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Intermediate-filament expression in thyroid gland carcinomas*

Sören Schröder[1], **Barbara Dockhorn-Dworniczak**[2], **Hartwig Kastendieck**[3], **Werner Böcker**[4], **and Werner W. Franke**[2]

[1] Institute of Pathology (Director: Prof. Dr. G. Seifert), University of Hamburg, Martinistrasse 52, D-2000 Hamburg 20,
[2] Division of Membrane Biology and Biochemistry (Head: Prof. Dr. Werner W. Franke), Institute of Cell and Tumour Biology, German Cancer Research Center, D-6900 Heidelberg
[3] Department of Pathology, General Hospital Hamburg-Harburg (Head: Prof. Dr. H. Kastendieck), D-2100 Hamburg 90
[4] Department of Pathology, General Hospital Hamburg-Altona (Head: Prof. Dr. W. Böcker), D-2000 Hamburg 50, Federal Republic of Germany

Summary. Paraffin-embedded specimens of 200 primary thyroid carcinomas were examined immunohistologically for the expression of intermediate-filament (IF) protein of the cytokeratin, vimentin and neurofilament type. In 36 cases, snap-frozen tissue was available, and double label immunofluorescence microscopy was performed in 23 of them.

Cytokeratin reactivity was found in all cells of all follicular, papillary and medullary carcinoma cases examined. Using a monoclonal vimentin antibody, positive staining was found in many, though not all cells of the papillary tumours and in approximately 50% of the follicular and the medullary carcinomas. Among anaplastic carcinomas, some tumours were positive for cytokeratins, with or without coexpression of vimentin. Neurofilaments could only be demonstrated in approximately 13% of medullary tumours which in general also exhibited vimentin positivity.

The differences of IF expression in follicle and C-cell thyroid carcinomas and the broad variation of cytokeratin and vimentin immunoreactivity among anaplastic tumours of this organ is discussed in relation to the possible intrinsic heterogeneity of these tumours and the diagnostic value of these markers.

Key words: Thyroid carcinoma – Intermediate filaments – Keratin – Vimentin – Neurofilaments

Introduction

Based on their immunoreactivity, intermediate filaments (IF) have been divisible into 5 major categories of constituent proteins: cytokeratins, found

* Dedicated to Prof. Dr. G. Seifert on the occasion of his 65th birthday

Offprint requests to: S. Schröder at the above address

in all epithelial cells; vimentin, characteristically but not exclusively found in mesenchymal cells; desmin, characteristic of smooth and striated muscle cells; glial filament protein, characteristic of astrocytic glial cells; and neurofilament proteins, typically found in neurons (Franke et al. 1978; Osborn and Weber 1983). Vimentin, desmin, glial filament protein and the neurofilament 68 kd polypeptide NF-L all can form IF consisting of only one type of subunit protein. In contrast, the cytokeratin filaments are obligatory heteropolymers and represent a complex family of at least 19 different polypeptides (Moll et al. 1982). Neurofilaments often appear as heteropolymers of three subunits of 68, 160 and 200 kd (NF-L, NF-M, NF-H; Osborn and Weber 1983).

Cell-type specific IF protein expression is generally maintained in neoplastic cells (Gabbiani et al. 1981; Moll et al. 1982, 1983; Osborn and Weber 1983), and human tumours can be subdivided according to the IF type present: For example, carcinomas can be identified by cytokeratin antibodies; melanomas, malignant lymphomas and non-muscular sarcomas by vimentin antibodies; myosarcomas by desmin antibodies; and diverse tumours of neural origin by neurofilament antibodies.

There is, however, an increasing number of reports presenting evidence of co-expression of two distinct classes of IF proteins in certain human tumours, which may reflect the continuation of a type of co-expression present in the normal, i.e. non-neoplastic cell (e.g., LaRocca and Rheinwald 1984; Czernobilsky et al. 1985; McNutt et al. 1985) or a change of expression of IF proteins in neoplastic cells as compared with their non-neoplastic counterparts (Gould 1985). This also applies to the thyroid gland for which Miettinen et al. (1984) have reported co-expression of cytokeratins and vimentin in all types of thyroid carcinoma. These findings, however, were based on a relatively small number of cases. Since some of these findings reported by Miettinen et al. (1984) were at variance with some of our own observations we wish to communicate our findings obtained from a large scale of organoid, anaplastic and medullary carcinomas.

Materials and methods

Paraffin blocks of 200 thyroid carcinomas (50 papillary, 50 follicular, 40 anaplastic, and 60 medullary tumours) were retrieved from the surgical pathology files of the Institute of Pathology, University of Hamburg, and of the Department of Pathology, General Hospital Hamburg-Harburg. All specimens had been fixed in 10% buffered formalin, i.e. approximately 3.7% formaldehyde, and processed for embedding in routine manner. In 36 cases, snap-frozen (isopentane cooled with liquid nitrogen) tumour tissue (stored at $-60°$ C until use) was also available for study.

Immunohistological studies on formalin-fixed material and acetone-fixed cryostat sections obtained from the frozen specimens were performed using the Avidin-Biotin-Peroxidase Complex (ABC)-system (Hsu et al. 1981). Indirect immunofluorescence microscopy was performed essentially as previously described (Franke et al. 1979a, b; 1980). For double immunofluorescence microscopy, both primary antibodies were applied simultaneously, as were the specific secondary antibodies (Schmid et al. 1980; Moll et al. 1984).

