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Co-expression of cytokeratin and vimentin intermediate-sized filaments in renal cell carcinomas*

Comparative study of the intermediate-sized filament distribution in renal cell carcinomas and normal human kidney

R. Waldherr and K. Schwechheimer

Institute of Pathology and Institute of Neuropathology, University of Heidelberg, D-6900 Heidelberg, Federal Republic of Germany

Summary. The expression of intermediate-sized filaments (IF) was examined by immunocytochemical methods in 40 primary renal cell carcinomas and compared with the IF distribution in the normal adult human kidney. All tumours stained positively with cytokeratin IF antibodies. Co-expression of cytokeratins and vimentin was observed in 21/40 (52,5%) renal carcinomas. Double immunofluorescence labelling demonstrated that in most of these cases tumour cells contained both cytokeratin and vimentin type IF. In normal human kidneys, cells of the various tubular segments disclosed a positive reaction with cytokeratin antibodies in a different intensity and intracellular localization. Co-expression of cytokeratin and vimentin IF in normal adult human kidneys has never been observed. From a histogenetic point of view, co-expression of cytokeratins and vimentin in renal cell carcinoma obviously represents an atavistic phenomenon since vimentin is re-expressed by these tumour cells during neoplastic transformation. This finding indicates the metanephric origin of the renal parenchyma. In surgical pathology the possibility of very rare co-expression of cytokeratin and vimentin IF within tumour cells should be considered, particularly in the differential diagnosis of clear cell carcinomas.

Key words: Human kidney – Renal cell carcinoma – Intermediate-sized filaments – Co-expression – Immunocytochemistry

Introduction

Intermediate-sized filaments (IF) are unbranched cytoplasmic filaments (7–11 nm in diameter) which participate in the formation of the cytoskeleton. IF are represented by five different protein types, i.e. cytokeratins, vimentin, desmin, glial fibrillary acidic proteins (GFAP), and neurofilament

* Dedicated to Prof. Dr. K. Goerttler on the occasion of his 60th birthday

Offprint requests to: R. Waldherr at the above address

proteins (for review see: Osborn and Weber 1983). Cytokeratins have been exclusively demonstrated in most epithelial cells (Moll et al. 1982, 1983; Osborn and Weber 1983), whereas vimentin has been found in many non-epithelial cell types, particularly mesenchymal cells. Desmin, the muscle type IF, is expressed in skeletal, cardiac and smooth muscle cells (Lazarides and Hubbard 1976; Small and Sobieszek 1977; Bennett et al. 1978). GFAP is specific for glial cells (Bignami and Dahl 1974; Liem et al. 1978; Schachner et al. 1978; Schnitzer et al. 1981; Roesmann et al. 1983), and the neurofilament proteins occur in many, but not all, neurons (Liem et al. 1978; Schachner et al. 1978; Schlaepfer and Lynch 1978; Shaw and Weber 1981).

IF are structural and higly conservative proteins. In tissues most cells produce only one cell-type characteristic IF protein. Specific IF protein expression is generally preserved in neoplastic cells (Franke et al. 1981; Moll et al. 1982, 1983). For these reasons immunocytochemical demonstration of IF has become a preferential tool in differential tumour diagnosis in surgical pathology (Schlegel et al. 1980; Gabbiani et al. 1981; Sieinski et al. 1981; Miettinen et al. 1982; Tascos et al. 1982; Denk et al. 1983; Evans et al. 1983; Nagle et al. 1983; Osborn and Weber 1983; Ramaekers et al. 1983a, 1983d; Trojanowski and Lee 1983). There are, however, some cell types which are capable of expressing two different IF types simultaneously. For example, vimentin and desmin IF are found in smooth muscle cells of vascular walls (Frank et al. 1982; Schmid et al. 1982), and the co-expression of both GFAP and vimentin is seen in glial cells (Schnitzer et al. 1981; Quinlan and Franke 1983).

IF expression in renal cell carcinomas is controversial. In earlier reports no IF proteins were demonstrated within tumour cells (Schlegel et al. 1980; Espinoza and Azar 1982; Nagle et al. 1983; Osborn and Weber 1983) but recent data show that either cytokeratin (Zerban et al. 1983; Makin et al. 1984) or vimentin, or both may be expressed in renal cell carcinomas (Herman et al. 1983; Holthöfer et al. 1983a, 1983b). The present study was carried out to clarify IF expression in a large series of renal cell carcinomas and to compare these data with the IF pattern in the normal human kidney. Special emphasis was laid on the question whether there is a coexpression of two IF types within the same tumour cell, and whether a counterpart in the non-neoplastic normal human kidney exists.

