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Keratin immunohistochemistry in renal cell carcinoma subtypes and renal oncocytomas: a systematic analysis of 233 tumors

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Abstract Keratin immunohistochemistry represents a widely applied differential diagnostic tool in surgical pathology. To investigate the value of keratin subtyping for the diagnosis among histological subtypes of renal cell carcinoma and oncocytomas, we performed a detailed immunohistochemical study, applying 22 different monoclonal keratin antibodies on a large series of 233 renal tumors [125 conventional, 22 chromophobe, and 20 papillary (12 type-1, 8 type-2 tumors) cancers and 66 oncocytomas] using a tissue microarray technique. Immunoreactivity for keratin 7, 8, 18, and 19 was present in all tumor entities, albeit in varying quantities. With antibodies directed against keratins 8 and 18, oncocytomas showed a distinct perinuclear and punctate dot-like pattern, which was not observed in renal cancer specimens. The only tumors showing immunoreactivity for keratin 20 were two type-2 papillary cancers. All other monospecific keratin antibodies yielded consistently negative results. Overall, in contrast to some recent publications, keratin subtyping generally appeared to be of additional value only for the differentiation of renal epithelial tumors. Hence, with respect to differential diagnostic value, Hale's colloidal iron stain and vimentin immunostaining are still the most useful tools in renal tumor pathology.

Keywords Renal cell carcinoma · Oncocytoma · Histological subtype · Keratin · Differential diagnosis

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Introduction

Renal cell carcinomas (RCCs) account for approximately 2% of annual new cancer cases worldwide, with men having a higher risk than women (male to female ratio = 1.5:1). As for the majority of cancers, tumor stage at presentation and histological tumor grade are the principal prognostic factors [20]. Prognosis is also related to histological subtypes, since patients with conventional RCCs have a poorer cancer-specific survival than patients with papillary or chromophobe tumors [1, 5, 18, 22]. Differential diagnosis among histological RCC subtypes, however, can be difficult in standard hematoxylin and eosin (H&E)-stained sections, especially in poorly differentiated cancers. In these cases, diagnosis is primarily based on the absence of vimentin immunostaining of the chromophobe subtype and its reticular cytoplasmic positivity with Hale's colloidal iron stain [15, 25, 34].

The first comprehensive description of the histopathological features of renal oncocytoma (RO) was presented by Klein and Valensi in 1976 [13]. Today, ROs account for approximately 3–7% of renal cell neoplasms in surgical series [15, 16, 29, 34]. Although a single well-documented case of metastasis to the liver has been reported [27], ROs generally are benign [1, 29]. Therefore, they have to be separated from renal cancer. However, differential diagnosis can, again, be difficult in standard H&E-stained sections, as has just recently been stressed in the review by Perez-Ordonez et al. [27].

In the last few years, keratin typing has been repeatedly applied to facilitate differential diagnosis among RCC histological subtypes [4, 10, 12, 17, 19, 28, 35] and between RCCs and ROs [6, 12, 17, 19, 28, 35, 38]. However, the results reported are, at least as far as the keratin expression profile of ROs is concerned, still limited and partly contradictory. For example, keratin 7 immunoreactivity has been detected in 8–100% [12, 17, 19, 33, 38] and keratin 20 in 0–80% of ROs [12, 33, 38]. Hence, we decided to apply a high throughput tissue microarray technique using 22 different mouse monoclonal antibodies to evaluate the keratin expression profiles

of RCC histological subtypes and ROs in a large series of patients to identify a potential basis for diagnosis and differential diagnosis of these tumor entities.

