Virchows Arch A (1986) 410:203–215

Virchows Archiv A

Evidence of vascular differentiation in anaplastic tumours of the thyroid – an immunohistological study

F. Eckert[1] *, U. Schmid[1] *, F. Gloor[1], and Chr. Hedinger[2]

[1] Institute of Pathology, Kantonsspital St. Gallen, CH-9007 St. Gallen
[2] Institute of Pathology, University of Zürich, University Hospital, CH-8091 Zürich, Switzerland

Summary. Sixteen cases of anaplastic carcinoma (ACA) and 4 cases of malignant haemangioendothelioma (HAE) of the thyroid were studied by light microscopy and immunohistochemistry.

Seven cases of ACA and 3 cases of HAE were characterized by coexpression of immunohistological features of epithelial and vascular endothelial cells.

Expression of vimentin was common to all tumours investigated. The present study provides evidence that ACA and HAE are partially closely related tumours showing alternating differentiation. This speaks in favour of a common neoplastic cell with the potential for epithelial and vascular endothelial differentiation.

Key words: Anaplastic carcinoma of the thyroid – Haemangioendothelioma – Immunohistology – Coexpression of epithelial and vascular antigens

Introduction

Anaplastic tumours of the thyroid can produce an array of appearances with transitions and intermediate forms. The wide range of histological and cytological features may lead to their confusion with a variety of neoplasms, including fibrosarcoma, malignant fibrous histiocytoma, rhabdomyosarcoma, osteogenic sarcoma, angiosarcoma, lymphoma and metastatic carcinoma (Hedinger 1969).

While it is generally accepted that small cell carcinoma should be assigned to other categories such as medullary carcinoma (Mendelsohn et al. 1980) and non-Hodgkin's lymphoma (Mambo et al. 1984) there are some controversies about the nature of tumours composed of spindle cells and/or giant cells (Carcangiu et al. 1985). Many authors support the hypothesis that spindle and giant cell carcinomas represent anaplastic transformation of pre-existing papillary (Aldinger et al. 1978; Albores et al. 1983) or follicular (Heitz et al. 1976) carcinoma. This was also documented by electron microscopy (Jao et al. 1975) and immunohistochemistry (Miettinen et al. 1984).

Histogenetic questions also concern the interrelations between anaplastic carcinoma (ACA) and malignant haemangioendothelioma (HAE) of the thyroid (Rosai and Carcangiu 1984). While some authors accept this tumour to be a distinct neoplasm of endothelial origin (Hedinger 1909, Wegelin 1926, Egloff 1977) it has been interpreted as a variant of undifferentiated carcinoma by others (Meissner and Warren 1969, Krisch et al. 1980). The tumour may display considerable morphological variation, often mimicking anaplastic carcinoma.

The development of immunohistological techniques has provided a new diagnostic tool in the evaluation of ACA and HAE and staining for intermediate filament proteins has proved to be of diagnostic value in thyroid tumors. Cytokeratin was detected in at least half of anaplastic thyroid tumours (Carcangiu et al. 1985) and some neoplastic cells coexpress cytokeratin type and vimentin type of intermediate filaments (Miettinen et al. 1984). Recent studies demonstrated F VIII-RAG in some of the tumour cells, establishing the endothelial origin of a number of tumours classified as malignant haemangioendothelioma (Schäffer and Ormanns 1983, Egloff 1983, Pfaltz et al. 1983 and Ruchti et al. 1984). However, inconsistancy

* F. Eckert was supported by the Dr. Mildred Scheel-Stiftung/Deutsche Stiftung für Krebshilfe. U. Schmid was supported by the Krebsliga St. Gallen/Appenzell