The following antibodies were used: (*1*) commercially available monoclonal broad range cytokeratin antibody (KL1, Dianova; cf. Viac et al. 1983); (*2*) murine monoclonal antibody

K_G 8.13 which reacts with a relatively large number of cytokeratins (Gigi et al. 1983); (*3*) vimentin monoclonal antibody (V9, Boehringer, Mannheim; cf. Osborn et al. 1984); (*4*) guinea pig antibodies to vimentin (Franke et al. 1979c); (*5*) a pool of monoclonal neurofilament antibodies constituted by equal ratios of antibodies to NF-L, NF-M and NF-H (Dianova); (*6*) guinea pig antibodies raised against gel electrophoretically separated polypeptide NF-H of porcine brain neurofilaments which also react with human neurofilaments (Denk and Franke 1982).

The immunoperoxidase techniques were performed with antibodies (*1*), (*3*) and (*5*) whereas antibodies (*2*), (*3*), (*4*) and (*6*) were used for the immunofluorescence procedures.

Results

Follicular carcinomas

Immunoperoxidase techniques. Immunoreactivity with the monoclonal cytokeratin antibody KL1 was found in each of the 50 paraffin-embedded tumours, and cytokeratin positivity was demonstrated for all neoplastic thyrocytes in these lesions. Twelve cases showed a predominantly diffuse cytoplasmic immunoreaction. The remaining 48 tumours showed an enrichment of the staining near the cell plasma membranes (Fig. 1 a). Vimentin immunoreactivity was demonstrated in 22/50 carcinomas; 13 of them displayed a cap-like positivity in the basal aspect of most of the tumour cells, and 9 showed more extended cytoplasmic staining in focal patterns. The remaining 28 tumours were devoid of vimentin immunoreaction (Fig. 1 b). No correlation was found between vimentin expression and the degree of differentiation or variation in growth pattern of the specific tumour. Neurofilament expression was not found in any of the follicular carcinomas.

In 5 of the cases, frozen material was also available, including 2 tumours that showed co-expression of vimentin and cytokeratins. In each tumour identical results were obtained with paraffin and cryostat sections.

Immunofluorescence microscopy. Three follicular carcinomas were studied by immunofluorescence microscopy. Each case showed intense cytoplasmic cytokeratin positivity. Positive vimentin immunostaining was seen with the monoclonal antibody used. With the guinea pig vimentin antisera, however, the tumour cells appeared to be devoid of immunoreaction whereas positive vimentin staining was seen in the tumour stroma. An example of double label immunofluorescence microscopy, which was performed in all 3 cases, is given in Fig. 2. No reaction was found with antibodies against neurofilaments.

Papillary carcinomas

Immunoperoxidase techniques. In each of the 50 tumours examined, cytokeratin and vimentin positivity was found. As with the follicular carcinomas, cytokeratin positivity was demonstrable for all tumour cells, usually (34/50) in a diffuse cytoplasmic staining pattern (Fig. 1 c). Vimentin was concen-