Materials and methods

Tumour ($n=40$) and normal ($n=5$) tissue of nephrectomy specimens from patients with renal cell carcinomas were snap frozen in isopentane-cooled liquid nitrogen and stored at $-70°$ C until use. *Cryostat sections* were cut at 4–5 µm and dried for 2–3 h at room temperature. After fixation in cold acetone ($+4°$ C) for 10 min and washing with phosphate buffered saline (PBS) the sections were incubated with the primary antisera (monoclonal antibodies to cytokeratin and vimentin, Labsystems Helsinki; polyclonal rabbit antibody to desmin, Dakopatts) for 60 min at room temperature or overnight at $+4°$ C. Indirect immunofluorescence was performed by using fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgG, $F(ab)_2$, or FITC-labelled goat anti-rabbit IgG as second antibodies. In addition, selected sections from normal and tumour tissue were tested with a two-step peroxidase (PO) or alkaline phosphatase (AP) method using PO or AP conjugated goat anti-mouse IgG as second antibody, and PO or AP labelled rabbit anti-goat IgG as a third antibody (Dakopatts; Tago Inc., Burlingame).

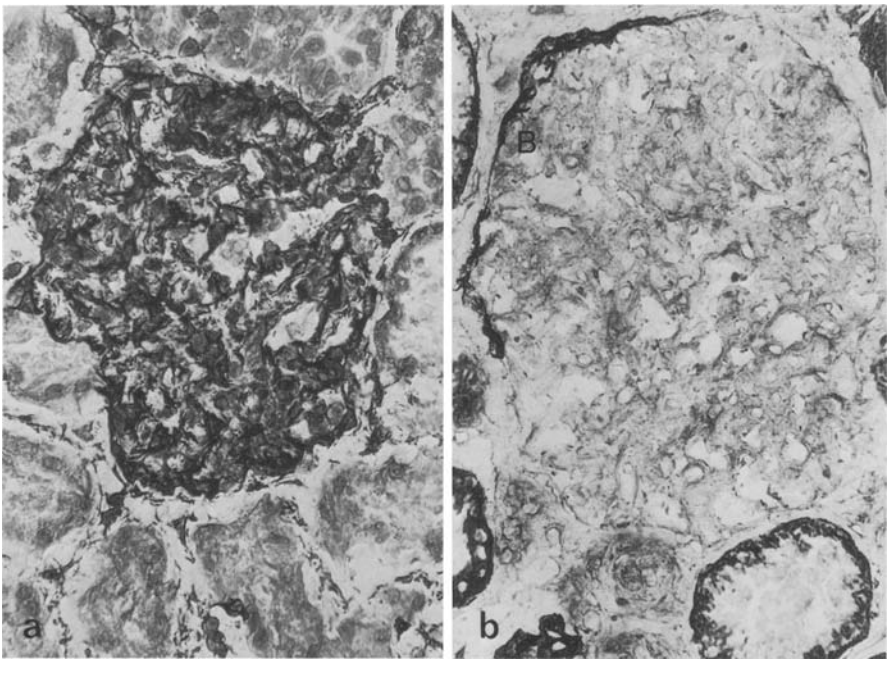

Fig. 1. Staining reaction for vimentin (**a**) and cytokeratin (**b**) in the glomeruli of the normal human kidney. **a** Vimentin is positive in the perikaryon of podocytes, endothelial and mesangial cells, a few cells of Bowman's capsule as well as in interstitial fibroblasts and endothelial cells. Alkaline phosphatase reaction, × 300. **b** Cells of the glomerular tuft are completely negative for cytokeratin. A positive reaction, however, is observed in a part of the parietal epithelium of Bowman's capsule (**b**). Peroxidase reaction. × 300

Incubation of the second and third antibodies was carried out for 30 min at room temperature. A positive reaction was visualized with aminoethyl-carbazole (AEC) or naphtol AS phosphate/ Fast Red, respectively. Nuclei were counterstained with Mayer's haematoxylin. Double immunofluorescence was done by successive incubation of two monoclonal antibodies (anti-cytokeratin and anti-vimentin), or a monoclonal (anti-cytokeratin or anti-vimentin) and a polyclonal antibody to desmin raised in rabbit. Each step was followed by FITC or rhodamine-conjugated anti-mouse IgG or anti-rabbit IgG as second antibodies. Control experiments yielded completely negative results with second and/or third antibodies alone. Tumours which exclusively expressed cytokeratin were negative for vimentin on double immunostaining.

