Cytokeratins 7 and 20 Immunoreactivity in Chromophobe Renal Cell Carcinomas and Renal Oncocytomas

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Chromophobe renal cell carcinomas and renal oncocytomas share morphologic similarities and may present a diagnostic challenge on routine hematoxylin–eosin staining. Currently recommended additional studies of Hale's colloidal iron staining and electron microscopy are often difficult to interpret and technically challenging and may not be readily available. Previous studies have reported conflicting results with regard to the cytokeratin 7 staining pattern in chromophobe renal cell carcinomas and renal oncocytomas. Cytokeratin 20 expression in chromophobe renal cell carcinomas has not previously been studied. Formalin-fixed paraffin-embedded tissue of 11 chromophobe renal cell carcinomas and 21 renal oncocytomas were retrieved from the archived files (1984–2000) of four teaching hospitals. Of the 11 chromophobe renal cell carcinomas, eight stained positive (73%) for cytokeratin 7, one stained focally positive (9%), and two cases (18%) were completely negative. Cytokeratin 7 staining of the 21 oncocytomas revealed 4 positive (19%), 7 focally positive (33%), and 10 negative cases (48%). Cytokeratin 20 was uniformly negative on all 11 cases of chromophobe renal cell carcinomas and all 21 cases of oncocytomas. Cytokeratin 7 does not appear to show the consistent immunoreactivity in chromophobe renal cell carcinomas and renal oncocytomas, as has been previously suggested. Cytokeratin 20 immunostaining in chromophobe renal cell carcinomas and renal oncocytomas is uniformly negative. Despite the technical and interpretive challenges of Hale's colloidal iron, it is still the most useful stain in differentiating chromophobe renal cell carcinomas from renal oncocytomas.

KEY WORDS: Chromophobe renal cell carcinoma, Cytokeratin 7, Cytokeratin 20, Immunohistochemistry, Renal oncocytoma.

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Chromophobe renal cell carcinoma is a distinct subtype of renal cell carcinoma first described in humans by Thoenes in 1985 (1). Chromophobe renal cell carcinomas have characteristic morphologic features of broad trabeculae of polygonal cells with clear to granular cytoplasm, prominent cell borders, wrinkled nuclear membranes, and frequent multinucleation (2–4). Ultrastructural examination of chromophobe cells containing prominent intracytoplasmic microvesicles between 250–400 nm in diameter represents the traditional gold standard for diagnosis (5). Chromophobe renal cell carcinomas, particularly the eosinophilic variants, may resemble renal oncocytomas on routine hematoxylin-eosin stains. Accurate distinction of chromophobe renal cell carcinomas from renal oncocytomas has significant prognostic implications because aggressive behavior of chromophobe renal cell carcinomas has been reported, whereas an overwhelming majority of oncocytomas are benign and do not metastasize (6–8).

In recent years, multiple studies have explored the potential utility of special staining techniques and immunohistochemistry in differentiating renal neoplasms with eosinophilic, or "granular," cytoplasm (9–18). Early enthusiasm for Hale's colloidal iron resulted from the strong and diffusely positive reticular staining pattern reported in chromophobe

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renal cell carcinomas. However, further elucidation of the Hale's colloidal iron stain reveals variable positivity and staining patterns in oncocytomas and other renal cell carcinomas. Therefore, proper interpretation of Hale's colloidal iron requires experience in recognizing different staining patterns rather than simply identifying positivity (14). Technical difficulties, variable staining, and poor correlation among different laboratories also complicate the overall utility of Hale's colloidal iron.

Subsequently, the possible role of immunohistochemistry in distinguishing chromophobe renal cell carcinomas from renal oncocytomas has been explored. In particular, cytokeratins (CKs) 7 and 20 have generated interest because of their well-recognized utility in determining the site of origin of metastatic carcinomas of unknown primary origin (19–21). Published reports on the CK 7 immunostaining of chromophobe renal cell carcinomas have produced conflicting results. Leroy *et al.* (17) concluded that CK 7 may be useful in the differential diagnosis of chromophobe renal cell carcinomas and renal oncocytomas, whereas Taki *et al.* (16) reported that their cytokeratin profile (including CK 7) was inconsistent and therefore not useful in distinguishing the two entities. The CK 20 profile for chromophobe renal cell carcinomas has not previously been studied. A recent brief report on CK 20 staining in oncocytomas demonstrated 80% positivity with variable patterns and distributions (18).