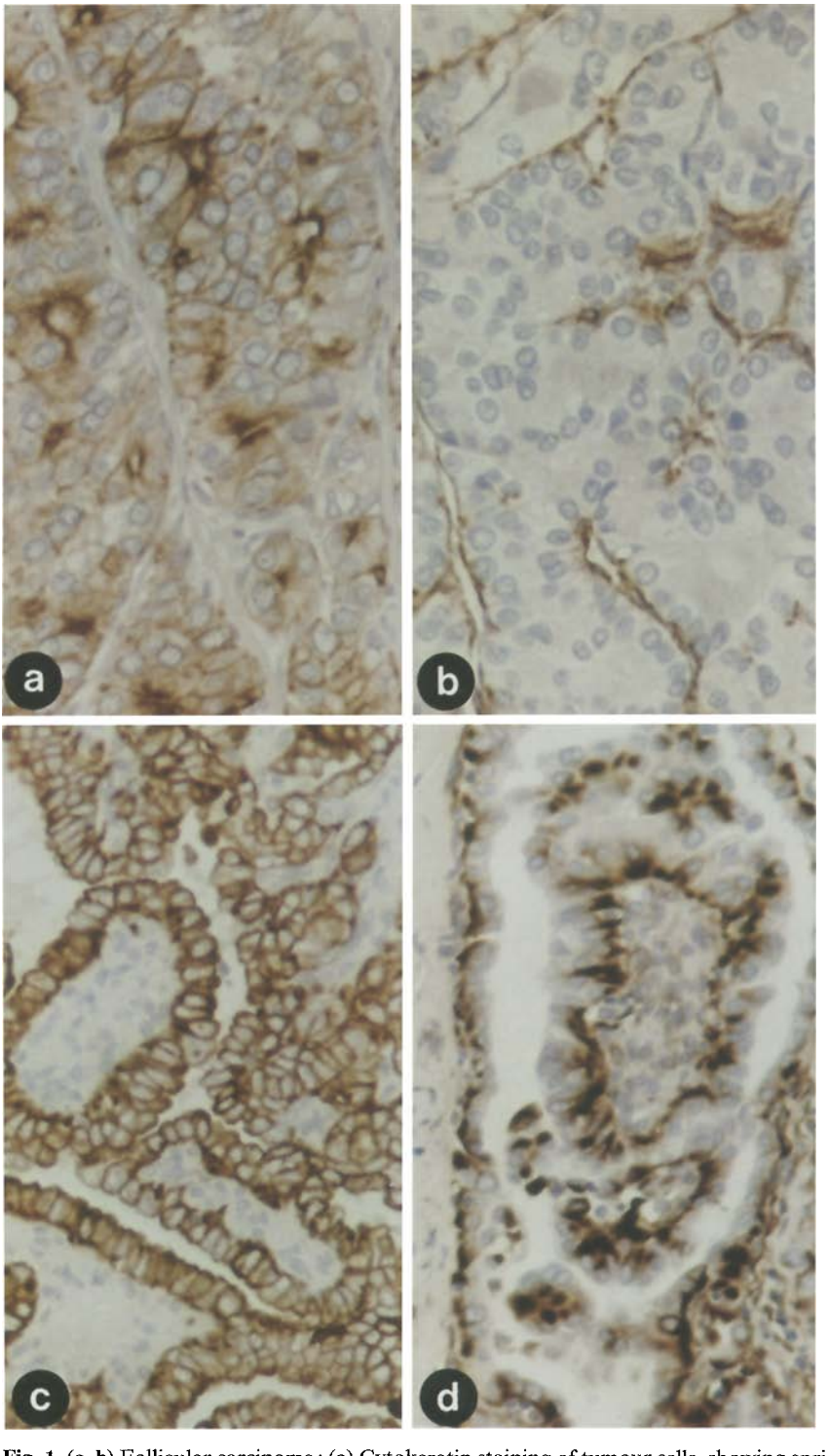


Fig. 1. (**a, b**) Follicular carcinoma: (**a**) Cytokeratin staining of tumour cells, showing enrichment near cell membranes; (**b**) vimentin positivity only seen in stromal cells. (**c, d**) Papillary carcinoma: (**c**) diffuse cytoplasmic keratin positivity; (**d**) cap-like vimentin positivity in the basal cytoplasm of tumour epithelium (all magn., ×288)

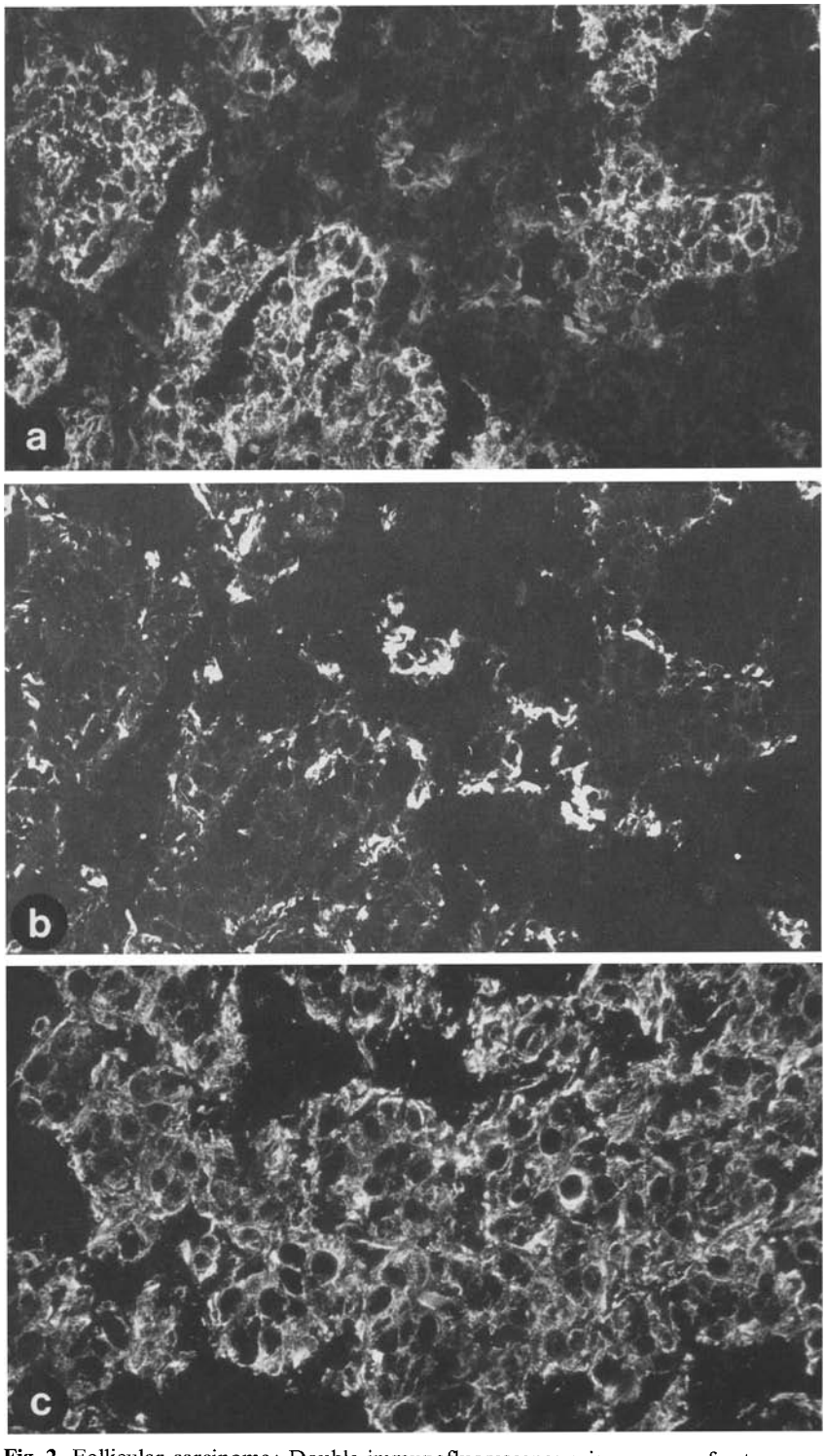


Fig. 2. Follicular carcinoma: Double immunofluorescence miscroscopy of a tumour showing **(a)** positivity of tumour cells for cytokeratin, whereas **(b)** vimentin can only be detected in the stroma (guinea pig vimentin antiserum). In contrast, **(c)** strong vimentin positivity of tumour epithelial cells is seen after application of the monoclonal vimentin antibody (each $\times 250$)

