

Immunohistochemical and Lectin-Histochemical Patterns of Renal Non-Neoplastic and Neoplastic Epithelium: Utility of the Patterns in the Differential Diagnosis of Renal Epithelial Tumors

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Renal tumors are considered to be composed of several distinct subtypes with different cell origin. In this study, we tried to find useful immunohistochemical and lectin histochemical patterns for the differential diagnosis and the origin of the tumor relative to the components of the nephron. Typical cases of clear cell carcinoma, granular cell carcinoma, chromophobe cell carcinoma, collecting-duct carcinoma, papillary carcinoma and oncocytoma, as well as sufficient margin of normal renal parenchyma were stained immunohistochemically and lectin-histochemically, using antibodies and lectins reported to show differences in reactivity. Non-neoplastic epithelial cells showed specific reactivity according to the nephron segments. Some of our staining results tended to differ from those reported earlier by other investigators. Neoplastic lesions showed specific immunoreactivities and the staining results were similar to those of the normal nephron segment, suggesting the origin of the respective tumors. Our results suggest that testing the immunoreactivity pattern using a panel of antibodies and lectins is useful for the differential diagnosis of renal neoplastic lesions.

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Introduction

The origin of renal cell carcinoma (RCC) has still been in controversy. According to the second edition of the Fascicle of the AFIP,¹ Sudeck was the first to propose that the proximal convoluted tubules is the origin of RCC in 1893.² Since then, electron microscopic,³ immunohistochemical⁴⁻⁶ and lectin histochemical⁵⁻⁸ evidence for the origin of the RCC has been reported. In addition, new entities of tumor originating from the distal renal tubules such as oncocytoma, chromophobe cell carcinoma and collecting-duct carcinoma have been recognized. They are sometimes difficult to differentiate from the conventional RCC.

Immunohistochemical and lectin histochemical reactivity of the nephron should provide us with useful information for the differential diagnosis of renal epithelial neoplasms as well as for speculat-

ing on the cell origin of such tumors. The aim of the present study was to define the immunohistochemical and lectin histochemical patterns that are suitable for the differential diagnosis as well as for speculating on the cell origin of the tumors.

Materials and Methods

We selected typical cases from the renal neoplastic lesions filed in the Department of Pathology, Nagasaki University Hospital. The examined cases were 11 cases of RCC-clear cell carcinoma, 11 cases of RCC-granular cell carcinoma, 4 cases of RCC-chromophobe cell carcinoma, 5 cases of collecting-duct carcinoma, 4 cases of papillary RCC, and 4 cases of oncocytoma (Table 1). Each of the selected tumors contained sufficient amount of normal renal parenchyma and

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Table 1. Renal lesions examined

Diagnosis ^a	No. of cases	Male/Female	Age (years)
RCC, clear cell carcinoma	11	11/0	56.3±8.6 ^b
RCC, granular cell carcinoma	11	9/2	55.6±13.8
RCC, chromophobe cell carcinoma	4	1/3	50.8±7.5
Collecting duct ca.	5	5/0	64.8±13.3
RCC, papillary renal cell carcinoma	4	4/0	54.5±30.3
Oncocytoma	4	2/2	66.8±5.1

^aRCC=Renal cell carcinoma.

^bMean±standard deviation.

both of these areas were stained immunohistochemically and lectin-histochemically. The antibodies and lectins were selected based on the information available in the literature.⁵⁻⁸ The antibodies used were epithelial membrane antigen (EMA), AE1/AE3, cytokeratin (CK) 7, CK 19, Tamm-horsfall protein (THP), carcinoembryonic antigen (CEA) and vimentin. The lectins used were *Ulex Europaeus* agglutinin-1 (UEA-1), *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), *Lotus tetragonolobus* agglutinin (LTA) and peanut agglutinin (PNA) (Table 2).