MATERIAL AND METHODS

Formalin-fixed paraffin-embedded tissue of 11 chromophobe renal cell carcinomas and 21 renal oncocytomas were retrieved from the archived files of the following four teaching hospitals in Houston, Texas: Memorial Hermann Hospital (1984–2000), St. Luke's Episcopal Hospital (1993–2000), Methodist Hospital (1997–2000), and Lyndon B. Johnson General Hospital (1999–2000).

Consecutive paraffin sections were cut at 4 μm and placed on poly-L-lysine slides. The sections were sequentially treated with a primary monoclonal antiserum, mouse biotinylated antibody, labeling reagent (avidin-biotin complex), and a chromogenic substrate system (3,3' diaminobenzidine). All 32 cases were stained with CK 7 (DAKO Corporation, clone 12/30, 1:100 dilution), CK 20 (DAKO, clone 20.8, 1:100 dilution), and Hale's colloidal iron with slight modifications (sections were treated with 12% acetic acid before adding the colloidal iron solution; 14). Appropriate positive and negative controls were performed. Two of the authors (SLW and JHC) reviewed all slides. The percentage of reactive neoplastic cells was quantified as 0% (negative), $<$5% (focally positive), or $>$5% (positive), consistent with the values established by previous immunohistochemical studies (19–21).

Electron microscopy was performed in 10 cases (five chromophobe renal cell carcinomas and five renal oncocytomas) to establish a diagnostic gold standard in selected cases. Specimens were deparaffinized with xylene and fixed with 3% glutaraldehyde. Thin sections were taken and stained with uranyl acetate and lead nitrate, and subsequent photographs were taken on the JEOL 1200EX electron microscope.

RESULTS

Of the 11 chromophobe renal cell carcinomas, 8 stained positive (73%) for CK 7 (Fig. 1), 1 stained focally positive (9%), and 2 cases (18%) were completely negative (Fig. 2). CK 7 staining of the 21 renal oncocytomas revealed 4 positive (19%; Fig. 3), 7 focally positive (33%; Fig. 4), and 10 negative (48%) cases. In chromophobe renal cell carcinomas, positivity was diffuse throughout the cytoplasm of the tumor cells with accentuated cell membrane intensity. CK 7 positivity in renal oncocytomas exhibits similar cytoplasmic intensity without peripheral accentuation. The prominent cytoplasmic CK 7 staining of the normal glomerular and tubular structures served as appropriate internal controls.

CK 20 was uniformly negative on all 11 cases of chromophobe renal cell carcinomas (Fig. 5) and all 21 cases of renal oncocytomas (Fig. 6). All glomeruli and tubules of the adjacent uninvolved kidney also stained negative for CK 20.

Hale's colloidal iron showed cytoplasmic positivity in 10 of 11 chromophobe renal cell carcinomas (91%) and exhibited the diffuse, meshwork-like reticular pattern described by Tickoo *et al.* (15). The remaining case was completely negative (Table 1). Fourteen of the 21 renal oncocytomas stained positive or focally positive for Hale's colloidal iron (67%). The staining pattern in renal oncocytomas consisted of fine, dustlike granules in the cytoplasm or concentrated cytoplasmic staining in the luminal aspect of the tumor cells, as previously described. The remaining seven oncocytomas were negative for Hale's colloidal iron (Table 2).

Ultrastructural studies were performed on 10 random cases, including the two cases of CK 7–negative chromophobe renal cell carcinomas and the four cases of CK 7–positive renal oncocytomas. All five cases of chromophobe renal cell carcinomas revealed distinct foci of characteristic cytoplasmic microvesicles varying between 250–400 nm with occasional inner vesicles. The five cases of renal oncocytomas revealed abundant mitochondria with lamellar cristae.