Materials and methods

Formalin-fixed and paraffin-embedded specimens of 167 RCCs from 167 patients (99 males, 68 females; ratio 1.5:1) and 66 ROs from 63 patients (34 females, 29 males; ratio 1.2:1) operated on between November 1988 and August 2002 were chosen for further analysis. Surgical treatment included either radical nephrectomy (RCC larger than 4 cm) or partial nephrectomy (peripheral tumors smaller than 4 cm and presumed oncocytomas). In the RCC group, mean and median age of patients at operation was 62.3 years and 63.1 years (range 28–85 years), in the RO group, mean and median age was 66.3 years and 69.0 years (range 11–88 years), respectively. All original H&E sections were independently reevaluated by two pathologists (C.L. and M.R.), and discrepancies were resolved by simultaneous reexamination of the slides by both investigators using a double-headed microscope. In selected cases, additional vimentin immunostaining and Hale's colloidal iron stain were applied. In four cases, the original diagnoses of RCC had to be revised and changed into either RO (two cases) or transitional cell carcinoma of the renal pelvis (two cases). Cancer specimens were classified according to the consensus classification of renal cell neoplasia [15, 34]: 125 conventional, 22 chromophobe and 20 papillary (including 12 type-1 and 8 type-2 tumors) RCCs. RO specimens were classified according to the World Health Organization criteria [25] and previously described light microscopic features [16, 27]. After a mean follow-up of 2.2 years, progressive disease was observed in 34 of 167 (20.3%) RCC patients, including 16 patients who died from cancer and 18 patients who currently are alive with metastatic disease. Three patients had died from causes unrelated to RCC. In the RO group, after a mean follow-up of 6.8 years, 57 of 63 patients (90.5%) were alive with no evidence of tumor, while the remaining 6 patients had died of other causes without evidence of tumor recurrence or metastatic disease. All procedures were in accord with the ethical standards established by our institution and approved by the ethics committee.

For immunohistochemical evaluation, a tissue microarray technique was used, which allows staining of a large number of specimens on one slide. Tissue microarrays (TMAs) were prepared using a manual tissue-arraying instrument (Beecher, Silver Spring, MD, USA). The details of this technique have been described previously [14]. At least three cylindrical core biopsies, 0.6 mm in diameter, were taken from different areas of each tumor and arrayed in a recipient paraffin TMA block. Sections from tissue array blocks 4-μm thick were mounted on Superfrost slides for immunohistochemical analysis using automated immunostainers (Dako-Autostainer, Universal Staining System, Dako, Glostrup, Denmark; for pancytokeratin MNF116 VENTANA ES, Ventana, Strasbourg, France). Briefly, TMA sections were deparaffinized, rehydrated in graded alcohols and treated for 5 min with 1% H_2O_2. Thereafter, sections were subjected to antigen retrieval with either microwave treatment (30 min 160 W in 0.01 M sodium citrate buffer pH 7.3), protease digestion (10 min room temperature 0.1% protease type XXIV, Sigma-Aldrich, Steinheim, Germany) or Epitope Retrieval Solution (Dako, code no. K 5205, 40 min 98°C) and subsequently incubated for 30 min with 22 different mouse monoclonal anti-human keratin antibodies. For antibody specificity, dilution, source and positive controls used, see Table 1. Binding of the primary antibody was usually assessed by the Dako LSAB2 System HRP (AEC) Detection kit, for pancytokeratin MNF116, the Ventana Basic DAB Detection Kit was used and for the keratin 14 antibody, both the Dako LSAB2 System HRP (AEC) Detection kit and the Dako EnVision+System (Peroxidase, DAB) were used. Negative controls were performed by substitution of the primary antibody by the Dako ChemMate antibody diluent code no. S 2022. Staining results were assessed in a semi-quantitative fashion independently by two pathologists (C.L. and M.R.). Discrepancies were resolved by simultaneous reexamination of the slides by both investigators using a double-headed microscope. Keratin immunoreactivity was documented in categories as follows: no reactivity; "weak," <10% of cancer cells positive; "moderate," 10–50% of cancer cells positive; "strong," >50% of cancer cells positive. Finally, with the help of the tissue microarray technique, it was ensured that chromophobe RCCs were, in fact, negative for vimentin and positive with Hale's colloidal iron stain, whereas

Table 1 List of antibodies used. *MW* microwave treatment 30 min 160 W in 0.01 M sodium citrate buffer pH 7.3, *P* protease digestion 10 min at room temperature (0.1% protease type XXIV, Sigma-Aldrich, Steinheim, Germany), *ER* epitope retrieval solution (Dako, Glostrup, Denmark, Code No. K 5205) 40 min at 98°C