Characterization of monoclonal antibodies (Labsystems, Helsinki). Immunoblotting experiments demonstrated a specific reaction of the monoclonal antibody to cytokeratins with all cytokeratins of HeLa cells, i.e. of 44, 46, 52, 54 KD molecular weight. According to Moll's catalogue of human cytokeratin polypeptides (1982) these correspond to cytokeratins 17, 18, 7 and 8. Anti-vimentin reacted in different cell lines (humen fibroblasts, cultured human rhabdomyosarcoma cells) with the 58 KD polypeptide only.

Results

Normal human kidney

Glomeruli. Vimentin was strongly positive within the podocytes and their primary processes (Fig. 1a). A weaker reaction was present in endothelial

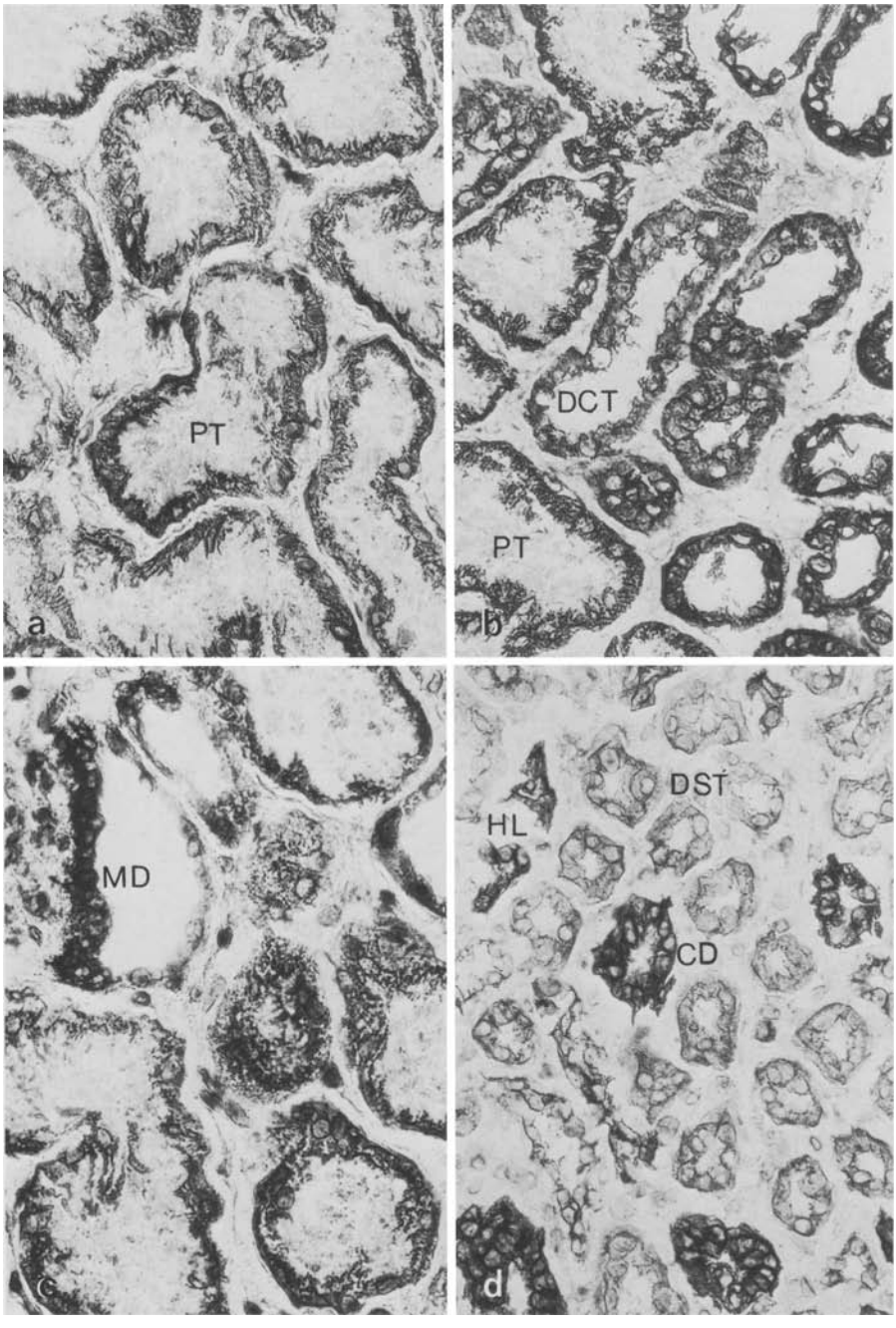

Fig. 2. Cytokeratin positivity in different tubular segments of the normal human kidney. Proximal tubules (*PT*) with subapical and basolateral staining (**a, b**), distal convoluted tubules (*DCT*) with subapical and lateral staining (**b**), and distal straight tubules (*DST*) with a faint subapical positive reaction (**d**). In cells of the macula densa (*MD*), collecting ducts (*CD*) and thin limbs of Henle's loops (*HL*) cytokeratin is evenly distributed throughout the cytoplasm (**c, d**). Peroxidase reaction. × 300

