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Distribution of cytokeratins, vimentin and desmoplakins in normal renal tissue, renal cell carcinomas and oncocytoma as revealed by immunofluorescence microscopy

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Summary. Forty-two renal cell carcinomas, one oncocytoma and normal renal tissue were studied for the presence of cytokeratins and vimentin. The investigations were performed by immunofluorescence microscopy applying a panel of mono- and polyclonal antibodies to intermediate filament proteins. In all tumours except chromophobic renal cell carcinoma (CRCC) and oncocytoma a co-expression of cytokeratins and vimentin could be shown. The intermediate filament expression was often, however, very heterogeneous particularly with respect to the distribution of cytokeratins and vimentin, to the clonality of the antibodies used and to the tumour areas studied. The latter could be impressively demonstrated by examining a whole tumour. In CRCC and oncocytoma all tumour cells expressed cytokeratins and, in addition, single tumour cells also expressed vimentin. In normal renal tissue we could show vimentin-positive epithelia of proximal and distal tubules, which is reported for the first time.

Key words: Normal renal tissue – Renal cell carcinoma – Cytokeratin – Vimentin – Desmoplakin

Introduction

In recent years some papers dealing with the intermediate filament typing of renal cell carcinomas (RCC) have been published, revealing a co-expression of cytokeratins and vimentin in tumours ranging from 52.5% (Waldherr and Schwechheimer 1985) to 90% (Herman et al. 1983). Moreover, in addition to the conventional histological subtypes of RCC a distinct entity, chromophobic cell renal carcinoma (CRCC), was introduced, which was described as being completely negative when tested with vimentin antibodies (Pitz et al. 1987; Thoenes et al. 1988). Interestingly, oncocytic tumours were also reported to lack vimentin and to express only cytokeratins (Pitz et al. 1987). Thus, it was found that RCC differ widely regarding co-expression of cytokeratins and vimentin. Desmosomal plaque proteins (desmoplakins) are proteins occurring mainly but not exclusively in epithelial cells with strong correlation to cytokeratins (see Moll et al. 1986 for further information). Therefore, desmosomal plaque proteins are commonly regarded as epithelial cell markers and could be found in all RCC investigated (Moll et al. 1986).

We examined 42 RCC and 1 oncocytoma by immunohistochemistry using mono- and polyclonal antibodies to cytokeratins, vimentin and desmoplakins in order to study (1) if the application of different cytokeratin and vimentin antibodies of various types provides additional information concerning the heterogeneity of RCC and (2) the distribution of desmosomes in different histological subtypes of RCC as well as in oncocytomas. For comparison normal renal tissue of 6 patients was also studied.

Materials and methods

Tissues were obtained immediately after surgery, frozen in isopentane cooled with liquid nitrogen and stored at −70° C for further processing. The histological classification of renal neoplasms was established according to the criteria of Thoenes et al. (1986) and is given in Table 1.

Table 1. Histological diagnoses and number of cases investigated

Normal renal tissue		6
RCC, clear cell type		32
typical variant	20	
eosinophilic variant	12	
RCC, chromophilic type		5
RCC, chromophobic cell type		3
RCC, spindle cell type		2
Oncocytoma		1