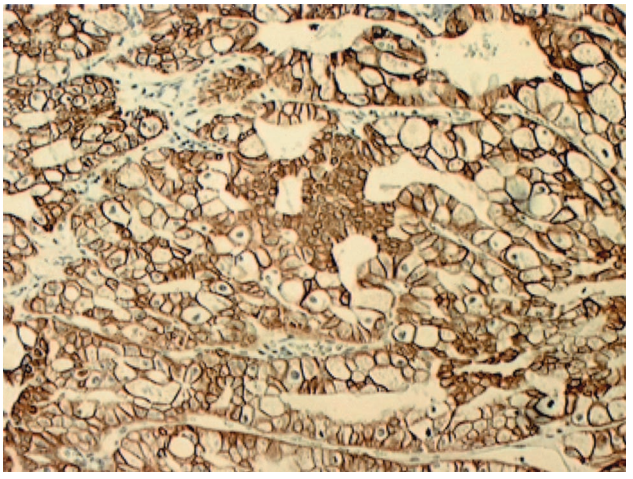

FIGURE 1. Chromophobe renal cell carcinoma (Case 9) exhibits diffuse cytokeratin 7 (CK 7) positivity with accentuated cell membrane intensity (CK 7, clone 12/30, 100×).

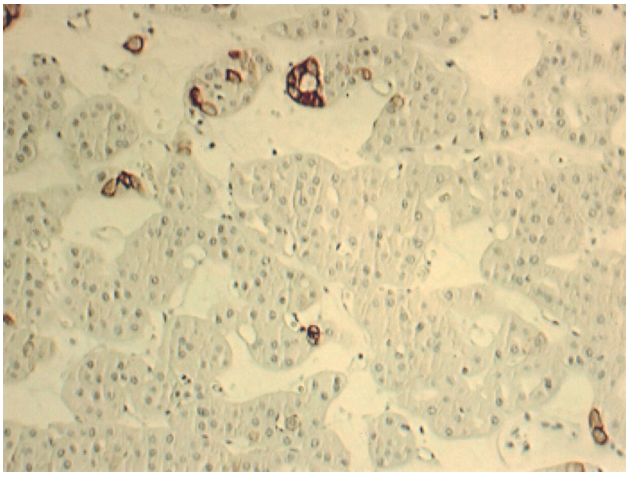

FIGURE 4. Renal oncocytoma (Case 19) exhibits focal positivity for cytokeratin 7 (CK 7, clone 12/30, 100×).

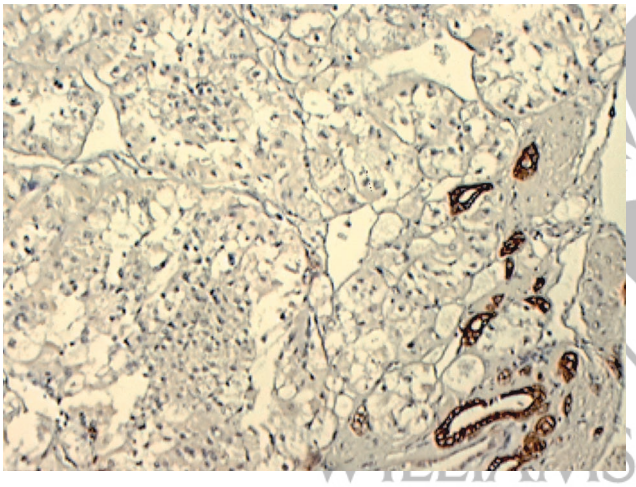

FIGURE 2. Chromophobe renal cell carcinoma (Case 1) is completely negative for cytokeratin 7, with normal tubular epithelium as internal positive control (CK 7, clone 12/30, 100×).