Offprint requests to: U. Schmid at the above adress

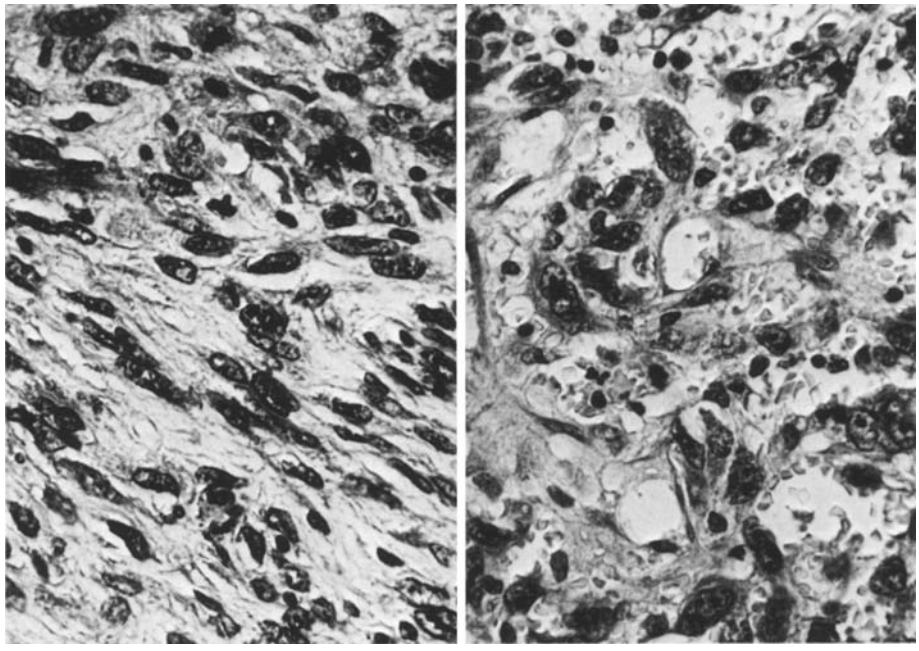


Fig. 1 a–b. Anaplastic carcinoma of spindle cell type with haemangioendotheliomatous features. **a** Spindle cell component; **b** angiosarcoma-like component (H+E, ×350)

in reacting with malignant tumours and great variation in intensity of staining indicate limitations to the method (Burgdorf et al. 1981, Guarda et al. 1982). Ordonez and Batsakis (1984) and Stephenson et al. (1986) showed that UEA I lectin is a more sensitive marker for the endothelial cells than FVIII-RAG.

The aim of this study was to characterize the nature of tumour cells in ACA and HAE and to elucidate possible interrelations between these two entities. We applied antibodies to thyroglobulin, epithelial membrane antigen (EMA), Vimentin and a new panepithelial antibody (mAB lu-5) (von Overbeck et al. 1985). To determine their utility in the assessment of ACA and HAE we used a panel of vascular markers including FVIII-RAG, UEA I and a recently published new endothelial marker, BMA 120 (Alles and Bosslet 1985).

Material and methods

Material. The cases studied in this article were drawn from the files of the Institute of Pathology, St. Gallen. The surgical material investigated comprised 25 primary tumours of the thyroid. All were reviewed and classified according to the WHO classification (Hedinger Chr, Sobin LH 1974).

These tumours consisted of 5 anaplastic carcinomas of the spindle cell type (male:female=2:3, average age: 76.8), 11 anaplastic carcinomas of the giant cell type (male:female=6:5, average age: 69.4) and 4 malignant haemangioendotheliomas (male:female=1:3, average age: 72.8). Three follicular and 2 papillary carcinomas were investigated as controls (male:female=0:5, average age:68.4).

Formalin-fixed and paraffin-embedded specimens had been stored for periods ranging from months to more than 10 years. Autopsy material was not used.

Table 1. Immunohistological results in anaplastic carcinoma of spindle cell type

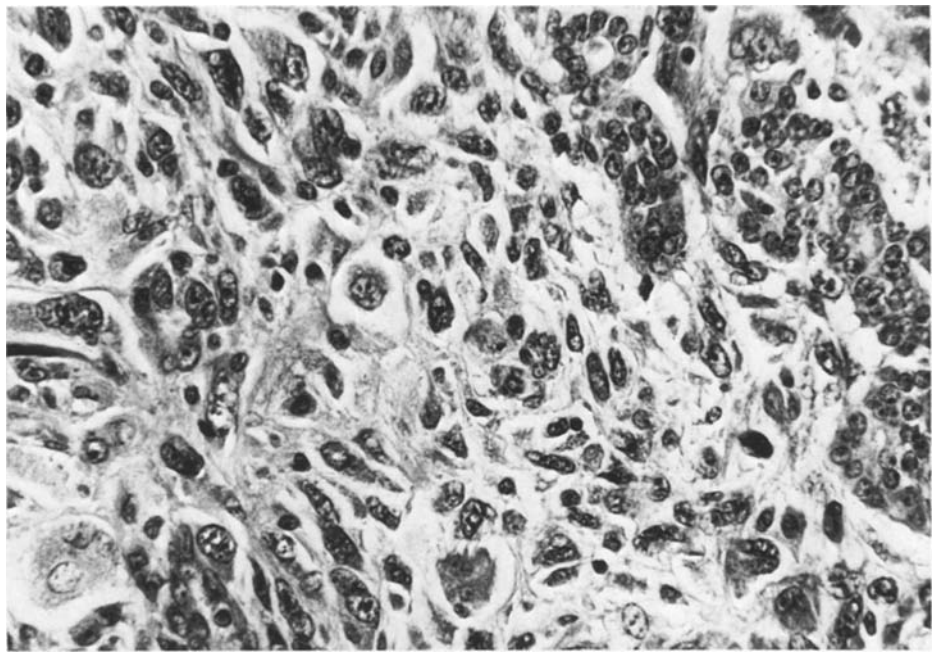
case	THY	EMA	LU-5	VIM	FVIII	UEA I	BMA
1	–	(+)	+++	+++	–	–	–
2	–	–	+++	++	–	–	0
3	–	–	+	++	–	–	0
4	–	–	–	++	–	–	–
5[a]	–	(+)	++	+++	–	–	–