RCC, Renal cell carcinoma

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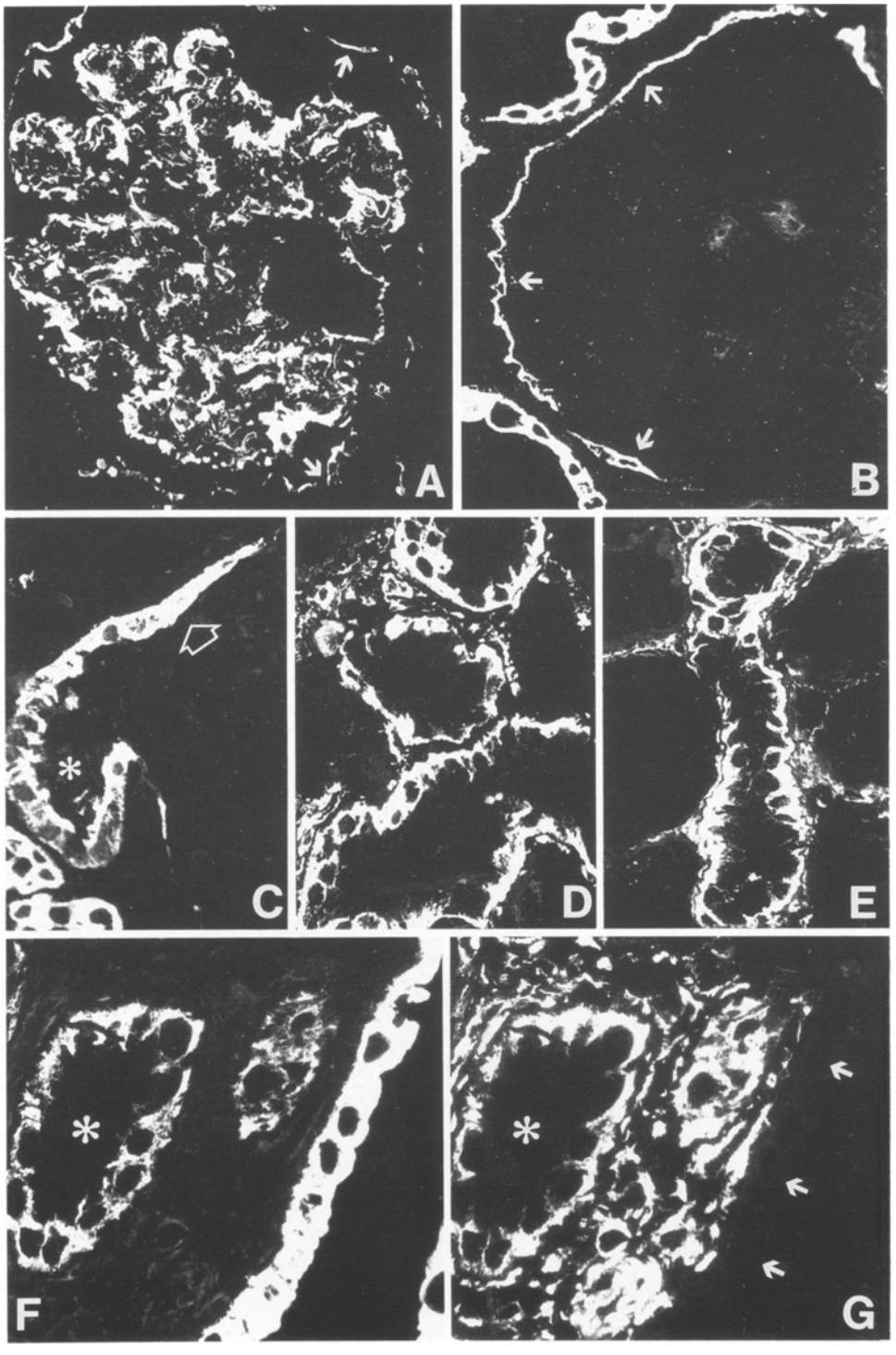

Fig. 1 A–G. Normal renal tissue. All glomerular cells are reactive for vimentin (**A**) but not for cytokeratin (**B**). The parietal cells of the Bowman's capsule (*arrows*), however, show positive staining for both vimentin (**A**) and cytokeratin (**B**). At the urinary pole cytokeratin-positive parietal cells of the Bowman's capsule (*arrow*) pass into cytokeratin positive cells of a proximal tubule (*asterisk*) (**C**). Vimentin expression in epithelia of proximal tubules is demonstrable by monoclonal (**D**) as well as polyclonal (**E**) antibodies. Epithelia of a collecting duct exhibit an intensive staining reaction for cytokeratin (**F**), but are not reactive for vimentin (**G**, *arrows*). Note both cytokeratin (**F**) and vimentin (**G**) positive epithelia of distal tubules (*asterisks*) nearby the collecting duct. **A–E** Immunofluorescence, ×125. **F, G** Double immunofluorescence, ×200. **A, D** Monoclonal vimentin antibody; **B** polyclonal cytokeratin antibody; **C** monoclonal cytokeratin antibody; **E** polyclonal vimentin antibody; **F** polyclonal cytokeratin antibody; **G** monoclonal vimentin antibody

Frozen sections, 4 μm thick, were fixed in acetone at −20° C, air-dried, and then incubated with primary and secondary antibodies as described previously (Denk et al. 1985). Indirect immunofluorescence microscopy (including double immunofluorescence) was performed. The following antibodies were used:

1. Polyclonal antibodies to mouse liver cytokeratin component D (anti-D from guinea pig); these antibodies detect a wide range of cytokeratins (Denk et al. 1981).