Table 1. Intermediate-sized filament expression (cytokeratin, vimentin) in renal cell carcinomas according to the predominant histological cell type

Histology	Cytokeratin	Cytokeratin + vimentin
Clear cell carcinomas[a]	13	16
Granular Cell carcinomas[a]	2	4
Anaplastic/sarcomatoid carcinomas	4	1
	19/40	21/40

[a] Tubular, acinar, solid or papillary

and mesangial cells. This reaction extended from the tuft to the afferent and efferent arterioles of the vascular pole. Double immunofluorescence studies showed that mesangial cells also expressed intermediate filaments of the desmin type. Goormaghtigh cells were faintly positive for vimentin. All cells of the glomerular tuft were negative for cytokeratin, whereas cytokeratin was strongly positive within a few cells of Bowman's capsule, forming a short rim or cap along the inner surface of Bowman's capsular membrane (Fig. 1b). In serial sections, these cells were not always contiguous to the urinary pole. A few – even if not all – of the remaining cells of Bowman's capsule disclosed positive staining for vimentin.

Tubules. All tubular epithelia were negative for vimentin but showed a positive reaction of different intensity and intracellular localization with cytokeratin antibodies. In proximal tubules, a subapical and basolateral positivity was observed in cells of the convoluted as well as of the straight part of this segment (Fig. 2a, b). In the straight part of the distal tubules (Fig. 2d), the subapical region of the cytoplasm showed a faint reaction with cytokeratin antibodies, whereas in the macula densa a strong staining was observed throughout the cytoplasm of these cells (Fig. 2c). In the distal convoluted tubules a subapical and lateral reaction predominated (Fig. 2b). The cells of the thin limbs of Henle's loops, connecting tubules and collecing ducts displayed a strongly positive staining evenly distributed throughout their cytoplasm (Fig. 2d).

Vessels and interstitium. All arterioles, arteries and veins were strongly stained by vimentin antibodies within their endothelial cells and less intensively within the smooth muscle cells of the tunica media. The latter were also positive for desmin. Pericytes, capillary endothelial cells and interstitial fibroblasts reacted with anti-vimentin antibodies.

Renal cell carcinomas. Cytokeratin IF were expressed in all renal cell carcinomas (Table 1). In carcinomas of the clear cell type (Figs. 3, 4a) staining predominated along the inner aspect of the cell membranes, whereas a more diffuse cytoplasmic staining pattern except for the perinuclear space was seen in granular cell tumours (Fig. 5a). In addition to cytokeratin, vimentin was expressed in 21 (52,5%) samples studied including typical clear cell,