trated in the basal cytoplasm in the majority of the tumour cells, resulting in the appearance of basal cap-like aggregates (Fig. 1 d). None of the 50 papillary carcinomas revealed neurofilament reactivity.

Immunofluorescence procedures. Immunofluorescence microscopy of 10 papillary carcinomas gave identical results to the 3 follicular tumours: Strong positivity was found for cytokeratin, whereas significant vimentin reaction in tumour cells was only obtained with the monoclonal antibody. Double immunofluorescence using the guinea pig antisera gave no significant vimentin immunoreaction. Likewise, neurofilaments were not detected in these neoplasias.

Anaplastic tumours

Immunoperoxidase techniques. Of 40 anaplastic tumours, 16 gave immunoreaction for cytokeratin and 31 for vimentin, all displaying a rather disperse cytoplasmic staining. Seven cases exhibited positivity for both cytokeratin and vimentin. Vimentin generally was demonstrable in the majority of tumour cells, whereas cytokeratin-positive cells were found only focally. Neurofilament-positive cells were not seen in any of these tumours.

Figure 3 shows the local recurrence of a papillary carcinoma 4 years after surgery. With the cytokeratin antibodies used in this study pronounced immunoreaction is seen only within residual regions of papillary differentiation. In contrast, vimentin was the only IF protein detected in areas of anaplastic appearance.

Examination of frozen sections gave similar results. All cases that were positive for cytokeratins in paraffin sections were also positive in frozen sections, when available. However, of the 5 tumours negative for cytokeratin in paraffin sections 3 turned out to be positively stained with cytokeratin antibodies when examined on frozen sections. The remaining 2 cases were also not significantly stained with cytokeratin antibodies in frozen sections although they were positive with vimentin antibodies.

Immunofluorescence procedures. Double label immunofluorescence microscopy was performed on a total of 5 tumours. Of these, 4 tumours revealed co-expression of cytokeratins and vimentin in some but not all tumour cells (Fig. 4), whereas vimentin positivity only was observed in the one remaining case. Neurofilament-positive cells were not detected.

Medullary carcinomas

Immunoperoxidase techniques. Cytokeratin immunoreaction was demonstrated in all 60 medullary carcinomas of which paraffin material was available, displaying an intense and general cytoplasmic positivity in all tumour cells (Fig. 5a). Vimentin could be detected in a variable proportion of tumour cells of 32 and neurofilament proteins in some tumour cells of