2. Monoclonal mouse antibodies to human cytokeratin polypeptide component no. 18 (anticytokeratin no. 18; Boehringer, Mannheim, FRG).

3. Polyclonal rabbit antibodies to vimentin from fibroblasts of the human aorta (Denk et al. 1985).

4. Monoclonal mouse antibodies to vimentin from a pig kidney epithelial cell line (Labsystems, Helsinki, Finland).

5. Monoclonal mouse antibodies to vimentin of porcine eye lens (clone V9, Boehringer).

6. Monoclonal rabbit antibodies to vimentin of calf lens (Eurodiagnostics, Apeldoorn, Netherlands).

7. Monoclonal antibodies (IgG) to desmoplakins I and II (Boehringer).

8. Fluorescence isothiocyanate or TRITC-coupled (Tetrameth-

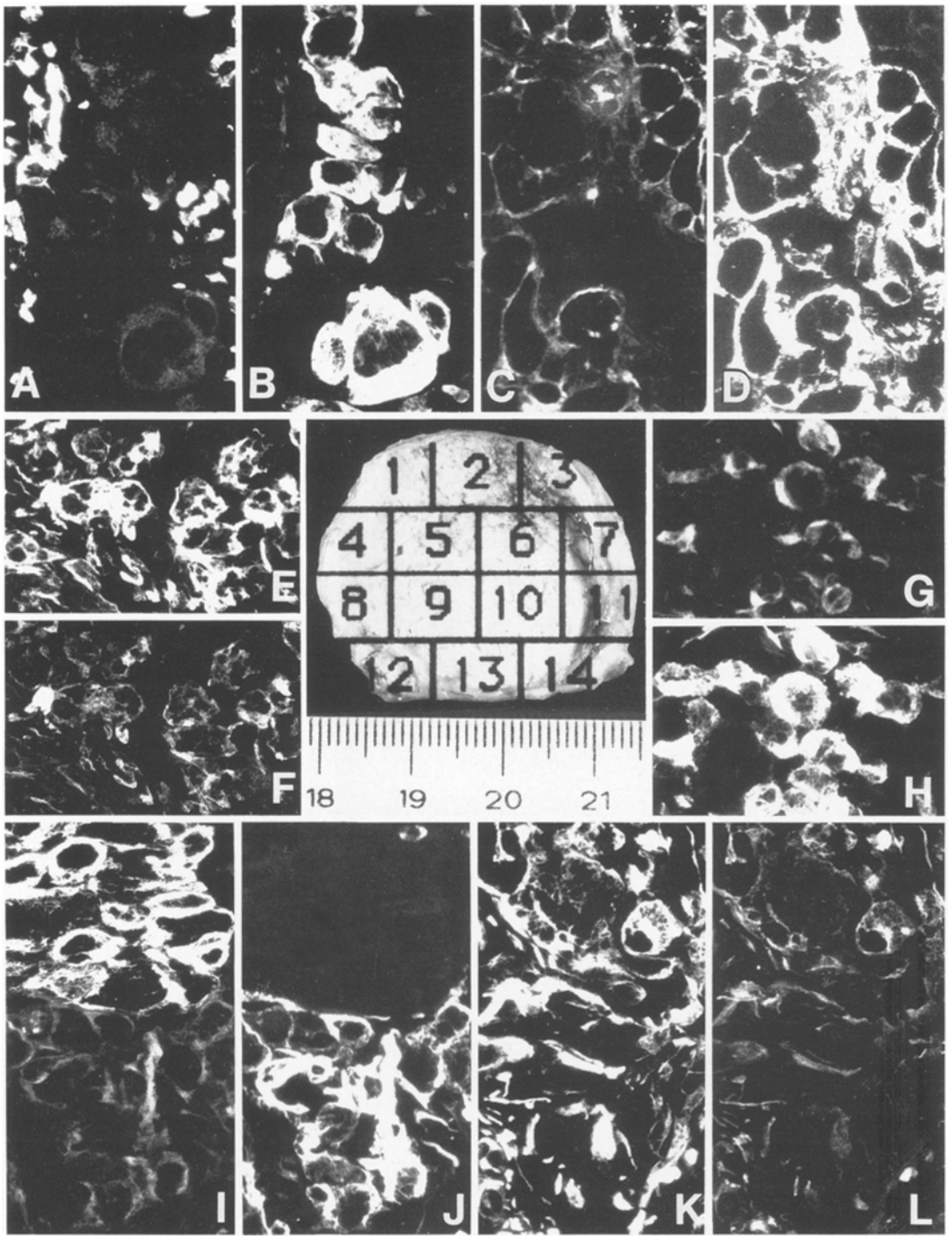

Fig. 2A–L. Immunophenotypic heterogeneity of renal cell carcinoma, clear cell type. Mode of working up the tumour: separation into 14 areas (*centre*). Vimentin-negative (**A**, monoclonal antibody) and cytokeratin-positive (**B**, polyclonal antibody) tumour cells (area 2). Cytokeratin-negative (**C**, polyclonal antibody) and vimentin-positive (**D**, monoclonal antibody) tumour cells (also area 2). Dividing area 6 into smaller subareas shows immunopositive cells for a polyclonal (**E**) and immunonegative ones for a monoclonal (**F**) vimentin-antibody in one subarea, whereas the tumour cells are negative or weakly positive for a polyclonal (**G**) and positive for a monoclonal (**H**) vimentin antibody in another subarea. Heterogeneity in intermediate filament expression (area 9) as demonstrated by cytokeratin-positive (*upper part*) and cytokeratin-negative cells (*lower part*) (**I**, polyclonal antibody) and the reverse for vimentin (**J**, monoclonal antibody). Tumour cells are positive for a monoclonal (**K**) and negative or weakly positive for a polyclonal (**L**) antibody to vimentin (area 14). **A, B** ×200; **C, D** ×200; **E, F** ×250; **G, H** ×250; **I, J** ×200; **K, L** ×200; double immunofluorescence