Antibody specificity	Dilution, antigen retrieval	Positive control	Source
K 1 (Clone 34βB4)	1:10, MW	Skin	Novocastra, Newcastle upon Tyne, UK
K 4 (Clone 6B10)	1:5, MW	Cervix	Monosan, Uden, The Netherlands
K 5/6 (Clone D5/16 B4)	1:50, MW	Breast	Dako, Glostrup, Denmark
K 6 (Clone Ks6.KA12)	Ready to use, MW	Cervix	Progen, Heidelberg, Germany
K 7 (Clone OV-TL 12/30)	1:100, P	Breast	Dako
K 8 (Clone RCK 102)	1:10, P	Breast	ICN, Aurora, USA
K 8/18, LMW (Clone 5D3)	1:50, P	Skin	Novocastra
K 9 (Clones Ks 9.70 and Ks 9.216)	1:20, MW	Palmoplantar Skin	Progen
K 10 (Clone DE-K 10)	1:100 (no retrieval)	Skin	Dako
K 13 (Clone DE-K 13)	1:50, MW	Cervix	Dako
K 14 (Clone LL002)	1:50, MW	Skin	Novocastra
K 15 (Clone LHK 15)	1:50, MW	Skin	NeoMarkers, Fremont, CA, USA
K 16 (Clone LL025)	1:10, MW	Cervix	Novocastra
K 17 (Clone E3)	1:20, MW	Breast	Dako
K 18 (Clone DC 10)	1:10, MW	Breast	Dako
K 19 (Clone RCK 108)	1:100, P	Breast	Dako
K 20 (Clone K_S 20.8)	1:100, P	Colon	Dako
HMW (Clone 34βE12, K 1, 5, 10, 14)	1:50, ER	Prostate	Dako
KL1 (Pan-Keratin)	1:50, ER	Skin	Immunotech, Marseille, France
MNF116 (Pan-Keratin)	1:75, P	Skin	Dako
AE1/AE3 (Pan-Keratin)	1:50, P	Skin	Dako
Lu5 (Pan-Keratin)	1:50, P	Skin	Biocarta, Hamburg, Germany

ROs were negative for vimentin and negative with Hale's colloidal iron stain.

Results

Tumor tissue sufficient for a reliable evaluation of all markers was present in all 167 RCCs and 66 ROs, respectively. The keratin expression profiles of RCC histological subtypes and ROs are summarized in Table 2.

Regarding RCCs, expression of keratins 7, 8, 18, 19, low molecular weight keratin (LMW, 8/18), and the four pankeratin antibodies was noted in all histological subtypes, albeit in varying quantities. For example, keratin 7 was more common in chromophobe (72.7%) and papillary (80.0%) subtypes, but was also found in conventional (13.6%) tumors (Fig. 1a). Regarding only strong keratin 7 expression, the difference was more pronounced, but still 4 of 125 (3.2%) conventional tumors showed keratin 7 immunostaining of more than 50% of cancer cells. With respect to the different types of papillary cancer, keratin 7 immunostaining was seen in all 12 type-1 tumors (always >50% of cancer cells; Fig. 1b), whereas only 4 of 8 (50.0%) type-2 tumors showed keratin 7 immunostaining (always $\leq$50% of cancer cells). Furthermore, 2 of 8 (25%) type-2 papillary cancers were the only tumors in our series showing strong, though heterogeneous, immunoreactivity for keratin 20 (Fig. 1c). With pankeratin antibodies, conventional RCCs showed a predominantly membranous immunoreactivity (Fig. 1d), while cytoplasmic staining was rare and primarily noted in poorly differentiated tumors (Fig. 1e). In contrast, chromophobe tumors showed a characteristic mixture of large cells with circumferential membranous staining (corresponding to the nearly transparent and ballooned cells in H&E-stained sections) and small cells with diffuse cytoplasmic staining, leaving a small perinuclear rim unstained (corresponding to the granular and eosinophilic cells in H&E-stained sections; Fig. 1f).