[a] revealing angiosarcoma-like features

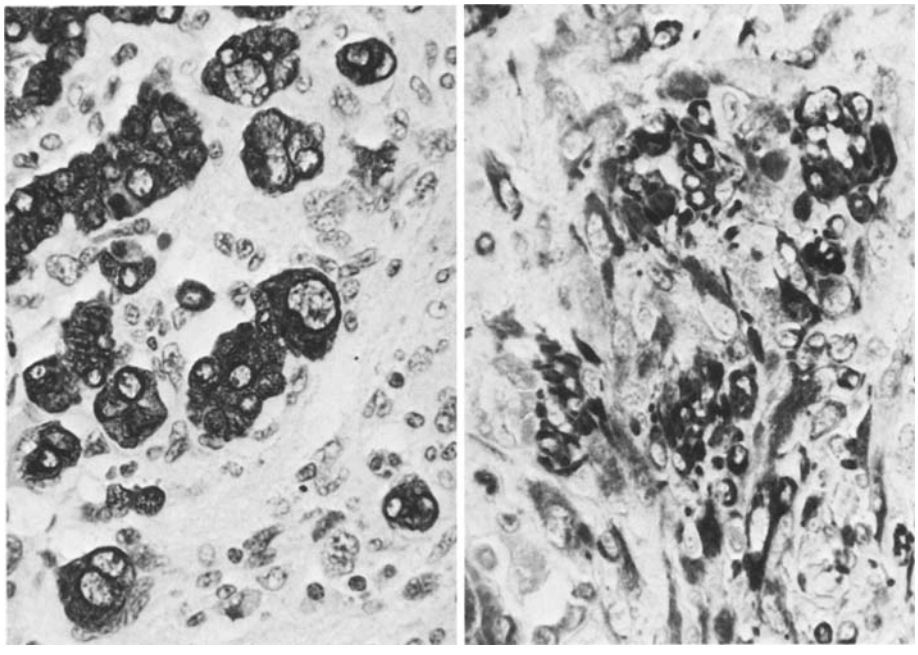
+++ = almost all cells stained; ++ = approximately 50% of cells stained; + = few cells stained; (+) = only single cells stained weakly; – = no reaction; 0 = no evaluation possible

Serial sections (3–4 μm) were stained with H&E, Elastic van Gieson, and Prussian Blue. For immunohistochemistry sections were deparaffinized in xylene and taken through alcohol series to water. In each case the same panel of antibodies was applied. Antibodies to Thyroglobulin, Epithelial Membrane Antigen, Factor VIII-related antigen, Ulex europaeus I lectin, Vimentin and the antibodies mAB lu-5 and BMA 120 were used. A minimum of 2 sections of different sites was labelled for each marker.

Reagents. Polyclonal antisera against thyroglobulin, factor VIII-related antigen (F VIII RAG) and ulex europaeus I lectin (UEA I lectin), swine anti rabbit IgG and the PAP-complex were purchased from Dako, Copenhagen, Denmark. UEA I lectin was obtained from Boehringer, Mannheim, FRG. Monoclonal antibodies to Epithelial Membrane Antigen (EMA) and Vimentin were purchased from Dako, BMA 120 from Behring, Marburg, FRG. The latter antibody reacts with an antigen located in the cell membrane and cytoplasm of human vascular endothelial cells (Alles and Bosslet 1985). Dr. C. Stähli, Central Research Division, F. Hoffmann-La Roche & Co, Ltd., Basel,



a



b c

Fig. 2a–c. Anaplastic carcinoma of giant cell type showing no coexpression. **a** H+E ×350; **b** Demonstration of mAB lu-5 reacting with tumour cells of the follicular carcinoma component (PAP ×350); **c** Demonstration of vimentin reacting with anaplastic tumour cells (IAP, ×350)

Switzerland generously supplied us with the monoclonal antibody mAB lu-5, which has been published to stain most epithelial and mesothelial cells, but not mesenchymal cells (von Overbeck et al. 1985). Rabbit antiserum against mouse immunoglobulin was purchased from DAKO, PAP-conjugated goat antiserum against rabbit IgG from DIANOVA, Hamburg, FRG and alkaline phosphatase conjugated goat antiserum against rabbit IgG from Sigma, St. Louis, MO, USA.