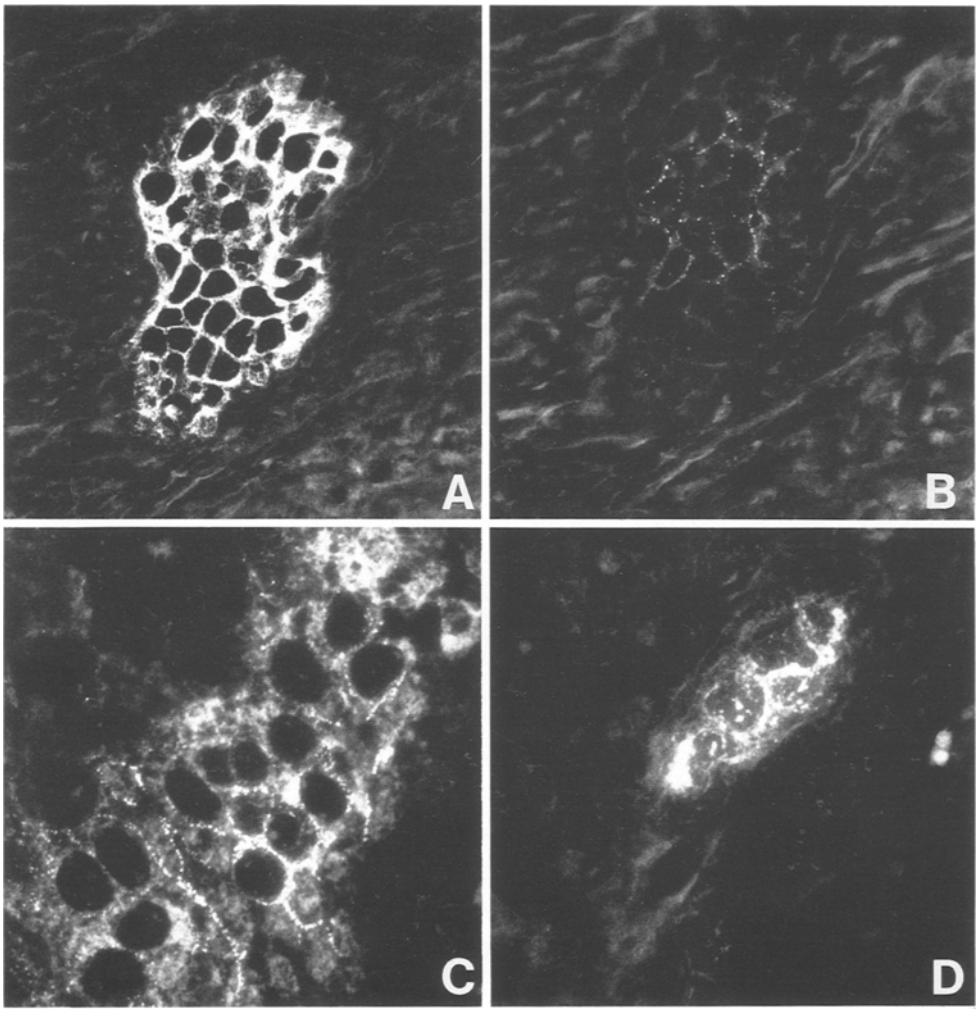

Fig. 3A–D. Desmoplakin reactivity of renal cell carcinoma, clear cell type. Only some cytokeratin-positive tumour cells (**A**) show a desmoplakin reactivity characterized by a punctate staining pattern at the cell periphery (**B**). In some areas the staining pattern for desmosomal plaque proteins exhibits in addition to a delicate punctation plumper immunoreactive spots (**C**) or an irregular staining pattern (**D**) quite different from the characteristic pattern (**B**). **A, B** ×200, double immunofluorescence; **C** ×400; **D** ×360

ylrhodamineisothiocyanate) antibodies to rabbit and guinea pig IgG from pig as well as to mouse IgG from rabbit (Dako, Denmark).

For control purposes, specific primary antibodies were substituted by pre-immune sera of the respective species or sera from non-immunized animals. Specimens were viewed and photographed using a fluorescence microscope with epi-illumination (Zeiss Photomikroskop III) and Kodak Tri-X-Pan black and white films.

Results

In all specimens of normal renal tissue ($n=6$; Fig. 1) a similar immunoreactivity could be seen. The glomeruli were entirely negative for cytokeratins. However, cells of the parietal Bowman's capsule, closely situated to the urinary pole, showed a positive staining reaction with both antibodies to cytokeratin. Thus, a hemispheric pattern of cytokeratin-positive cells of the Bowman's capsule could be demonstrated. Moreover, these cells were also decorated by antibodies to desmoplakins in a punctate pattern. All vimentin antibodies strongly