Table 2. List of the antibodies and lectins used in the present study

	Name	Dilution	Name, place and country of the maker
Antibody	EMA	1: 100	DAKO, Glostrup, Denmark
	AE1/AE3	1: 100	DAKO, Glostrup, Denmark
	CK7	1: 50	DAKO, Glostrup, Denmark
	CK19	1: 50	DAKO, Glostrup, Denmark
	CEA	1: 400	TAKARA, Ohtsu, Shiga, Japan
	Vimentin	1: 200	DAKO, Glostrup, Denmark
	THP	1: 150	CEDARLANE Laboratories, Hornby, Ontario, Canada
Lectin	UEA-1	1: 20	SEIKAGAKU Corporation, Tokyo, Japan
	LTA	1: 20	HONEN, Tokyo, Japan
	PNA	1: 100	HONEN, Tokyo, Japan
	SBA	1: 50	EY Laboratories, San Mateo, CA, USA
	DBA	1: 20	EY Laboratories, San Mateo, CA, USA

EMA=Epithelial membrane antigen; UEA-1=*Ulex Europaeus* agglutinin I; CK7=Cytokeratin 7; CK19=Cytokeratin 19; CEA=carcinoembryonic antigen; THP=Tamm-horsfall protein; LTA=*Lotus tetragonolobus* agglutinin; PNA=Peanut agglutinin; SBA=Soybean agglutinin; DBA=*Dolichos biflorus* agglutinin.

Non-neoplastic renal parenchyma was divided into the following 7 parts: (1) epithelial cells covering the inner side of Bowman's capsule; (2) proximal convoluted tubules (ProxCT) located near the glomerulus with a brush border in the luminal side; (3) small caliber Henle's loop located in the corticomedullary interface; (4) distal convoluted tubules (DistCT) located near the renal cortex and composed of more flat epithelial cells than ProxCT and inconspicuous brush border; (5) collecting ducts with distinct cell

membrane and perinuclear cytoplasmic halo located in the medulla; (6) epithelial cells located at the transitional area from the collecting duct to the pelvic surface; and (7) epithelial cells of the renal calices (Figure 1).

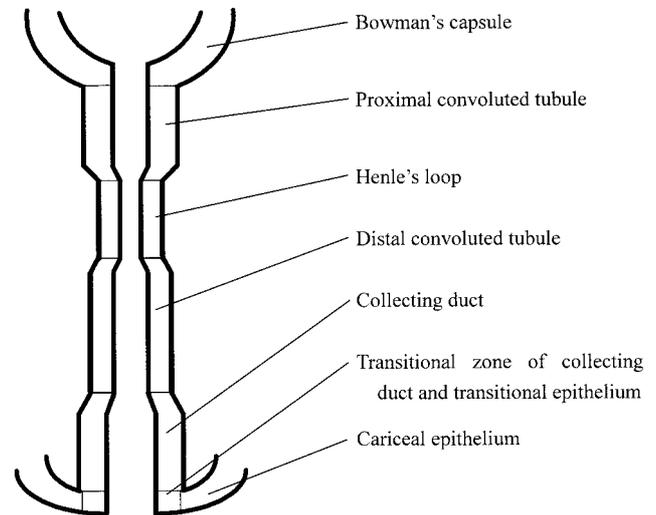


Figure 1. Scheme of the 7 segments of the epithelium in the nephron. (Modified Figure 5 of Rumpelt et al.⁶). Immunohistochemical patterns were evaluated based on these 7 parts.

We classified the tumors on the basis of *Histological Typing of Renal Tumors of the World Health Organization, 2nd ed.*,⁹ and compared their reactivity with previous reports.⁵⁻⁸ The reactivity of tumors was schematically visualized by the frequency of cases showing positive reaction.

Results

Non-neoplastic renal epithelium

The results are summarized in Figure 2. Bowman's capsule showed positive reaction to keratin and vimentin. Proximal convoluted tubules only reacted to vimentin and LTA. The Henle's loop and the distal convoluted tubule showed similar reactivity, that is, positive to EMA, keratin, DBA, SBA, PNA and THP. Especially, the reaction to THP was strong in these regions. The collecting ducts showed specific reactivity to UEA-1 as well as EMA, keratin, vimentin, DBA, SBA and PNA (Figure 3). LTA was also positive in the medullary collecting duct. The reaction profile of the transitional area between the collecting ducts and pelvic transitional epithelium was similar to that of the collecting duct. The transitional epithelium of the pelvis was positive to EMA, keratin and DBA. Comparison of our results with previous reports⁵⁻⁸ indicates that the reactivity in nephron area differed between the present and previous studies and that it also varied among previous studies (Figure 2).