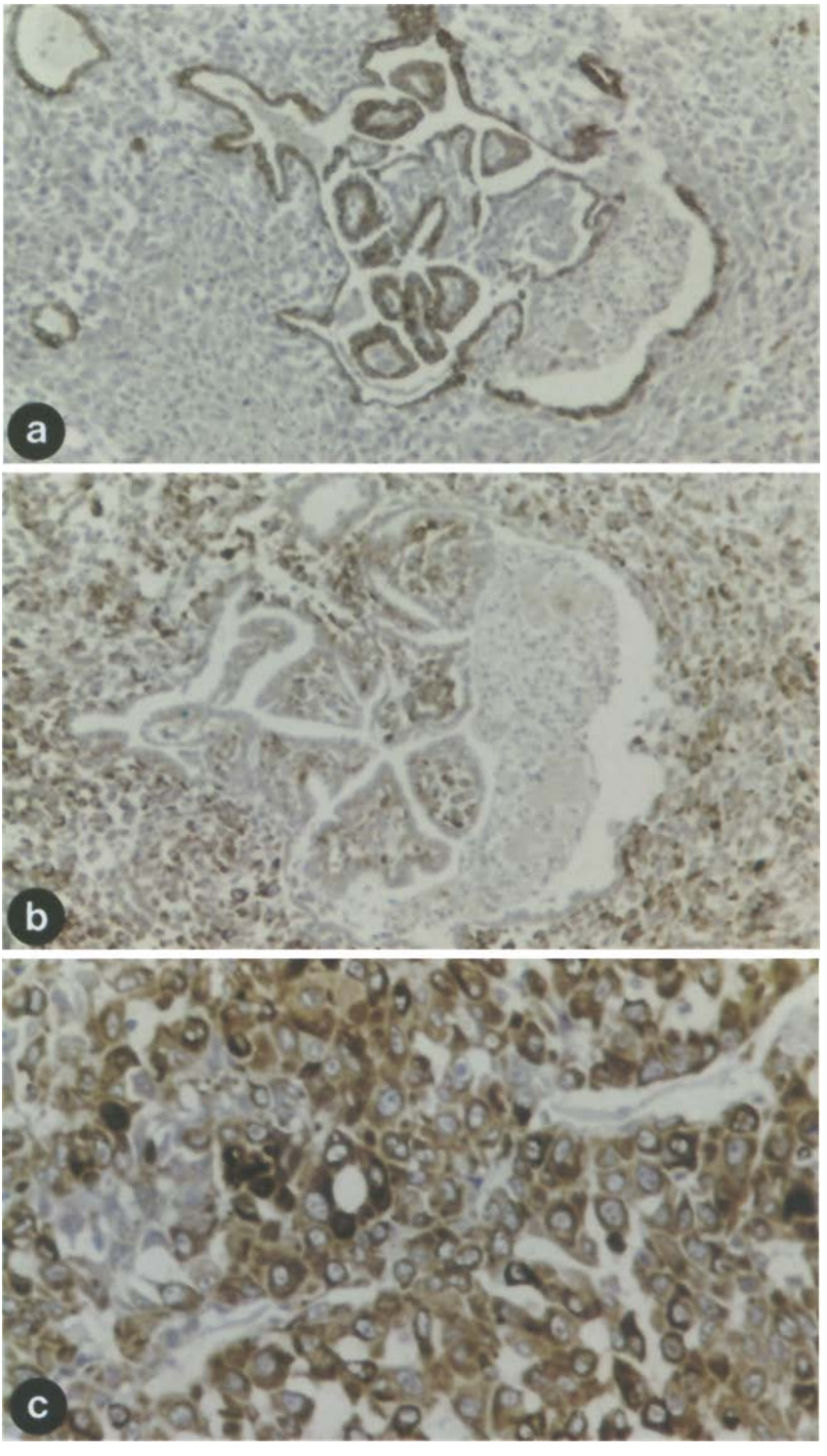


Fig. 3. Papillary carcinoma with local recurrence of anaplastic histology: (**a**) Markedly positive cells for cytokeratin are seen only in cells of regions showing residues of papillary differentiation ($\times 115$); (**b**) shows coexpression of vimentin ($\times 115$); (**c**) strong diffuse cytoplasmic vimentin positivity within anaplastic areas ($\times 288$)