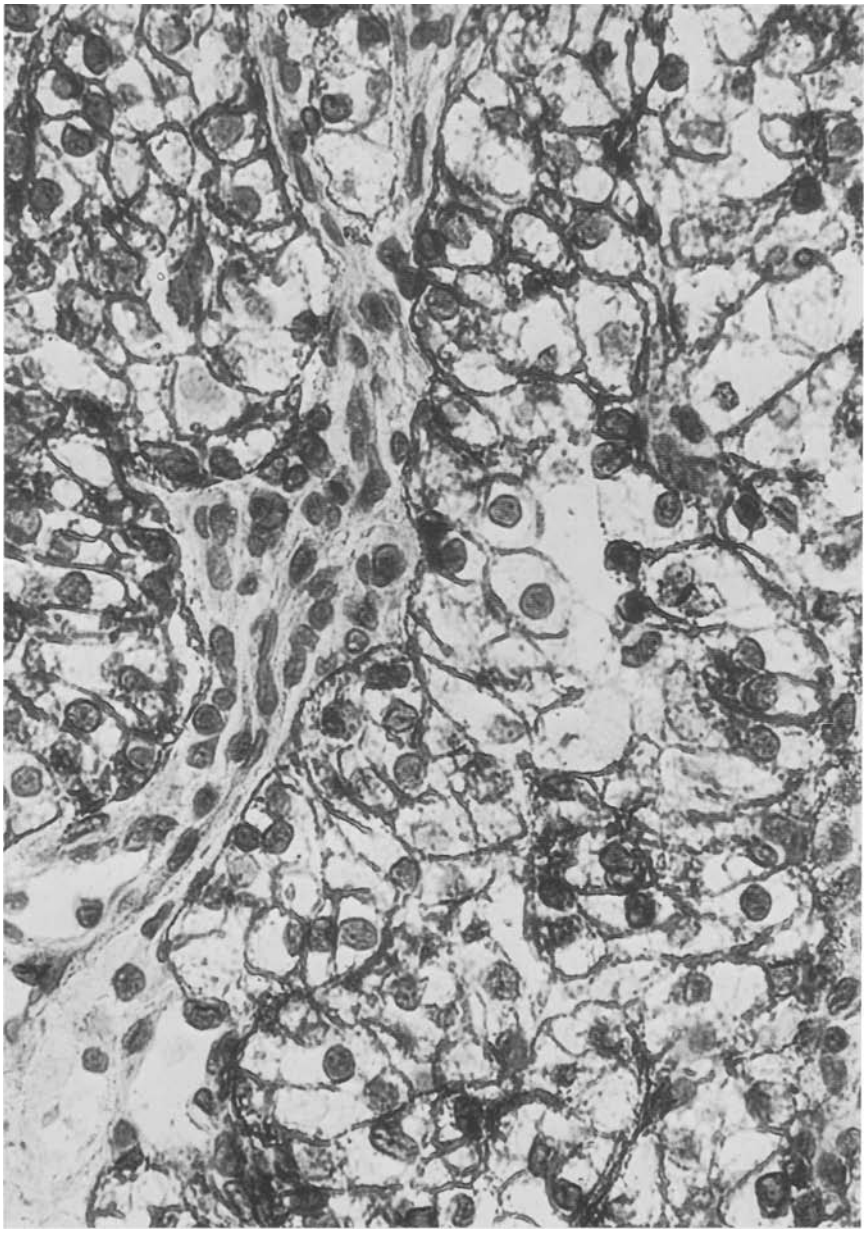

Fig. 3. Renal cell carcinoma of the clear cell type. Note the cytokeratin staining at the inner aspect of the tumour cell membranes. Peroxidase reaction. × 650