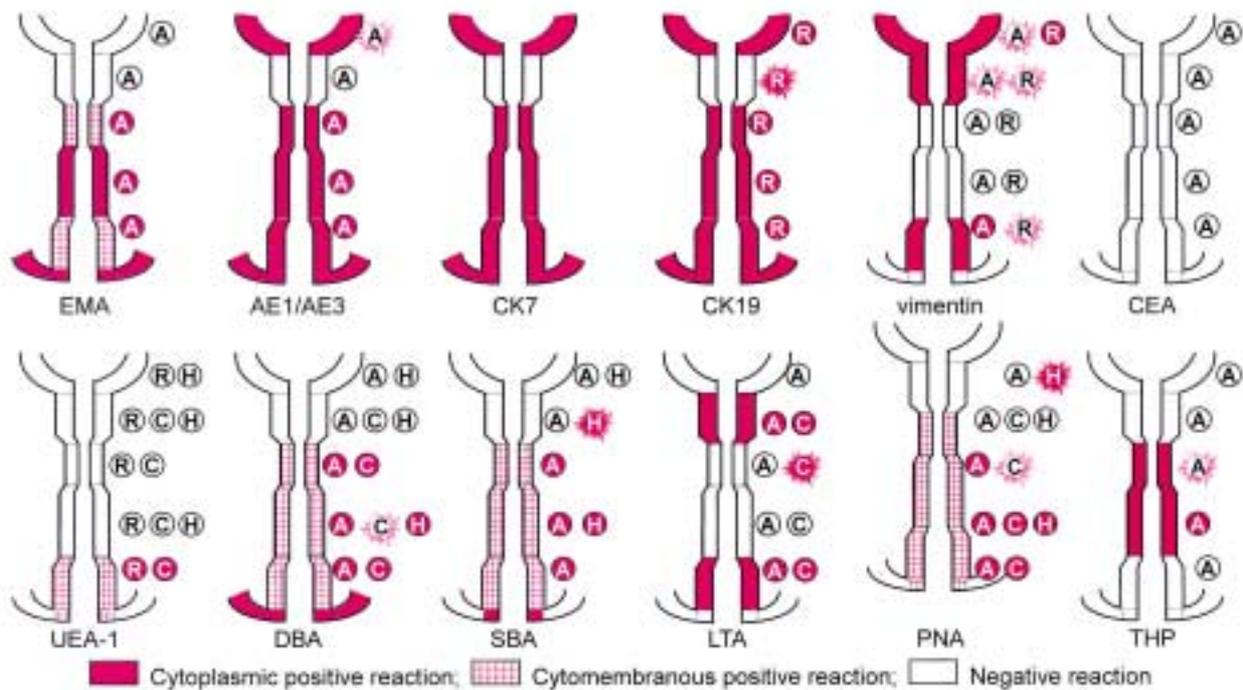


Figure 2. Immunohistochemical and lectin-histochemical reactivity of the normal nephron. Comparison with previous reports: our data and the reported data were both positive; our data and the reported data were both negative; our data were negative, while reported data were positive; our data were positive, while reported data were negative; No mark indicates that data were not available in the previous reports. Capital letter refers to the initial of the first author's name; C=Chan⁸; H=Holthofer et al.⁷; A=Aizawa et al.⁵; R=Rumpelt et al.⁶