decorated podocytes, endothelial cells of capillaries and mesangial cells of the glomeruli as well as cells of the Bowman's capsule. The epithelia of most tubules revealed a positive immunoreaction with the cytokeratin antibodies mainly at the basolateral aspect of the cells. However, differences were found in staining intensity from weak to moderate. In some tubules, apparently of collecting ducts, all cells showed strong cytokeratin reactivity. The epithelia of some proximal and distal tubules, except the collecting ducts, were also decorated by mono- and polyclonal vimentin antibodies. All tubular epithelia showed a positive reaction with the antibodies to desmoplakins exhibiting a punctate staining pattern at the laterobasal aspect of each cell.

Most tumours of the conventional clear cell type ($n=32$) showed a moderate to strong immunoreaction with the cytokeratin antibodies used but in one case the cells were entirely negative for both cytokeratin antibodies but positive for vimentin antibodies. The vimentin antibodies revealed a weak to strong immunoreaction in most cases; in some cases a pronounced heterogeneity of antigen expression was seen and negative and positive cells co-existed in the same area. In two cases the polyclonal vimentin antibody revealed a negative result, whereas a positive immunoreaction could be demonstrated with monoclonal vimentin antibodies. Tissue of one case was divided in 14 blocks and incubated with all antibodies. It was found that all tumour cells reacted with the cytokeratin antibodies but staining intensity dif-