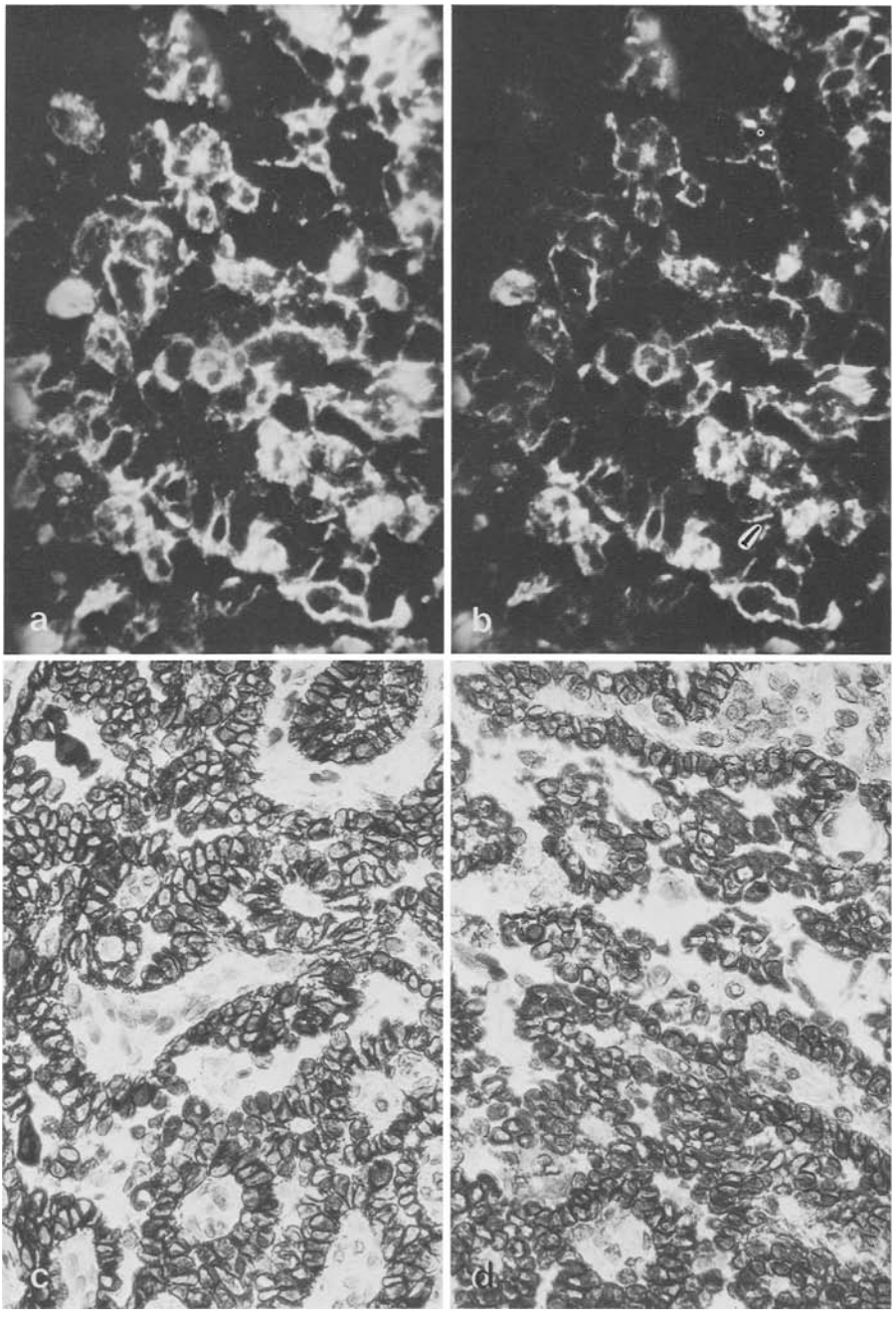

Fig. 4a–d. Co-expression of cytokeratin and vimentin in renal cell carcinomas. **a, b** Double immunofluorescence labelling of a clear cell carcinoma. Most tumour cells co-express cytokeratin (**a**) and vimentin (**b**). ×300. **c, d** Co-expression of cytokeratin (**c**) and vimentin (**d**) in a papillary renal cell carcinoma. Peroxidase reaction. ×300