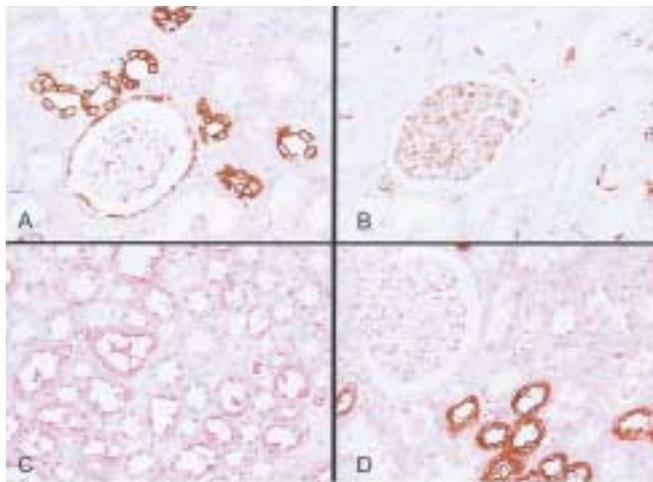


Figure 3. Typical staining patterns of the nephron. **A.** AE1/AE3. Epithelium of Bowman's capsule and distal convoluted tubules were positive. **B.** Vimentin. Epithelium of Bowman's capsule was positive. Some cells in the proximal convoluted tubules were also positive. **C.** LTA was positive mainly in the proximal convoluted tubules. **D.** Tamm Hallsfol protein was specifically positive in the distal convoluted tubules ($\times 100$). (Immunohistochemical sections were weakly stained with eosin for recognizing morphology better.)

Neoplastic lesions

(1) Clear cell carcinoma

EMA was negative or positive in the cell surface. Half of the cases

showed cell surface reaction to vimentin and the others showed cytoplasmic positivity. The reactivity to keratins was in general negative or weakly positive. There were some cases with positive reaction to AE1/AE3 and CK19, while few cases showed positive reaction to CK7. More than half of the cases showed positive reaction to LTA. These results were similar to those of ProxCT. Some cases were reactive to UEA-1, to which collecting ducts in the non-neoplastic renal tubules showed specific reaction. More than half of the cases showed cytomembranous weak reaction to PNA that was positive in Henle's loop, DistCT and collecting ducts (Figure 4).

(2) Granular cell carcinoma

This subtype showed similar reactivity to clear cell type with different cytoplasmic reactivity to EMA and positive reactivity to CK7 in some cases (Figure 5).

(3) Chromophobe cell carcinoma

EMA was strongly positive in the cytoplasm. Staining was positive with CK7, DBA and SBA, while it was negative with AE1/AE3, CK19 and vimentin. These results were similar to those of the DistCT except for the AE1/AE3 and CK19 reactivity. LTA was weakly positive in half of the cases (Figure 6).

(4) Papillary renal cell carcinoma

Cytoplasmic reaction was observed in 4 (100%) cases with vimentin, 3 (75%) with CK7, and 2 (50%) with AE1/AE3, CK19 and LTA.

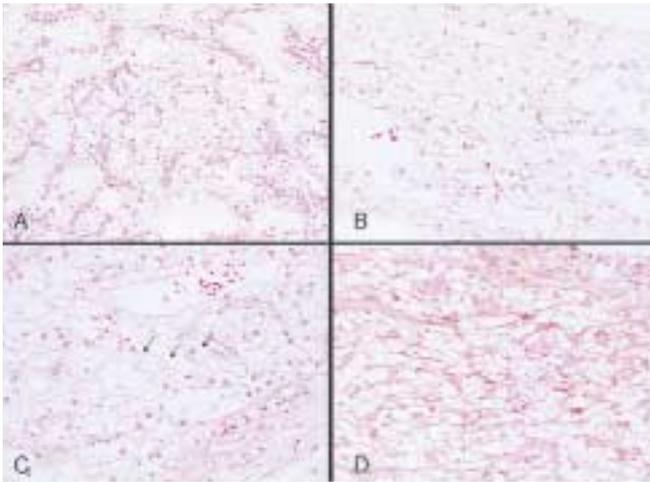


Figure 4. Clear cell carcinoma. **A.** Tumor cells with clear cytoplasm proliferated in alveolar and solid patterns (HE, $\times 50$). **B.** EMA showed cytomembranous positive reaction ($\times 100$). **C.** LTA was weakly positive in tumor cells (arrows) ($\times 100$). **D.** Vimentin was evidently positive ($\times 100$). (Immunohistochemical sections were weakly stained with eosin for recognizing morphology better.)