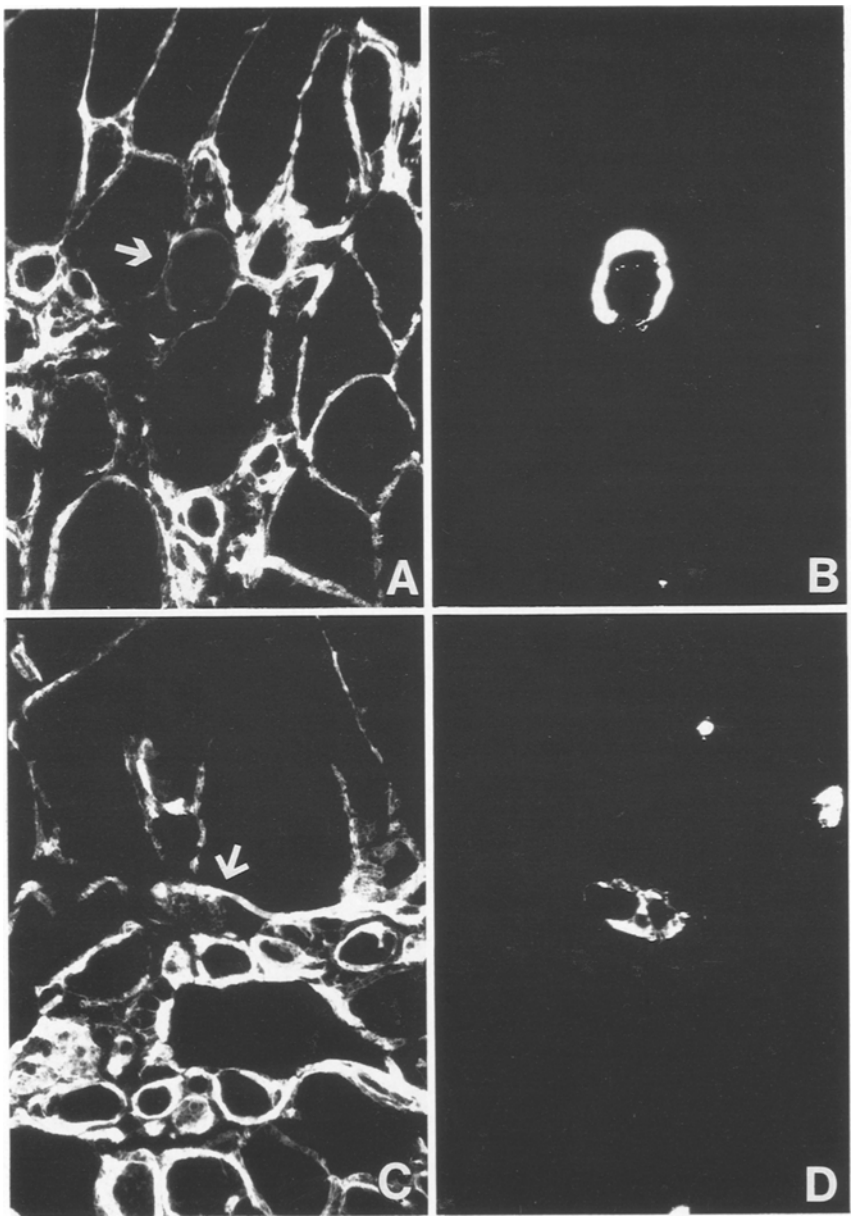

Fig. 4A–D. Vimentin-reactivity in chromophobic cell renal carcinoma. All tumour cells are cytokeratin-positive (**A, C**), whereas only single cells show a simultaneous immunoreactivity for vimentin (**B, D**). The reactivity for vimentin is different compared to that of cytokeratin (cytokeratin-positive cells are marked by *arrows*). **A, B** ×400, double immunofluorescence; **C, D** ×320, double immunofluorescence

fered not only from one area to another but also within one area. This was also true for the vimentin antibodies even in a more pronounced way. Moreover, there was also a difference between poly- and monoclonal vimentin antibodies in that sometimes the polyclonals revealed a negative or weak immunostaining, whereas the monoclonals showed moderate or even strong staining reaction (or vice versa) in the same cells as revealed by double immunofluorescence staining (Fig. 2).