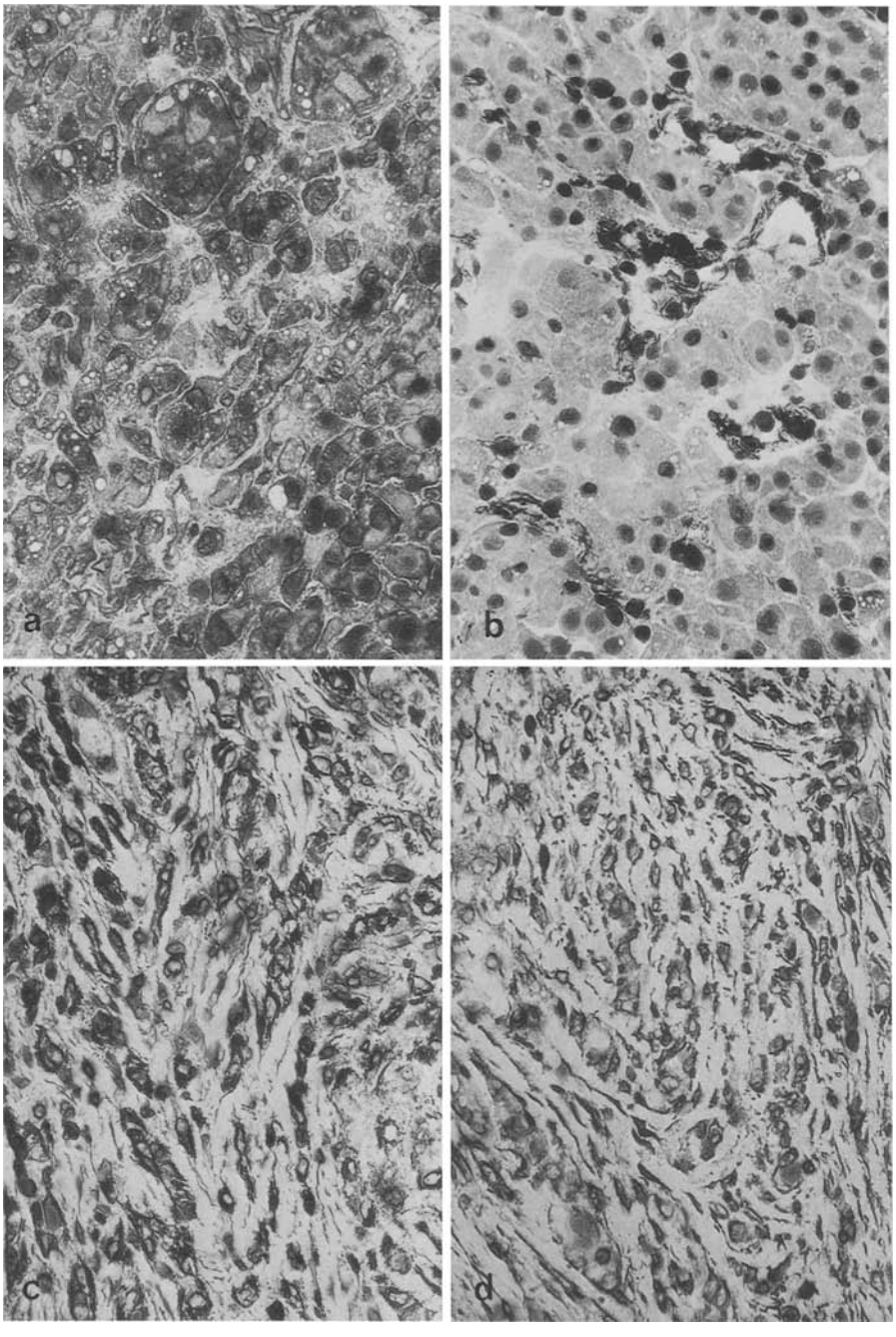

Fig. 5. Renal cell carcinoma of the granular cell type (**a, b**). Tumour cells are positive for cytokeratin (**a**) whereas vimentin (**b**) is exclusively present in stromal cells. Sarcomatoid renal cell carcinoma (**c, d**) with co-expression of cytokeratin (**c**) and vimentin (**d**). Peroxidase reaction. ×300

granular cell and sarcomatoid carcinomas (Table 1; Figs. 4; 5c, d). In about half of the tumour specimens vimentin was only focally positive whereas in others a diffuse positivity was observed. Double immunofluorescence staining showed that both IF types were present simultaneously within most of these tumours cells (Fig. 4a, b). Stromal tissue was stongly positive for vimentin but negative for cytokeratin. Desmin could only be detected in the walls of large stromal vessels.

Discussion

Intermediate filament (IF) analysis in renal cell carcinomas has yielded differing results. While in earlier series keratin antigens could not be demonstrated within tumour cells (Schlegel et al. 1980; Espinoza and Azar 1982; Nagle et al. 1983; Osborn and Weber 1983), recent reports show that most primary renal cell carcinomas do express cytokeratin IF (Herman et al. 1983; Holthöfer et al. 1983a, 1983b; Zerban et al. 1983; Makin et al. 1984). This discrepancy may be due to the use of formalin-fixed and paraplast embedded tissue which are known to alter the antigenicity of cytokeratin polypeptides in earlier series and the heterogeneity of the antisera applied (Ramaekers et al. 1983c; Holthöfer et al. 1983b). With the use of a monoclonal antibody to prekeratin on frozen material and a very sensitive immunohistochemical method we were able to demonstrate that all renal cell carcinomas studied express cytokeratin IF. A somewhat surprising fact was the obvious co-expression of cytokeratin and vimentin in a considerable number of tumours. Holthöfer et al. (1983b) noted the co-existence of these two IF types in 14/28 (50%) carcinomas, two samples being exclusively positive for vimentin. In the series of Herman et al. (1983) a co-expression of cytokeratin and vimentin was observed in 9/11 tumour specimens from 10 patients, whereas in two samples only vimentin could be demonstrated. In our material, 52,5% of the 40 primary renal carcinomas expressed both IF types. Double immunostaining experiments clearly showed that both filament proteins can be produced within the same cells.

Earlier reports on the IF distribution in the normal human kidney are not conclusive with respect to cytokeratin expression in the different tubular segments. Various studies show that antibodies against human epidermal prekeratin react with the epithelium of collecting ducts, but not with other epithelial structures of the kidney (Sun et al. 1979; Holthöfer et al. 1983a, 1983b). When antibodies to cytokeratin polypeptides isolated from MDCK cells, CK1-CK4, PKK1 or CAM 5.2 hybridoma antibodies are used, however, additional staining of proximal and distal tubules as well as of some glomerular parietal cells was observed (Debus et al. 1982; Holthöfer et al. 1983a, 1983b; Makin et al. 1984). Bachmann et al. (1983) examined frozen sections of rat and bovine kidneys with various antibody preparations in greater detail. Their results are very similar to those that we obtained with human material. While these authors, however, observed no significant staining in the proximal convoluted tubuli, clear-cut cytokeratin positivity was noted in our study. Furthermore, the two-step peroxidase or alkaline

phosphatase method enabled us to exactly localize the IF type expression in different parts of the nephron. In the *glomerulus*, podocytes, endothelial and mesangial cells as well as some cells of Bowman's capsule were positive for vimentin. Mesangial cells co-expressed desmin. Cytokeratin was only present within a few cells of Bowman's capsule, forming a characteristic rim at the inner aspect of the capsular membrane. The precise significance of this phenomenon is unknown. In contrast to Holthöfer et al. (1983b), we were not able to demonstrate a constant continuity of these cells to initial tubular cells at the urinary pole in all glomeruli. All *tubular segments* were stained by antibodies to cytokeratin and were negative for vimentin. While cytokeratin was homogeneously distributed in cells of thin limbs of Henle's loops, the macula densa, connecting tubules and collecting ducts, staining was subapical as well as basolateral in proximal tubules. In distal tubules, cells of the convoluted part showed a subapical and lateral staining reaction whereas a only weak subapical positivity was noted in the straight part of this segment.