Immunohistological staining procedure. Immunostaining for thyroglobulin and F VIII-RAG using the PAP-method was carried out by a modified technique after Sternberger et al. (1970).

Labelling for UEA I was performed by sequentially incubating the sections with UEA I (diluted to 20 μg/ml) for 30 min, 1:200 rabbit anti-UEA-1 for 30 min, 1:50 swine anti-rabbit IgG for 30 min and 1:100 PAP-complex for 30 min, each diluted in Tris-buffer (pH 7.4) with double washes in Tris-buffer between stages. Peroxidase reactivity was demonstrated with 3-amino-9-ethylcarbazole (Sigma), dissolved in dimethylformamide (DMF) (Merck, Darmstadt, FRG). The sections were then counterstained with haemalaun and covered.

Staining for mAB lu-5 was carried out by the following method: after blocking of endogenous peroxidase in a 1% H_2O_2 methanol solution for 30 min the sections were pretreated with fresh 0.1% solution of trypsin in 0.1% CaCl (pH 7.8)

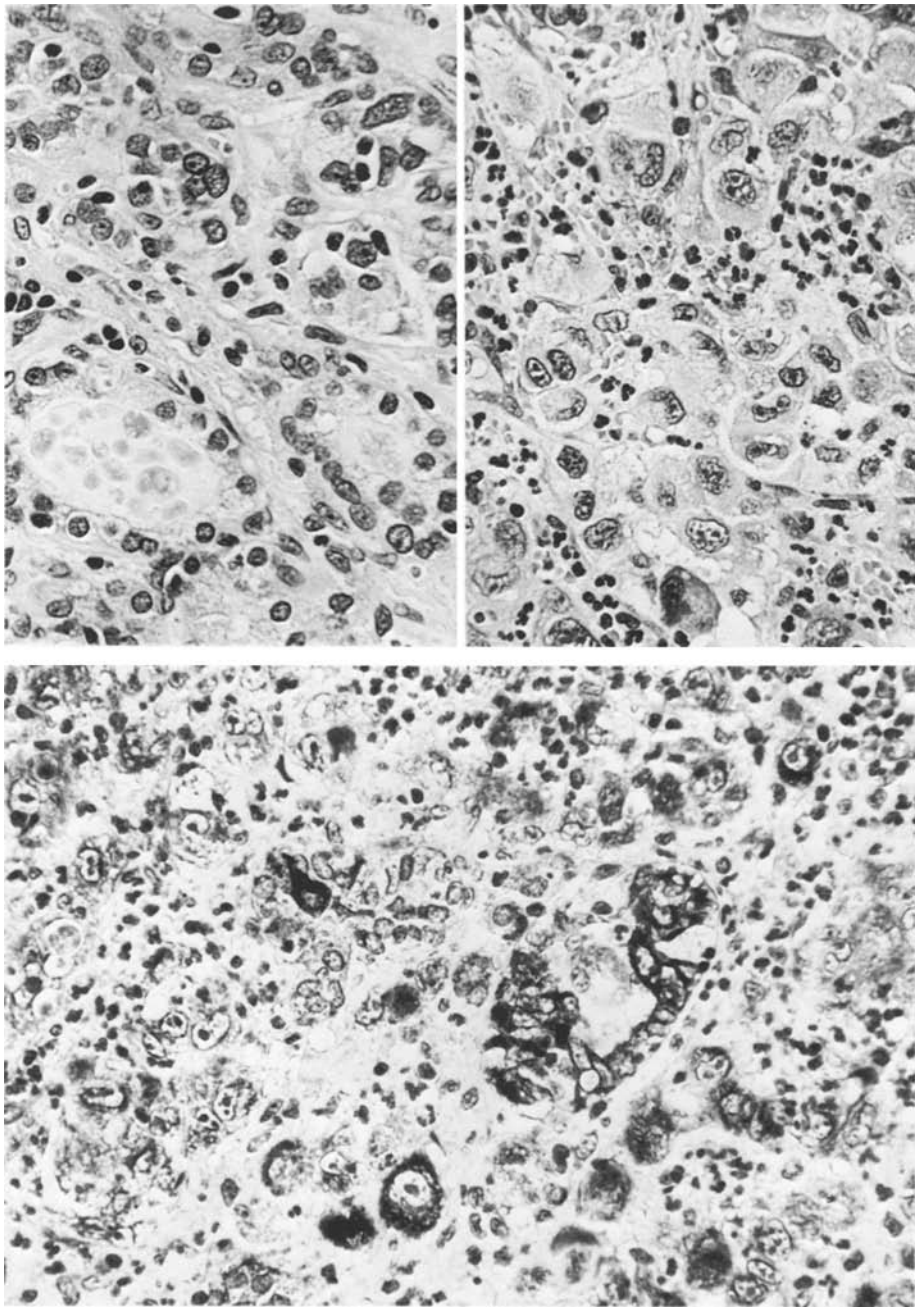
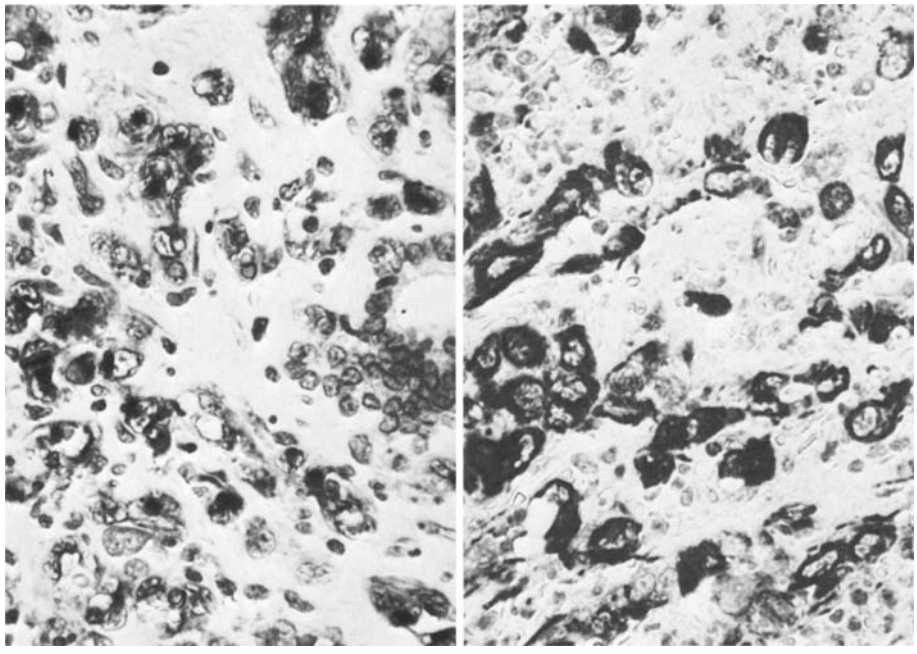