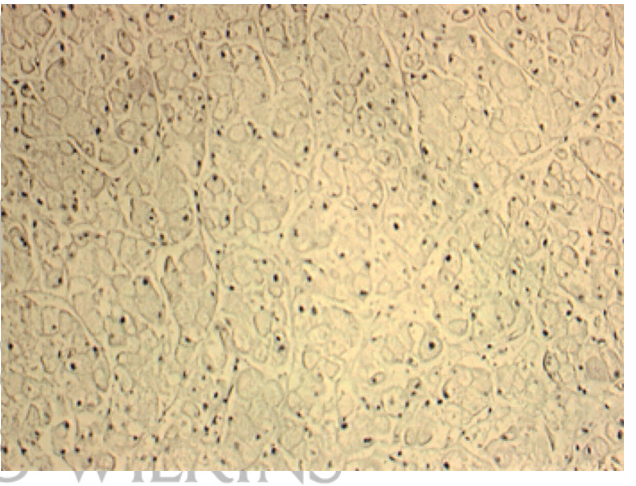

FIGURE 5. Chromophobe renal cell carcinoma (Case 5) is completely negative for cytokeratin 20 (CK 20, clone 20.8, 100×).

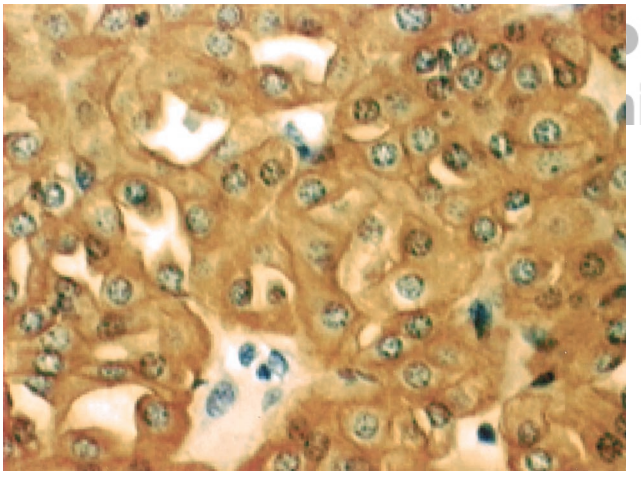

FIGURE 3. Renal oncocytoma (Case 5) shows strong cytoplasmic, granular positivity for cytokeratin 7 (CK 7, clone 12/30, 200×).

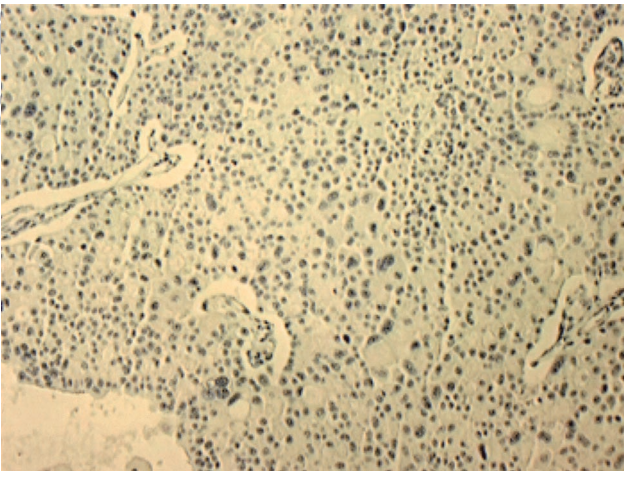

FIGURE 6. Renal oncocytoma is completely negative for cytokeratin 20 (CK 20, clone 20.8, 100×).

TABLE 1. Chromophobe Renal Cell Carcinomas

Case	Age (y)/Sex	Tumor Size (cm), Laterality	CK-7	CK-20	Hale's Colloidal Iron
1	65/F	2.3, R	−	−	+, reticular
2	39/F	9.3, R	+	−	+, reticular
3	53/F	8.7, L	+	−	+, reticular
4	36/M	5.2, R	+	−	−
5	62/F	13.0, R	+	−	+, reticular
6	81/F	16.0, R	−	−	+, reticular
7	60/M	3.0, L	Focal +	−	+, reticular
8	26/F	12.0, L	+	−	+, reticular
9	33/F	4.7, R	+	−	+, reticular
10	73/F	3.8, L	+	−	+, reticular
11	48/F	1.5, L	+	−	+, reticular

CK, cytokeratin; F, female; M, male; R, right; L, left; +, positive; −, negative.