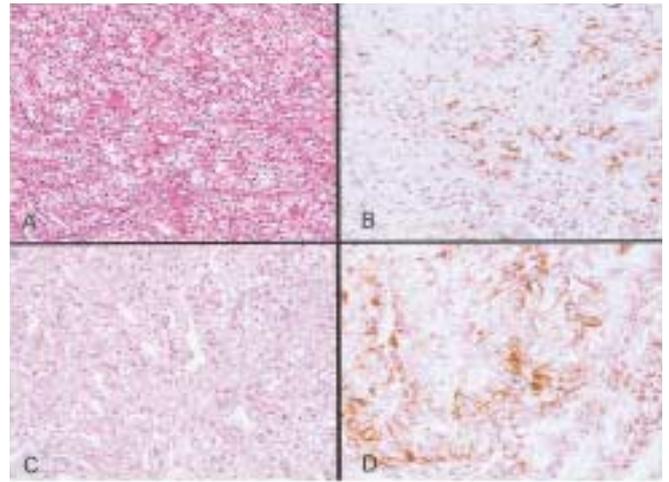


Figure 5. Granular cell carcinoma. **A.** Tumor cells with granular cytoplasm (HE, $\times 50$). **B.** EMA showed both membranous and cytoplasmic reaction ($\times 100$). **C.** LTA was positive ($\times 100$). **D.** Vimentin was evidently positive ($\times 100$). (Immunohistochemical sections were weakly stained with eosin for recognizing morphology better.)

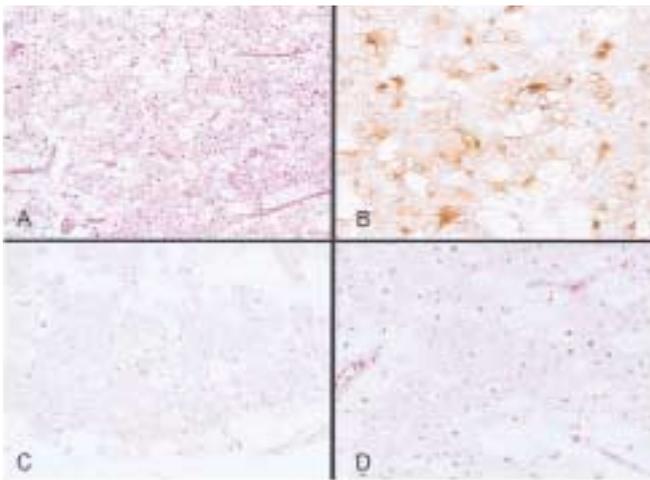


Figure 6. Chromophobe carcinoma. **A.** Tumor cells possessed plant-like cytoplasmic membrane (HE, $\times 50$). **B.** EMA showed strong reactivity ($\times 100$). **C.** Note the positive reactivity to CK7 ($\times 100$). **D.** Vimentin was negative ($\times 100$). (Immunohistochemical sections were weakly stained with eosin for recognizing morphology better.)