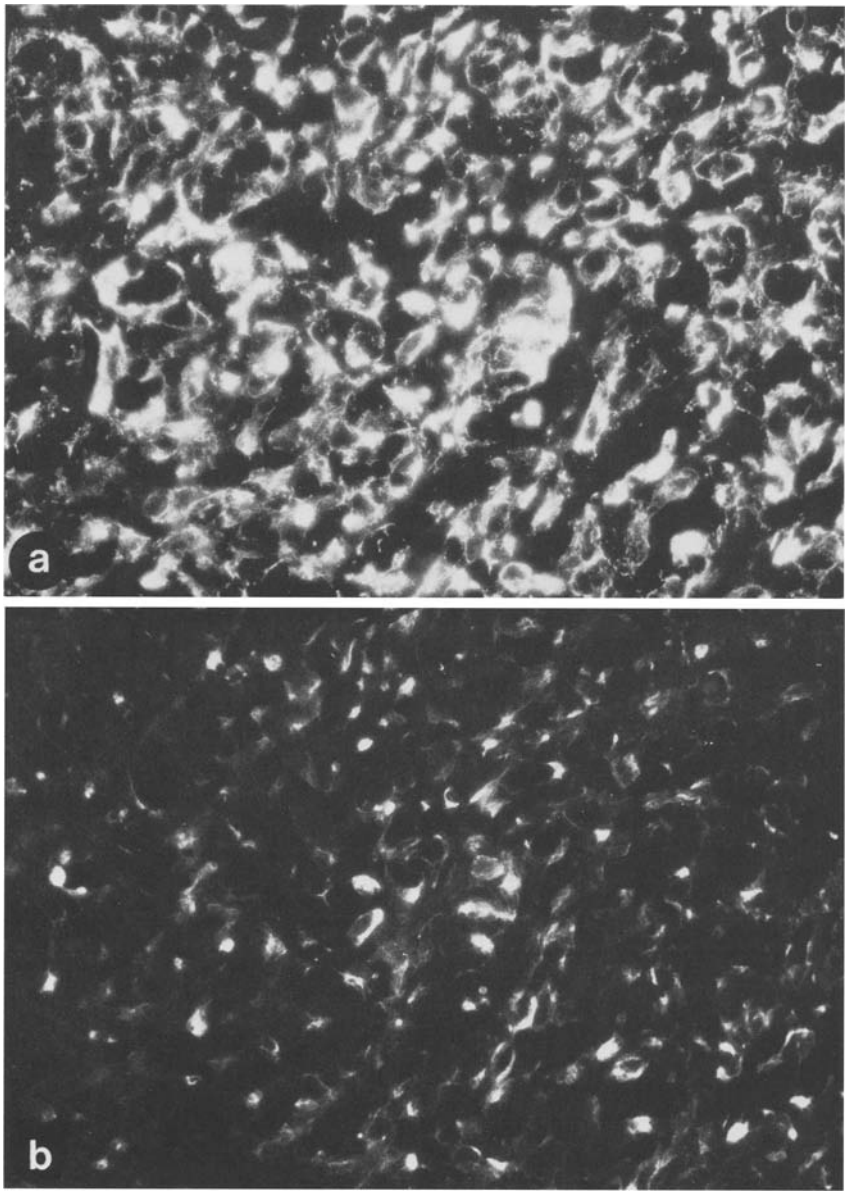
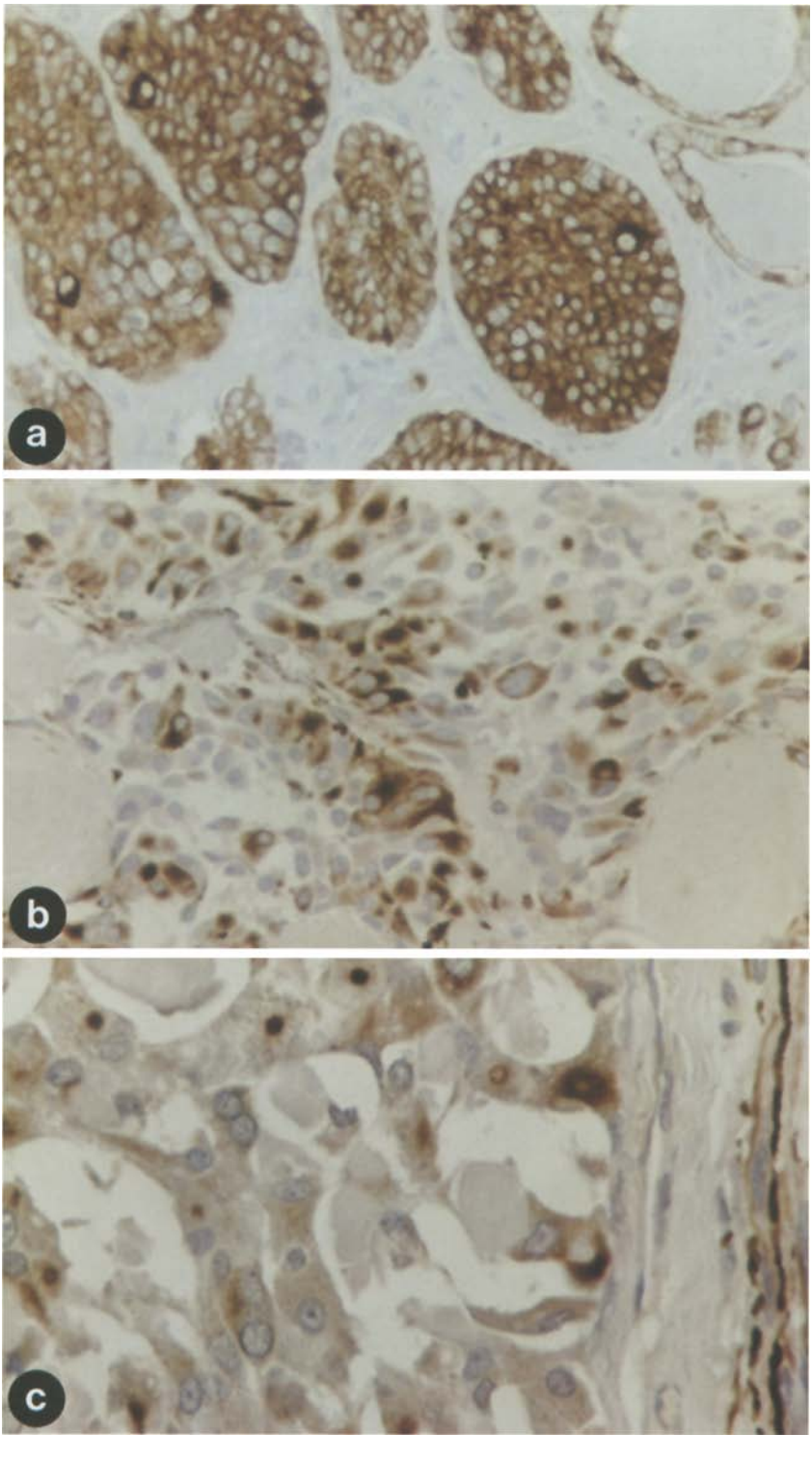


Fig. 4. Anaplastic carcinoma: Double immunofluorescence microscopy displays (**a**) strong cytokeratin positivity of all tumour cells, some of which also express vimentin (**b**; × 250)