Regarding ROs, staining with the pankeratin antibodies KL1, MNF116, and Lu5 specifically decorated the great majority of tumor cells and showed three distinct patterns of immunoreactivity (Fig. 2a, b): (i) perinuclear and punctate dot-like, (ii) membranous, and (iii) combination of both (mixed type). Comparable results were obtained with antibodies directed against keratins 8, 18, and 8/18 (LMW); details are given in Table 3. Interestingly, with the pankeratin antibody AE1/AE3, only 32 of 66 (48.5%) ROs were immunoreactive. In all these cases, a strong diffuse granular cytoplasmic staining of single tumor cells and small groups of tumor cells (<10%) was

Table 3 Immunohistochemical staining patterns of renal oncocytomas with antibodies directed against keratin 8 and/or 18

	Immunohistochemical staining patterns		
	Punctate (*n*)	Membranous (*n*)	Mixed (*n*)
KL1	14	32	19
MNF116	10	27	26
Lu5	15	26	21
K 8	16	7	15
K 18	17	34	15
K 8/18 (LMW)	17	14	19

Table 2 Keratin expression profiles (+ overall immunoreactivity; ++ immunostaining of more than 50% of tumor cells) of renal cell carcinomas (RCCs) related to histological subtypes and renal oncocytomas

Antibody specificity	Conventional RCCs (*n*=125)				Chromophobe RCCs (*n*=22)				Papillary RCCs (*n*=20)				Renal oncocytomas (*n*=66)			
	+	%	++	%	+	%	++	%	+	%	++	%	+	%	++	%
K 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 5/6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 7	17	13.6	4	3.2	16	72.7	13	59.1	16	80.0	12	60.0	42	63.6	1	1.5
K 8	18	14.4	3	2.4	11	50.0	5	22.7	10	50	6	30.0	38	57.6	11	16.7
K 8/18, LMW	35	28.0	8	6.4	13	59.1	6	27.3	17	85.0	10	50.0	50	83.3	19	28.8
K 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K 18	117	93.6	74	59.2	22	100.0	19	86.4	20	100.0	20	100.0	66	100.0	66	100.0
K 19	25	20	4	3.2	5	22.7	0	0	18	90.0	8	40.0	27	40.9	0	0
K 20	0	0	0	0	0	0	0	0	2	10.0	1	5.0	0	0	0	0
HMW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KL1	75	60.0	16	12.8	19	86.4	12	54.5	20	100.0	15	75.0	65	98.5	62	93.9
MNF116	98	78.4	62	49.6	22	100.0	18	81.8	20	100.0	19	95.0	63	95.5	61	92.4
AE1/AE3	97	77.6	55	44.0	16	72.7	5	22.7	19	95.0	18	90.0	32	48.5	0	0
Lu5	117	93.6	90	72.0	22	100.0	22	100.0	20	100.0	20	100.0	62	93.9	53	80.3