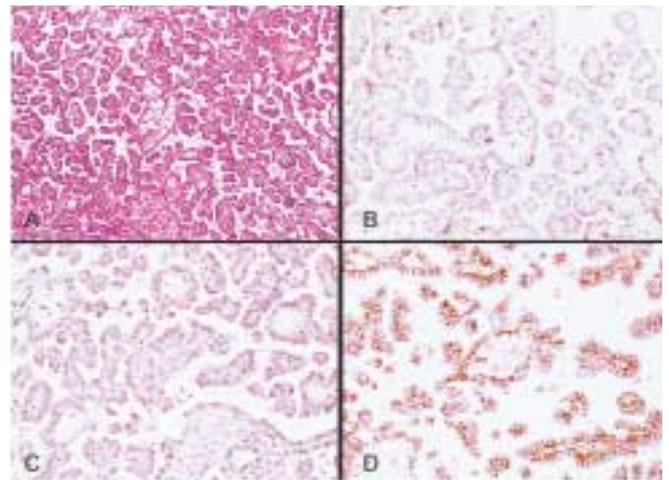


Figure 7. Papillary renal cell carcinoma. **A.** Tumor cells showed papillary growth with foam cell infiltration (HE, $\times 50$). **B.** EMA showed cytomembranous positive reaction ($\times 100$). **C.** LTA was weakly positive in tumor cells ($\times 100$). **D.** Vimentin was positive ($\times 100$). (Immunohistochemical sections were weakly stained with eosin for recognizing morphology better.)

Cytomembranous reaction was observed in 2 (50%) cases with EMA and 3 (75%) with DBA. Papillary renal cell carcinoma (RCC) showed similarity with conventional RCC in reactivity (Figure 7).

(5) Collecting-duct carcinoma

EMA was positive in the cytoplasm in two cases, positive in the cytomembrane in two cases and negative in one case. Differing from conventional RCC, AE1/AE3, CK7 and CK19 were positive in many cases, while LTA and PNA were negative. These results were similar to those of the collecting ducts. However, UEA-1, specific to the collecting ducts in normal nephron, was positive only in one

case. DBA and SBA were weakly positive in four cases (Figure 8).

(6) Oncocytoma

EMA was strongly positive in the cytoplasm, and DBA and SBA were also positive. Keratins and vimentin were all negative. The reactivity to EMA, DBA, SBA and vimentin was more similar to that of the DistCT than that of collecting ducts. Although oncocytoma and chromophobe cell carcinoma were similar in the reactivity to many antibodies and lectins, oncocytoma was negative to CK7, which was positive in chromophobe cell carcinoma (Figure 9).

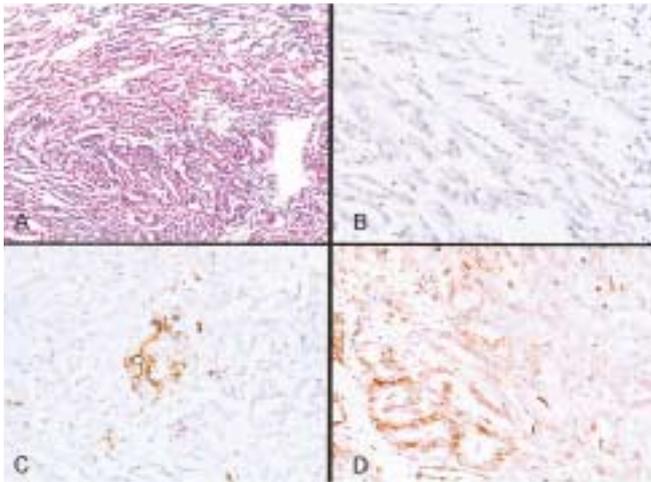


Figure 8. Collecting-duct carcinoma. **A.** Tumor cells showed maze-like slit of lumina (HE, $\times 50$). **B.** AE1/AE3 showed weakly positive reactivity ($\times 100$). **C.** Cytokeratin 7 was reactive ($\times 100$). **D.** Vimentin was strongly and diffusely positive ($\times 100$). (Immunohistochemical sections were weakly stained with eosin for recognizing morphology better.)