Fig. 5. Medullary carcinoma: (**a**) diffuse cytoplasmic cytokeratin positivity of all tumour cells (residual follicles also stained); (**b**) vimentin co-expression in many of the tumour cells (grey areas represent amyloid); (**c**) some tumour cells showing positivity for neurofilament proteins (nerve fibres are also seen on the right side) (each × 288)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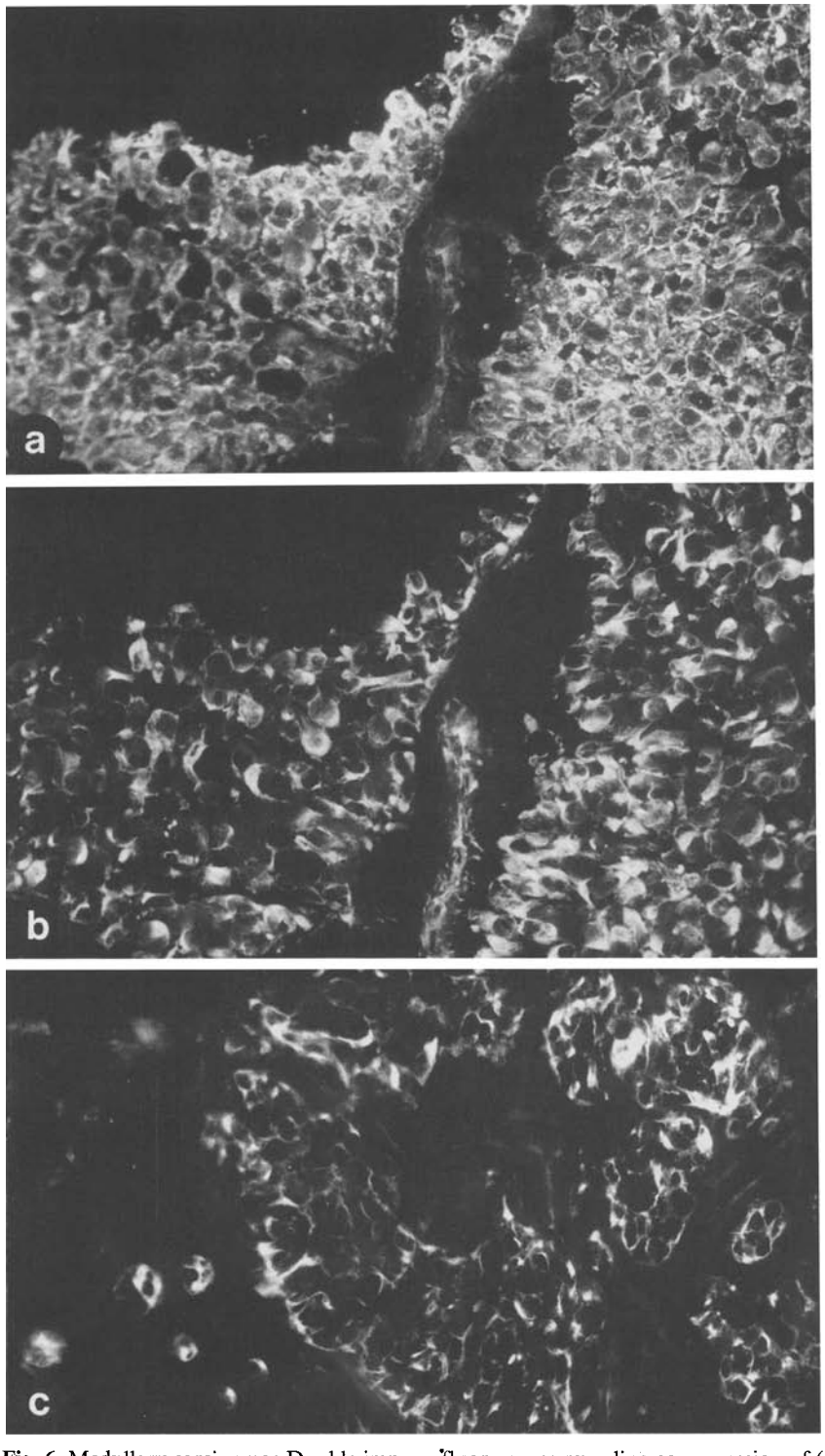

Fig. 6. Medullary carcinoma: Double immunofluorescence revealing co-expression of (**a**) cytokeratin and (**b**) vimentin; (**c**) positivity for neurofilaments seen in several tumour cells (each $\times 250$)

8 cases (Fig. 5b, c). Each of the tumours containing neurofilament-positive cells also contained a number of cells positive for vimentin. Identical results were obtained on cryostat sections in 2 of the tumours, both of them expressing only cytokeratin.

Immunofluorescence microscopy. Five cases were studied by double label immunofluorescence microscopy, and all cells were found to be cytokeratin-positive. Co-expression of vimentin was demonstrated in 3 of these carcinomas in which the majority of tumour cells was positive (Fig. 6a, b). One of these tumours also contained neurofilament-positive cells (Fig. 6c). The 2 tumours that did not show significant vimentin immunoreaction with the guinea pig antisera in double label immunofluorescence microscopy revealed a certain proportion of vimentin-positive cells when the monoclonal vimentin antibody was used.

Discussion

There are only few reports in the literature dealing with the expression of IF proteins in thyroid gland tumours, and all of them present only relatively low numbers of cases. Our present study is based on a large and representative number of diverse kinds of thyroid carcinomas and on the concerted application of different immunohistochemical techniques and different antibodies to IF proteins.

Basically, our results are in agreement in many respects with those of Miettinen et al. (1984). Specifically we show that cytokeratin antibodies are reliable markers for the identification of the epithelial nature of follicular, papillary and medullary carcinomas as well as of many of the anaplastic tumours. In our study, identical results were obtained by using two different monoclonal cytokeratin antibodies, which is most likely due to their broad range of cross-reactivity between different polypeptides of the cytokeratin family. Alternatively, antibodies to cytokeratins nos. 8 and/or 18 (cf. Moll et al. 1982) may be used in carcinomas of the thyroid gland as these are the major cytokeratins biochemically detected in these tumours (B. Dockhorn-Dworniczak and W.W. Franke, unpublished results). Permanetter et al. (1982) and Yagi et al. (1985) both reported cytokeratin negativity on paraffin-embedded follicular carcinomas, using insufficiently characterized antibodies. Similarly Miettinen et al. (1984) failed to demonstrate cross-reactivity with epidermal prekeratins in certain follicular thyroid tumours. Such negative results are easily understandable, considering the great differences between the cytokeratin polypeptide complements expressed in epidermis simple glandular epithelial cells such as thyrocytes (for review see Moll et al. 1982).