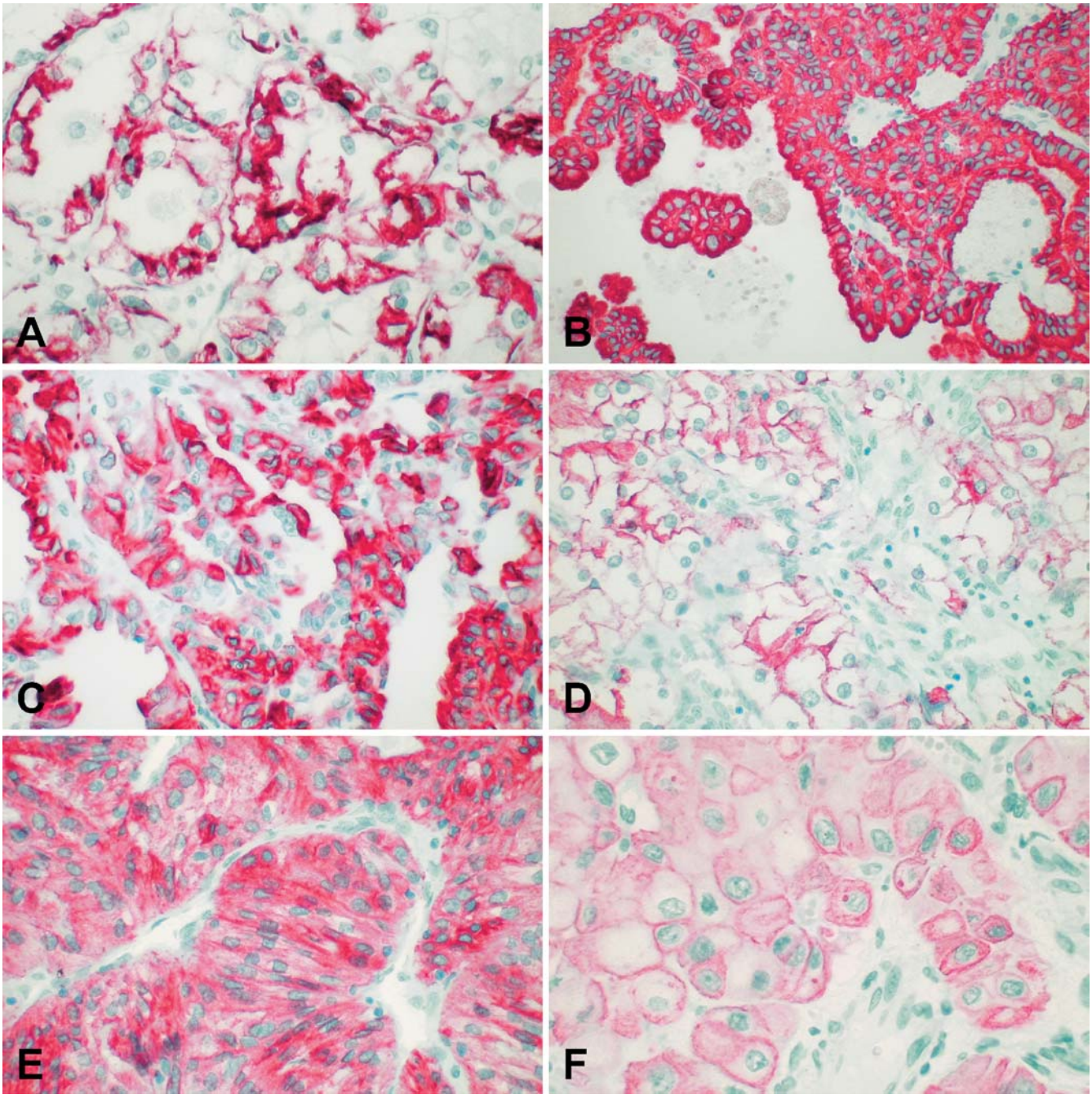

Fig. 1 Keratin immunohistochemistry in renal cell carcinomas (RCCs). Focal keratin 7 immunostaining in conventional (**A**) and strong diffuse staining in type-1 papillary (**B**) RCCs. Distinct keratin 20 immunoreactivity in type-2 papillary RCC (**C**). Strictly membranous immunostaining in a moderately differentiated (**D**) and additional diffuse cytoplasmic reactivity in poorly differentiated (**E**) conventional RCC using pankeratin antibody AE1/AE3. Characteristic heterogeneous immunostaining with a mixture of large cells with membranous staining and small cells with diffuse cytoplasmic staining in chromophobe RCC, using pankeratin antibody AE1/AE3 (**F**)

noted. A similar pattern of reactivity was noted for keratin 7 in 42 of 66 (63.6%) tumors and for keratin 19 in 27 of 66 (40.9%) tumors, respectively (Fig. 2c, d). In general, less than 10% of tumor cells were stained, mostly coexpressing keratins 7 and 19, but differences in immunoreactivity were additionally observed in a minority of cases (Fig. 2e, f). It is worth mentioning that both antibodies, which are known to decorate distal tubule epithelium, frequently disclosed non-neoplastic tubules entrapped, but not destroyed, by the tumor, especially in subcapsular regions. Using the Dako LSAB2 System HRP (AEC) detection kit, the keratin 14 antibody showed a weak diffuse cytoplasmic staining, lacking membranous immunoreactivity in the majority of the cases. By use of