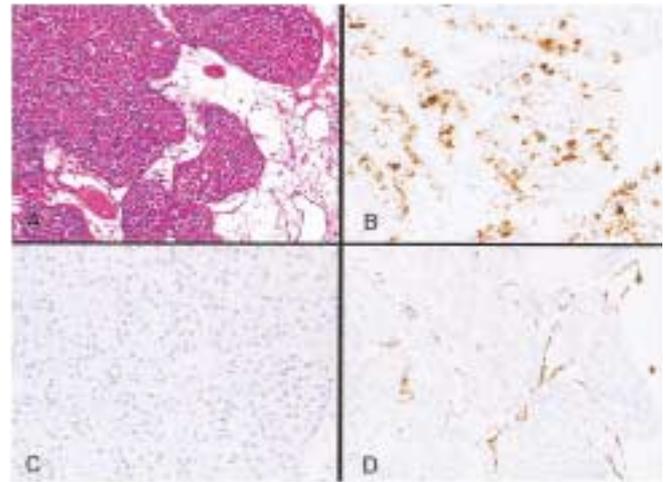


Figure 9. Oncocytoma. **A.** Small and regular tumor cells with eosinophilic granular cytoplasm proliferated in solid nests (HE, $\times 50$). **B.** Strong reactivity to EMA ($\times 100$). **C.** Cytokeratin 7 was faint and was evaluated as negative ($\times 100$). **D.** Vimentin was also negative ($\times 100$).

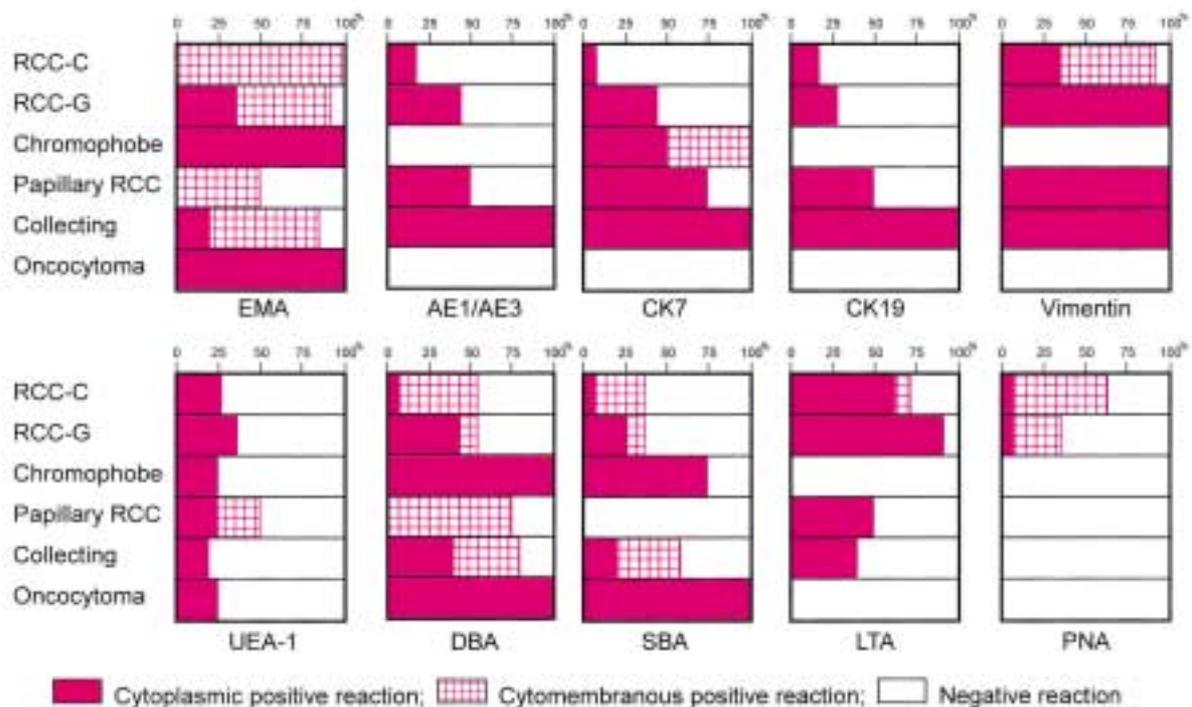


Figure 10. Summary of immunohistochemical and lectin-histochemical reactivity of renal epithelial tumors. RCC-C=RCC clear cell carcinoma; RCC-G=RCC-granular cell carcinoma; Chromophobe=RCC-chromophobe cell carcinoma; Collecting=Collecting-duct carcinoma.

The results of (1) through (6) are summarized and visualized in Figure 10.