Remarkably, a sizeable proportion of the anaplastic tumours examined in this study were not significantly stained with cytokeratin antibodies on paraffin sections. A similar observation has been reported by Carcangiu et al. (1985) who found positivity for cytokeratin in less than half of the anaplastic carcinomas tested. In the present study, of five tumours that

were cytokeratin-negative in paraffin sections three revealed a positive reaction when cryostat sections were examined, whereas two were negative even with this technique. This result provides a caveat in the interpretation of negative immunohistochemical results on anaplastic tumours when only paraffin sections are used, as obviously cytokeratin epitopes can be blocked during formalin fixation and/or paraffin embedding. However, our findings also point to the possible existence of certain anaplastic carcinomas which contain very little, if any, cytokeratin IF and thus are not readily identified as tumours of epithelial origin. We have good reasons to assume that the two cases that were negative for cytokeratins in both paraffin and cryostat sections were indeed true carcinomas as they displayed a histological appearance of typical polymorphic, non-spindle cell tumours which, in the case shown in Fig. 3, also contained residual structures of a preceeding papillary carcinoma. The cytokeratin-negative cases described here differ considerably from the single cytokeratin-negative case reported by Miettinen et al. (1984) which these authors then classified as "thyroid spindle cell sarcoma", also because it did not show electron microscopically identifiable desmosomes. Anaplastic tumours containing little cytokeratin – or no immunocytochemically detectable cytokeratin – have been observed sporadically in several laboratories, and totally negative immunocytochemical results with antibodies to all the diverse IF proteins have also been reported for various tumours (Gown and Vogel 1985), including small cell carcinomas of the lung (e.g., Blobel et al. 1985a), blastema cell structures of Wilms tumours (e.g., Denk et al. 1985) and certain cultured carcinoma cell lines (Venetianer et al. 1983; Hedberg and Chen 1986). It is presently not clear whether the absence of immunocytoreactivity in these two tumours is due to the presence of very low contents of cytokeratin that escape detection by the method used, or to the specific inaccessibility of an epitope. It may represent one of those situations in which cytokeratin synthesis is diminished and vimentin represents the major, if not exclusive IF protein present. As recently shown for blastema cells of nephroblastomas (Denk et al. 1985), the use of antibodies to desmoplakins, and probably also other desmosomal markers, can provide a valuable additional tool in characterizing the cell type of such cytokeratin-negative tumours (for review see Moll et al. 1986).

In contrast to Miettinen et al. (1984), we also noted substantial differences when comparing the immunoreactivity of certain monoclonal antibodies and a specific vimentin antiserum. In 3 follicular and 10 papillary carcinomas investigated with both probes, vimentin was visualized only with the monoclonal antibody in the tumour cells. These different vimentin antibodies produced identical results in 5 anaplastic and 5 medullary carcinomas of the study. The reason for these differences, and the differences of vimentin reactivity observed between tumour and stromal cells in the same tumours, is not understood. The differences may reflect differences of accessibility of a specific epitope within the vimentin molecule as this has been reported for different states of cell cycle and cell density and metabolism (Dulbecco et al. 1983; Franke et al. 1984).

The positive neurofilament immunoreaction observed in a total of 9

medullary carcinomas confirms earlier findings (Droese et al. 1984; Miettinen et al. 1984; McNutt et al. 1985; Altmannsberger et al. 1986; Wiedenmann et al. 1986) that this IF protein can be expressed in C-cell neoplasms. Altmannsberger et al. (1986) described immunostaining for both cytokeratins and neurofilament proteins in cytological specimens of 6 tumours, 4 of which also contained cells positive for vimentin. In the cases reported here, co-expression of vimentin and cytokeratin was found only in about half of the medullary carcinomas, and the presence of cells positive for neurofilaments could be demonstrated in an even smaller proportion, i.e. approximately 13%. Whether these differences represent true differences in the expression patterns of the specific tumours or are due to restrictions in the accessibility of the epitopes of the different antibodies used is presently not clear.

We do not concur, however, with the suggestion of Droese et al. (1984) and Altmannsberger et al. (1986) that the demonstration of co-expression of vimentin and cytokeratin adds support to the hypothesis of a common origin of follicular and parafollicular cell carcinomas (Ljungberg et al. 1983, 1984). The same mode of IF protein co-expression has been described in cultured epithelial and carcinoma cells (Franke et al. 1979a, c; Osborn and Weber 1983), as well as in a variety of tumours such as pleomorphic adenomas and adenoid cystic carcinomas of salivary glands (Caselitz et al. 1981, 1984; Krepler et al. 1982), certain renal cell carcinomas (Holthoefer et al. 1983; McNutt et al. 1985; Waldherr and Schwechheimer 1985) and nephroblastomas (Altmannsberger et al. 1984; Denk et al. 1985), mesotheliomas (Blobel et al. 1985b), and endometrial carcinomas (McNutt et al. 1985); tumours which differ widely in their histogenic derivation.

As judged from the frequent, although not obligatory co-expression of cytokeratins IF with neurofilaments, medullary carcinomas resemble other neuroendocrine neoplasms such as bronchopulmonary carcinoids, Merkel cell carcinomas of the skin, and certain tumours of the gastrointestinal tract (Kerl et al. 1984; Gould et al. 1985; Miettinen et al. 1985; Moll et al. 1985; Merot et al. 1986). Moreover, the demonstrated absence of neurofilaments in any of our over 100 papillary and follicular carcinomas provides yet another example of differences between follicle and C-cell neoplasms.

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