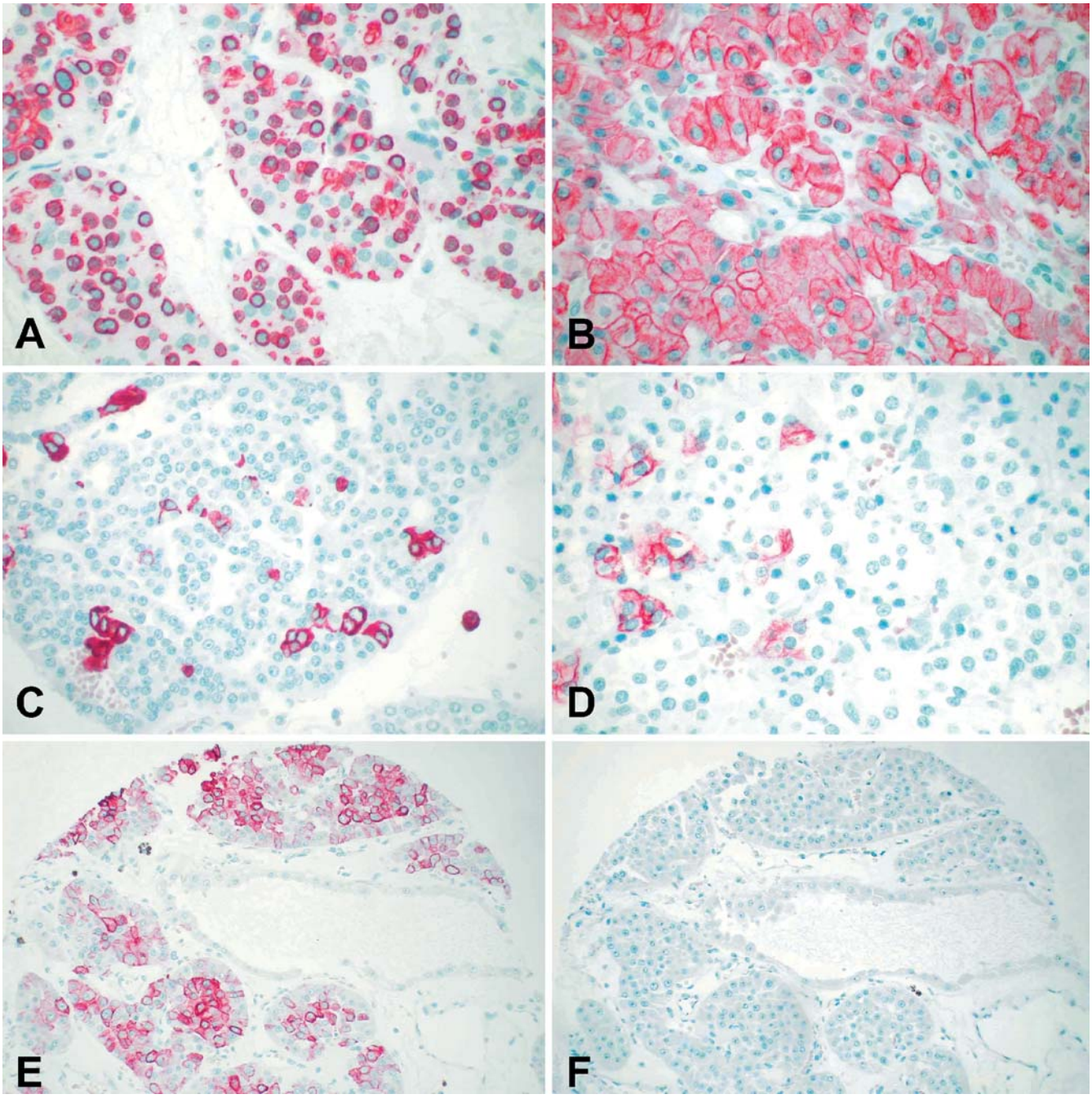

Fig. 2 Immunoreactivity of renal oncocytomas. Staining with pankeratin (KL1) shows a characteristic perinuclear and punctate dot-like (**A**) or membranous (**B**) pattern. Keratin 7 (**C**, **E**) and keratin 19 (**D**, **F**) immunoreactivity reveals a predominantly diffuse cytoplasmic staining in a minority of tumor cells. Mostly, a coexpression of these two keratin subtypes was found, but in few cases a marked difference in staining patterns was observed (**E**, **F**)

the biotin-free EnVision+System, staining for keratin 14 yielded unequivocally negative results. In contrast, a distinct strong immunoreactivity was seen in positive controls with both LSAB2 and EnVision detection systems.