In most cases of the eosinophilic variant of clear cell carcinoma all tumour cells were moderately to strongly decorated by the cytokeratin antibodies. However, in one case the tumour cells did not react with the cytokeratin antibodies used; in another case tumour cells showed a weak to moderate immunostaining with the broad range cytokeratin antibodies and a focally negative or weak one with the anticytokeratin no. 18 antibody. With the vimentin antibodies many tumours revealed moderate to strong staining. In some tumours, however, the cells were non-reactive with vimentin antibodies (in contrast to a strong cytokeratin expression) or showed a negative result with the polyclonal vimentin antibody and positive staining with the monoclonals, or revealed a heterogenous staining pattern with alternating negative and positive cells or cell groups.

In all cases of the clear cell variant desmosomal staining was very irregularly arranged (Fig. 3). Mostly a close association between cytokeratin and desmoplakin positivity of tumour cells could be demonstrated; in some cases, however, single cell groups displayed desmoplakin negativity in cytokeratin-positive cells, or vice versa cytokeratin-negative cells were positive with the antibodies to desmoplakins used. A similar situation was apparent between vimentin and desmoplakins.

All tumours of the chromophilic type ($n=5$) reacted with the cytokeratin antibodies used. The polyclonal cy-

tokeratin antibody usually revealed a strong reaction and the monoclonal a moderate staining reaction. However, in one case in some areas of the tumour a negative immunostaining with the monoclonal antibody could be demonstrated. Desmosomes were distributed on the surface of most tumour cells in an irregular pattern. All vimentin antibodies showed a positive immunoreaction with the tumour cells, but the staining differed in intensity from weak to strong.

All tumour cells of the chromophobic cell type ($n=3$) were moderately to strongly decorated by the antibodies to cytokeratins. Conversely, the neoplastic cells revealed no immunoreaction with the vimentin antibodies used, except a few tumour cells which showed a weak to moderate staining reaction. In double immunofluorescence microscopy the scarce vimentin-positive cells corresponded to cytokeratin-positive cells indicating a co-expression (Fig. 4). Desmosomes were demonstrable in almost all tumour cells, but in irregular distribution.

All the spindle cell type tumour cells ($n=2$) failed to react with the cytokeratin antibodies. By contrast, most neoplastic cells were moderately to strongly stained by all monoclonal vimentin antibodies. With the polyclonal vimentin antibody many tumour cells remained unstained; in some cells, however, a weak to moderate immunoreactivity could be demonstrated. Despite cytokeratin negativity, desmosomes were irregularly distributed; in some areas the neoplastic cells contained many desmosomes, in others only few or even no desmosomes were found.

In the one case of oncocytoma studied, all tumour cells were moderately to strongly decorated by antibodies to cytokeratins. Only scattered tumour cells co-expressed vimentin. With desmoplakin antibodies an irregular dot-like immunoreaction at the cell periphery could be demonstrated.