Discussion

Our examination revealed that LTA, THP and UEA-1 reacted

specifically in ProxCT, DistCT and collecting ducts, respectively, and that EMA, keratin, DBA, SBA and PNA reacted in the lower nephron. Interestingly, reactivity of LTA and vimentin was identified in ProxCT and collecting ducts.

With regard to the neoplastic lesions, the immunoreactivity in the tumors was similar to that in the corresponding original area of the nephron: the immunoreactivity of conventional RCC was similar to

that of the ProxCT; that of collecting-duct carcinoma was similar to that of collecting duct; and that of chromophobe cell carcinoma and oncocytoma was similar to that of DistCT. In addition, there were specific staining patterns which differed among the histopathological types of tumor.

In routine pathology work, immunohistochemical and lectin reaction will be helpful in the differential diagnosis in the following cases: (1) metastasis from renal cell carcinoma vs. other original adenocarcinoma; (2) chromophobe renal cell carcinoma vs. clear cell RCC; (3) oncocytoma vs. chromophobe renal cell carcinoma; (4) granular RCC vs. chromophobe RCC or oncocytoma; (5) papillary RCC vs. collecting-duct carcinoma; and (6) collecting-duct carcinoma vs. conventional RCC.

Reactivity to vimentin and EMA will be helpful in determining the tumor origin from the kidney or other locations. Conventional RCC, papillary RCC and collecting-duct carcinoma are known to show positive reaction to vimentin that does not appear in other adenocarcinoma free of sarcomatous change. EMA will show cytomembranous reaction to clear cell RCC and some cases of papillary RCC, cytoplasmic or cytomembranous reaction to granular RCC, and cytomembranous reaction to collecting-duct carcinoma.

Clear cell RCC will be positive to both vimentin and LTA, while they will be negative in chromophobe RCC. In addition, EMA will show cytomembranous reaction in clear cell RCC and cytoplasmic reaction in chromophobe RCC. Oncocytoma and chromophobe RCC will show a similar immunoreactive pattern except for the reactivity to CK7, which will be negative and positive in oncocytoma and chromophobe RCC, respectively. Granular RCC, but not chromophobe RCC or oncocytoma, will be positive to vimentin.

The pattern of immunoreactivity in papillary RCC was similar to that shown by both conventional RCC and collecting-duct carcinoma. This suggests that the papillary pattern will be observed both in conventional RCC and collecting-duct carcinoma. Collecting-duct carcinoma and conventional RCC showed a similar pattern in immunoreactivity except for that to LTA which was negative in collecting-duct carcinoma. Since a larger proportion of collecting-duct carcinomas was positive to keratins, the tumor cell showing strong immunoreactivity to epithelial markers such as EMA, AE1/AE3, CK7 and CK19, is highly likely to be collecting-duct carcinoma.

Therefore, making a diagnosis does not necessarily require to carry out all of the above staining procedures as we did in the present study. Pathologists should select several markers for the specific

purpose of the differential diagnosis as mentioned. These selected panels will be helpful in the differential diagnosis (Figure 10).

Our study showed that immunoreactivity of the epithelial cells in the nephron varies among institutions. Our results differed from those of the previous studies.⁵⁻⁸ The difference in the recognition of areas of the nephron, e.g. Henle's loop and collecting ducts, among the present and the previous studies may have resulted in the discrepancy of the results. However, we also noted differences in immunoreactivity of the cells of the Bowman's capsule that cannot be mistaken at all. We speculate that the variation in reactivity among studies may be due to the difference in laboratory work including tissue handling, fixation condition, staining protocol, reagents and antibodies. Furthermore, in the neoplastic lesions, a difference in reactivity was observed even in the same case, although we selected only typical cases.

Nevertheless, tumors of the same histopathological type tended to show similar staining patterns and we are confident that the immunohistochemical and lectin-histochemical staining panel could provide a better and easier differential diagnosis of renal neoplastic lesions. We recommend testing the immunoreactivity pattern using a panel of antibodies and lectins in each laboratory for the differential diagnosis of renal neoplastic lesions.

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