TABLE 2. Renal Oncocytomas

Case	Age (y)/Sex	Tumor Size (cm), Laterality	CK-7	CK-20	Hale's Colloidal Iron
1	64/M	3.5, L	+	−	+, luminal
2	62/M	4.0, L	Focal +	−	Focal +, dustlike
3	67/M	9.5, R	−	−	Focal +, dustlike
4	57/M	5.3, R	−	−	−
5	39/F	3.7, L	+	−	+, luminal
6	45/M	1.6, R	+	−	Focal +, dustlike
7	71/M	4.5, L	−	−	+, dustlike
8	70/M	2.9, L	−	−	−
9	58/M	5.0, R	Focal +	−	+, dustlike
10	72/M	11.5, R	−	−	−
11	90/M	2.1, R	Focal +	−	+, luminal
12	72/M	8.0, R	Focal +	−	Focal +, dustlike
13	52/M	7.6, R	+	−	−
14	83/F	8.5, R	−	−	Focal +, dustlike
15	65/M	1.7, R	−	−	−
16	61/M	1.7, R	−	−	−
17	84/M	2.5, R	Focal +	−	+, dustlike
18	79/M	3.2, L	Focal +	−	−
19	56/M	4.2, R	Focal +	−	+, dustlike
20	80/M	0.9, L	−	−	+, dustlike
21	69/M	5.5, L	−	−	Focal +, dustlike

CK, cytokeratin; F, female; M, male; R, right; L, left; +, positive; −, negative.

DISCUSSION

Cytokeratins are a class of intermediate filaments (7 to 11 nm) which form the major structural proteins in eukaryotic cells. Polyclonal and monoclonal antibodies to cytokeratins are widely used in the differential diagnosis of numerous carcinomas of epithelial origin. Recent literature has characterized the diverse and unique expression of CKs 7 and 20 in epithelial neoplasms from various organ systems (19–23). The correlation of CK 7 and CK 20 expression is particularly helpful in distinguishing primary breast, lung, and ovarian carcinomas (CK 7 positive, CK 20 negative) from primary colon and Merkel cell carcinomas (CK 7 negative, CK 20 positive). Pancreatic carcinomas and cholangiocarcinomas may coexpress CKs 7 and 20, whereas adrenal cortical carcinomas, prostatic carcinomas, and thymomas generally do not express either CK 7 or CK 20.

CK 7 has been reported to be generally negative in conventional renal cell carcinomas with positivity ranging from 4.8% to 10.5% (16). CK 7 immunoreactivity in papillary renal cell carcinomas is positive in low-grade tumors but is generally negative in tumors with high-grade nuclei (24). In chromophobe renal cell carcinomas, however, two previous studies have reported disparate results (16, 17). Taki *et al.* (16) reported that 43% of chromophobe renal cell carcinomas (9/21 cases) were positive for CK 7, whereas Leroy *et al.* (17) reported 100% positivity (6/6 cases). Both studies reported a characteristic cytoplasmic staining for CK 7 with frequent peripheral accentuation. The two studies also reported conflicting results for CK 7 expression in renal oncocytomas; Taki *et al.* (16) reported focal CK 7 positivity in all three cases of oncocytomas, whereas Leroy *et al.* (17) reported focal positivity in only 3/11 cases. Leroy *et al.* (17) proceeded to conclude that immunohistochemical staining for CK 7 might be useful in the differential diagnosis of chromophobe renal cell carcinomas and renal oncocytomas. In our study, however, we are unable to arrive

at the same conclusion because of the following observations: (1) two cases of chromophobe renal cell carcinomas in our study are completely negative for CK 7; (2) 4 of 21 renal oncocytomas are positive for CK 7, with seven additional cases exhibiting focal positivity; and (3) electron microscopy confirms the diagnosis in the two cases of CK 7–negative chromophobe renal cell carcinomas and the four cases of CK 7–positive oncocytomas. A comparison of the materials and methods of each study reveals relative consistency; both aforementioned studies and our study used the CK 7 antibody (clone OV-TL12/30) manufactured by DAKO Corporation. Possible factors contributing to the variability of CK 7 staining reported in chromophobe renal cell carcinomas and renal oncocytomas include the relative small number of total cases, interobserver variability in interpreting positivity, inadequate sampling, and inaccurate initial diagnosis. A recent study using comparative genomic hybridization also suggested that chromophobe renal cell carcinomas and renal oncocytomas represent a morphologic and genetic continuum with a subset of cases having overlapping phenotypic features, which may also contribute to variable immunohistochemical expression (25). Furthermore, it is important to recognize inherent limitations of immunohistochemical studies on paraffin-embedded chromophobe renal cell carcinoma tissue caused by the partial destruction of microvesicles by dehydrating solvents (26).