The co-expression of cytokeratin and vimentin in renal cell carcinomas has never been found in the mature human kidney. The various tubular segments, particularly proximal tubules – from which most renal cell carcinomas are thought to originate – are exclusively positive for cytokeratin. The co-existence of two classes of IF is a regular feature of cultured cells (Franke et al. 1978, 1981; Sharp et al. 1982; Osborn and Weber 1983; Ramaekers et al. 1983d), however, it is thought to be exceptional in epithelia in situ. Cytokeratin and vimentin co-expression has recently been reported from plemorphic adenomas of the parotid gland (Caselitz et al. 1981; Krepler et al. 1982), in parietal endoderm cells of early mouse embryo (Lane et al. 1983), within certain human metastatic carcinoma cells in ascites or pleura fluid (Ramaekers et al. 1983b), in carcinomas of the thyroid gland (Droese et al. 1984; Miettinen et al. 1984) and in blastema cells of some nephroblastomas (Altmannsberger et al. 1984). In rhabdomyomatous and leiomyomatous tumours desmin and vimentin may be co-expressed. (Gabbiani et al. 1981; Altmannsberger et al. 1982; Miettinen et al. 1982; Denk et al. 1983; Evans et al. 1983), and in gliomas GFAP and vimentin appear together (K. Schwechheimer, unpublished data).

A better understanding of variable IF expression in renal cell carcinomas is achieved by knowledge of IF occurrence in the developing kidney. Undifferentiated cells of the metanephric mesenchyma express vimentin only (Franke et al. 1982; Holthöfer et al. 1984) whereas only cytokeratins can be visualized in cells of induced renal vesicles (Holthöfer et al. 1984). At the S-shaped body stage of nephrogenesis, visceral and parietal cells of the developing glomerulus contain neither vimentin nor cytokeratins, whereas cells at the tubular pole are positive for cytokeratins. At a later stage, cuboidal visceral epithelial cells display strong staining for vimentin which is maintained in the mature kidney. Only a few parietal glomerular cells demonstrate cytokeratin-specific staining. In proximal and distal tubules as well as in collecting ducts of fetal kidneys, epithelial cells are positive with monoclonal antibodies to cytokeratins (PKK1 and PKK2), whereas

in adult kidneys the PKK1 antibody reacts only with cells of collecting ducts. Vimentin is not observed in proximal or distal tubular epithelial cells at any developmental stage, whereas collecting ducts show a transient expression of vimentin in fetal kidneys (Holthöfer et al. 1984).

From a histogenetic point of view, the co-existence of vimentin and cytokeratins in renal cell carcinomas is of particular interest, since it suggests that some tumour cells re-express, in addition to their "mature" IF type (cytokeratins), the original IF protein present in the embryonal mesenchyma namely vimentin. This interpretation is favored by the co-expression of cytokeratins and vimentin in blastema cells of nephroblastomas in children (Altmannsberger et al. 1984). At present, however, there is no evidence that the additional expression of vimentin in renal cell carcinomas is a sign of its biological potential, i.e. that renal carcinoma should be considered as a carcinosarcoma, as proposed by Herman et al. (1983). In our series, we were not able to differentiate between carcinomas expressing only cytokeratins, or both cytokeratins and vimentin on histological grounds. Furthermore, co-expression of both IF types was not correlated to cytological grading, tumour size, or to vascular invasion and the TNM-state.

For practical reasons, the variable expression of IF proteins in renal cell carcinomas should be considered in surgical pathology when antibodies to IF are applied to determine the origin of metastases of clear cell tumours, i.e. pulmonary carcinomas, carcinomas of the thyroid gland, the endometrium, the kidney, or even sarcomas. Even though it was not observed in our study, it should be borne in mind that there might be isolated cases of renal cell carcinomas expressing vimentin IF proteins exclusively (Holthöfer et al. 1983b; Herman et al. 1983). We must therefore conclude that the demonstration of vimentin IF alone does not exclude an epithelial neoplasm (renal cell carcinoma). In our experience, antibodies to proximal tubular antigens, particularly brush-border antigens, are helpful in diagnosis of such doubtful cases (Waldherr et al., in preparation).

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