Discussion

It is well known that RCC contain cytokeratin- and vimentin-type intermediate filaments and this is confirmed by our present study. However, there is still controversy about the number of cytokeratin- and/or vimentin-positive tumours. Thus, cytokeratin-positive tumours were reported to range from 78% (Cohen et al. 1988) to 100% (Waldherr and Schwechheimer 1985; Fleming and Symes 1987), vimentin-positive tumours from 53% (Holthöfer et al. 1983) to 100% (Herman et al. 1983; Oosterwijk et al. 1990) and tumours with co-expression of these intermediate filaments from 52.5% (Waldherr and Schwechheimer 1985) to 90% (Herman et al. 1983). When reviewing the respective reports in detail the given figures were found to be based on studies with different methodology. Most of the authors performed their investigations on sections of liquid-nitrogen-frozen material, but some on formalin-fixed, paraffin-embedded tissues (Fleming and Symes 1987; Cohen et al. 1988; Donhuijsen and Schulz 1989). In this context it must be emphasized that the latter procedures may adversely influence antigenicity (Altmannsberger et al. 1981). For example, investigating frozen material Herman et al. (1983) found 92% of all RCC to be vimentin positive, whereas Donhuijsen and Schulz (1989) reported 30% vimentin-positive tumours examining formalin-fixed, paraffin-embedded tissue. Moreover, different antibodies, particularly to cytokeratins, with different antigen specificities were used. Waldherr and Schwechheimer (1985), for instance, used a monoclonal antibody recognizing epitopes on cytokeratins with molecular weights of 44, 46, 52 and 54 kDa, Fleming and Symes (1987) a monoclonal antibody (CAM 5.2) reacting with cytokeratins with molecular weights of 39, 43 and 50 kDa. Holthöfer et al. (1983) applied antibodies to human epidermal prekeratins, whereas Herman et al. (1983) used antibodies to cytokeratins of glandular epithelium. In addition, the antibodies used were either monoclonal or polyclonal (Herman et al. 1983; Donhuijsen and Schulz 1989). Therefore, the results reported could hardly be compared with each other. The same holds true for oncocytomas exhibiting no immunoreactivity for cytokeratins and vimentin in paraffin-embedded material (Holthöfer et al. 1987), but displaying a strong reactivity with antibodies to cytokeratins in frozen tissues (Pitz et al. 1987 and present study). Moreover, the immunoreaction was negative with antibody PKK1 (Holthöfer 1987) and positive with CAM 5.2 (Fleming and Symes 1987).

In 1987 Pitz et al. published a seminal paper on the expression of intermediate filaments in RCC and oncocytomas (including cytokeratin subtyping) demonstrating that generally all RCC co-express cytokeratins and vimentin. However, in some histological subtypes of RCC, as well as in oncocytomas, the tumour cells were found to contain only cytokeratins (CRCC) or vimentin (pleomorphic carcinoma respectively). These results are confirmed by our study but, additionally, we were able to show single cells of CRCC and oncocytomas with co-expression of cytokeratin and vimentin which, to our knowledge is reported for the first time. In view of vimentin expression of collecting duct epithelia in fetal kidneys (Moll et al. 1991), these findings may be explained as tumour phenomenon showing recurrence of intermediate filaments of fetal stage.

By examining a large number of block samples of RCC, clear cell type, we detected a considerable heterogeneity of the tumours with respect to the intermediate filament expression. Thus, we demonstrated different immunoreactivity when using mono- and polyclonal antibodies to the same intermediate filament protein. Interestingly, in general immunostaining was much more pronounced when the polyclonal cytokeratin antibody was used as compared with the monoclonal cytokeratin antibody, but the reverse was true with the antibodies to vimentin. However, the monoclonal cytokeratin antibody used was directed to cytokeratin no.18, whereas the polyclonal one was a broad range antibody recognizing all cytokeratins found in RCC by cytokeratin mapping (namely nos. 7, 8, 18, 19; Pitz et al. 1987). By working up a whole histologically homogenous tumour in numerous tissue blocks we could not only demonstrate a heterogeneous intermediate filament expression with regard to the distribution of cytokeratins and vimentin,

and to the clonality of the antibodies used but, most strikingly, it became evident that the immunoreaction depended largely on the tumour areas investigated. Thus, when compared to each other one could find areas positive or negative for cytokeratins and/or vimentin without any regularity which could also be detected in a similar way within one area. This has been reported for the first time in the literature and is important because it may be another explanation for the varying number of cytokeratin- and/or vimentin-positive tumours reported. It is obvious, therefore, that the number of tissue blocks examined could significantly influence the end result.

By examining normal renal tissue for comparison we found some interesting details. The epithelia or some proximal and distal tubules, except collecting ducts, immunoreacted with mono- and polyclonal antibodies to vimentin. This is demonstrated for the first time in the literature and is in contrast to previous investigations (Bachmann et al. 1983; Holthöfer et al. 1984; Oosterwijk et al. 1990). However, our findings parallel the expression of vimentin in the tubular system of fetal kidneys (Moll et al. 1991) and may be an explanation for the vimentin positivity of conventional RCC, since they are thought to originate from the proximal tubular system (Holthöfer et al. 1983). On the other hand, the collecting ducts, commonly regarded as histogenetic origin for CRCC and oncocytomas (Zerban et al. 1987; Ortmann et al. 1988; Thoenes et al. 1988), reflected the respective tumours in the absence of vimentin.

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