Discussion

Keratins are intermediate filament proteins, which are constituents of the mammalian epithelial cytoskeleton. At least 20 different types have been discriminated in various tissues on the basis of molecular weight and isoelectric pH values. In intermediate filaments, they are present as pairs of type-I (acidic) and type-II (neutral to basic)

proteins. Different subsets of keratins are expressed by different epithelia, and these patterns are largely retained during neoplastic transformation. Hence, epithelia (simple and complex) and epithelial tumors can be classified on the basis of keratin protein expression. Keratin typing has, therefore, been widely applied in surgical pathology as a differential diagnostic tool [7, 23, 24].

With pankeratin antibody cocktails, both RCCs and ROs were stained in the majority of cases. In RO specimens with antibodies recognizing keratins 8 and/or 18 (including the pankeratin reagents KL1, MNF116, and Lu5), distinct patterns of immunoreactivity (membranous or perinuclear/punctate dot-like) were found, thus confirming the results by Bonsib et al. [2, 3], who identified the punctate pattern on the ultrastructural level as paranuclear keratin-containing globular filamentous bodies. It is important to note that with AE1/AE3, a pankeratin antibody preparation without keratin 8 and/or 18 immunoreactivity, only about half of the ROs were stained with less than 10% of tumor cells reacting diffusely without the typical perinuclear/punctate dot-like pattern. Moreover, the characteristic perinuclear/punctate dot-like pattern was absent in all RCCs investigated in our series, thus confirming the results by Bosib et al. [2, 3] and providing a valid basis for differential diagnosis.

Published results on keratin 7 immunostaining in renal epithelial tumors are conflicting. Generally, keratin 7 expression is thought to be infrequent in renal cancer [8, 11, 37]. Keratin 7 is, however, commonly present in the papillary subtype [10, 17, 30]. The strong keratin 7 expression in the relatively benign type 1 compared with the weak expression in the more aggressive type 2 of papillary RCC in our series confirms a previous study by Delahunt and Eble [9], reporting strong or moderate keratin 7 immunoreactivity in 48 of 61 (78.7%) type-1 compared with 3 of 30 (10%) type-2 tumors. Recently, some authors have recommended keratin 7 as a characteristic marker of chromophobe RCCs and ROs, differentiating them from conventional cancers [19]. Others, however, concluded that keratin 7 may be useful in the differential diagnosis of chromophobe RCCs and ROs [12, 17]. In our series, keratin 7 was present in the majority of chromophobe and papillary RCCs, as well as ROs. The low percentage of immunoreactive tumor cells in ROs and their heterogeneous distribution might, however, explain the conflicting results [12, 17, 19, 33, 38]. Although the differential diagnostic value of keratin 7 appears to be limited, it is important to note that, in our series, keratin 7 expression in more than 50% of tumor cells was seen in only 1 of 66 (1.5%) ROs. Therefore, strong keratin 7 immunoreactivity makes the diagnosis of RO very unlikely, whereas it does not exclude conventional cancer, since almost every seventh conventional RCC showed keratin 7 expression in more than 50% of cancer cells. Another feature, that to the best of our knowledge has not been well recognized, is the incorporation of non-neoplastic tubules within ROs, which can easily be detected by their immunoreactivity for keratin 7. In some cases, these tubules can even be traced from the periphery to the center of the tumor by serial sectioning. In two cases, we have also noticed incorporated papillary adenomas at the subcapsular border of the tumors, which were well preserved.