CK 20 expression in renal neoplasms has generated limited initial interest. Early studies have indicated that CK 20 immunoreactivity in renal cell carcinomas is almost always negative (19–21). All of these studies involved conventional renal cell carcinomas, and CK 20 immunoreactivity in chromophobe renal cell carcinomas has not previously been described. We report 0 of 11 cases of chromophobe renal cell carcinomas staining positive for CK 20.

Interestingly, a recent brief report demonstrated 80% (12/15 cases) CK 20 positivity in renal oncocytomas (18). Their results appear contradictory to our findings of 0% (0/21 cases) CK 20 positivity in renal oncocytomas. Subtle differences in the manufacturers' antibodies used in the two studies (clone K5 20.8, Ventana *versus* clone 20.8, DAKO), variable selection criteria, and a variable threshold for interpreting immunoreactivity may contribute to the apparent discrepancy. Another possible explanation of the dotlike CK 20 reactivity observed by Stoprya *et al.* (18) may involve anomalous antigen expression. This pattern of aberrant expression is observed with desmin in nonmyogenic tumors and may be observed in other antibodies. Nevertheless, results from our study suggest that CK 20 is not diagnostically helpful in distinguishing chromophobe renal cell carcinomas from renal oncocytomas.

The diagnostic utility of either CK 7 or 20 in differentiating chromophobe renal cell carcinomas from renal oncocytomas remains somewhat controversial at this point in time. Further standardized studies with more cases are necessary to conclusively establish and confirm the CK 7–CK 20 profile of chromophobe renal cell carcinomas and renal oncocytomas.

Hale's colloidal iron positivity is recognized as a distinct feature of chromophobe renal cell carcinomas. Tickoo *et al.* (14) performed a comprehensive study on colloidal iron staining in renal epithelial neoplasms with emphasis on technique and staining pattern. They reported difficulty in distinguishing positivity in the traditional Hale's colloidal iron stain and recommended a slightly modified Hale's colloidal iron–staining technique (also known as modified Mowry's). We observed a similar pattern of staining with the modified Hale's colloidal iron as those described by Tickoo *et al.* (14). Intense cytoplasmic positivity in a diffuse meshwork-like pattern was observed in 10/11 cases of chromophobe renal cell carcinomas. Periluminal and cytoplasmic positivity in a dustlike, granular pattern was observed in 14 of 21 cases of renal oncocytomas. Despite possible slight improvements in the sharpness of the reticular staining with this modified Hale's technique, the overall technical challenges remain. Examples of such challenges include maintaining an optimal pH of <1.9 and requiring daily preparations of the stock colloidal iron solution to ensure freshness (14). With optimal staining and a clear understanding of characteristic staining patterns, Hale's colloidal iron may still be useful in distinguishing chromophobe renal cell carcinomas from renal oncocytomas.

A single discriminatory stain to confidently distinguish all chromophobe renal cell carcinomas from renal oncocytomas has not yet been identified. In light of recent evidence of their overlapping continuum of morphologic, histochemical, and genetic features, discovering a 100% discriminatory stain may be unrealistic.

CONCLUSION

CK 7 does not appear to show the consistent immunoreactivity in chromophobe renal cell carcinomas and renal oncocytomas as has been previously suggested. CK 20 immunostaining in chromophobe renal cell carcinomas and renal oncocytomas was uniformly negative in our study. Despite the technical and interpretive challenges of Hale's colloidal iron, it is more useful than CK 7 in differentiating chromophobe renal cell carcinomas from renal oncocytomas.

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