–504.
19. Miettinen M, Lasota J. Gastrointestinal stromal tumors—definition, clinical,

histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch.* 2001;438:1–12.

20. Petit A, Castillo M, Santos M, Mellado B, Alcover JB, Mallofre C. KIT expression in chromophobe renal cell carcinoma: comparative immunohistochemical analysis of KIT expression in different renal cell neoplasms. *Am J Surg Pathol.* 2004;28:676–678.

21. Wang HY, Mills SE. KIT and RCC are useful in distinguishing chromophobe renal cell carcinoma from the granular variant of clear cell renal cell carcinoma. *Am J Surg Pathol.* 2005;29:640–646.

22. Miliaras D, Karasavvidou F, Papanikolaou A, Sioutopoulou D. KIT expression in fetal, normal adult, and neoplastic renal tissues. *J Clin Pathol.* 2004;57:463–466.

23. Martignoni G, Pea M, Brunelli M, et al. CD10 is expressed in a subset of chromophobe renal cell carcinomas. *Mod Pathol.* 2004;17:1455–1463.

24. Mathers ME, Pollock AM, Marsh C, O'Donnell M. Cytokeratin 7: a useful adjunct in the diagnosis of chromophobe renal cell carcinoma. *Histopathology.* 2002;40:563–567.

25. Taki A, Nakatani Y, Misugi K, Yao M, Nagashima Y. Chromophobe renal cell carcinoma: an immunohistochemical study of 21 Japanese cases. *Mod Pathol.* 1999;12:310–317.

26. Stopyra GA, Warhol MJ, Mulhaupt HA. Cytokeratin 20 immunoreactivity in renal oncocytomas. *J Histochem Cytochem.* 2001;49:919–20.

27. Seligson DB, Pantuck AJ, Liu X, et al. Epithelial cell adhesion molecule (KSA) expression: pathobiology and its role as an independent predictor of survival in renal cell carcinoma. *Clin Cancer Res.* 2004;10:2659–2669.

28. Calabrese G, Crescenzi C, Morizio E, Palka G, Guerra E, Alberti S. Assignment of TACSTD1 (alias TROP1, M4S1) to human chromosome 2p21 and refinement of mapping of TACSTD2 (alias TROP2, M1S1) to human chromosome 1p32 by in situ hybridization. *Cytogenet Cell Genet.* 2001;92:164–165.

29. Shetye J, Christensson B, Rubio C, Rodensjo M, Biberfeld P, Mellstedt H. The tumor-associated antigens BR55-2, GA73-3 and GICA 19-9 in normal and corresponding neoplastic human tissues, especially gastrointestinal tissues. *Anti-cancer Res.* 1989;9:395–404.