Thus far, keratin 19 immunoreactivity has been systematically investigated only in conventional cancers [4, 12, 26]. The expression in 90% of papillary cancers in our study is in accordance with one recent report by Kim and Kim [12], who noted keratin 19 immunoreactivity in 14 of 20 (70%) papillary RCCs. Keratin 19 immunostaining might, therefore, be of additional value differentiating papillary RCC, especially the solid variant [30], from the eosinophilic variant of chromophobe RCCs [16], since, in our study, chromophobe tumors showed keratin immunostaining in only about 20% of cases. In ROs, keratin 19 immunoreactivity has, thus far, only anecdotally been reported; Pitz et al. [28] analyzed eight ROs and noticed only scattered keratin 19 positive tumor cells in "some cases". Taki et al. [35] found keratin 19 in 1 of 3 ROs, whereas according to Cao and Carsten [4] and Kim and Kim [12], keratin 19 was undetectable in 5 and 12 ROs, respectively. As shown by us, however, keratin 19 can be found in about 40% of RO cases, mostly coexpressed with keratin 7.

Keratin 20 immunoreactivity has been demonstrated only in single cases of renal tubular malignancies [8, 21, 32, 37]. In fact, the general lack of keratin 20 expression was suggested as criterion for differential diagnosis [11]. In our series, the only tumors showing keratin 20 immunoreactivity were two cases of type-2 papillary cancer. Our observation corresponds to a recent study by Kim et al. [12], who reported on 4 of 20 (20%) papillary RCCs showing keratin 20 expression. However, papillary carcinoma subtypes were not mentioned in that study, and additional data are needed to investigate the potential association of keratin 20 with type-2 papillary RCC. Keratin 20 was not detectable in our RO cases, thus confirming the reports obtained from other studies [12, 38]. The results by Stopyra et al. [33], who noticed a punctate dot-like keratin 20 immunoreactivity in as much as 12 of 15 (80%) ROs, could not be confirmed. Whether this discrepancy can be explained by differences in antibody reactivity, selection criteria, or variable thresholds in the interpretation of immunoreactivity, as proposed by Wu et al. [38], remains enigmatic. In any case, the punctate or dot-like pattern of RO produced by pankeratin antibody cocktails could, in our series, clearly be attributed to keratins 8 and 18.

Recently, Chu and Weiss introduced keratin 14 as a marker of RO in the distinction from RCC [6]. In our series, however, keratin 14 antibodies identical to those applied by Chu and Weiss [6] only produced a weak diffuse cytoplasmic staining that spared plasma membranes and lacked the staining intensity seen in positive controls. We regarded this reaction as nonspecific, since we saw a similar, yet somewhat weaker, reaction with other keratin antibodies and with antibodies exclusively recognizing nuclear antigens (p63, steroid hormone receptors; data not shown). This assumption is supported

by the negative staining obtained with the HMW-keratin antibody, which recognizes keratin subtypes 1, 5, 10, and 14. On the ultrastructural level, the cells of all oncocytic tumors are extremely rich in mitochondria [36], and the mitochondrial carboxylases are known to contain biotin as a co-enzyme [31]. Probably, this explains why Chu and Weiss, themselves [6], applying an avidin-biotin-complex method in their study, noticed a correlation between keratin 14 immunoreactivity and the concentration of mitochondria, which they tried to explain by cross-reactivity of the keratin 14 antibody with mitochondrial epitopes. To prove our hypothesis of non-specific staining due to endogenous mitochondrial biotin, we used the biotin-free EnVision+System, and the weak diffuse cytoplasmic staining, seen previously, disappeared.

In conclusion, our results demonstrate that due to lack of consistent differences in immunoreactivity, keratin subtyping only provides additional clues for the classification of renal epithelial tumors. The punctuate/dot-like pattern of ROs with antibodies recognizing keratins 8 and/or 18 appears to be the only useful criterion for differential diagnosis. However, its value is hampered by the fact that only about 50% of ROs show this characteristic morphology. Hence, despite technical and interpretative challenges of Hale's colloidal iron, it is still the most useful stain in differentiating chromophobe RCC from oncocytoma. Moreover, the combination of Hale's colloidal iron with vimentin immunostaining still represents the best way to differentiate chromophobe from conventional renal cancer. The potential value of keratin 20 as a marker for type-2 papillary cancer has to be addressed in future studies. Keratin 14 immunohistochemistry leads to nonspecific staining and cannot be recommended as a diagnostic tool.

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