Fig. 3a–e. Anaplastic carcinoma of giant cell type showing coexpression. **a** Remnants of thyroid follicles (H+E, ×350); **b** anaplastic tumour cells (H+E, ×350); **c** Demonstration of mAB lu-5 in tumour cells (PAP, ×350); **d** UEA I (PAP, ×350); **e** Vimentin (IAP, ×350)

at 37° C for 20 min (Mepham et al. 1979). After rinsing in Tris buffer (diluted 1:5 with NaCl) for 10 min, the slides were treated with normal human serum for 10 min and then incubated with mAB lu-5 (diluted 1:50) for 30 min. The sections were sequentially rinsed in Tris-buffer for 10 min, immersed in rabbit anti-mouse antiserum (1:15) for 30 min, rinsed again and incubated with PAP-conjugated goat anti-rabbit antiserum (1:200). Demonstration of bound peroxidase and counterstaining were as in the UEA I technique. Staining for EMA was carried out as for mAB lu-5 except prior trypsinization.

Labelling for Vimentin and BMA 120 was performed by an immunoalkaline phosphatase method (IAP) published and modified by Feller and Parwaresch (1983). Antisera were applied in the following order: monoclonal primary antibody (Vimentin 1:50, BMA 120 1:50), rabbit anti-mouse immunoglobulins and anti-rabbit IgG alkaline-phosphatase conjugated. Each incubation lasted 30 min and was followed by washing in Tris-buffer (pH 7.4). Alkaline phosphatase reactivity was demonstrated with fast-red TR salt (Sigma) and naphthol-AS-BJ-phosphatase (Sigma) as substrate, each dissolved in dimethylformamide (DMF) (Merck) and diluted with 0.05 M propandiol buffer, pH 9.75. Endogenous alkaline phosphatase was inhibited by adding 1 mM levamisole (Sigma) to the incubation medium. The slides were incubated at room temperature for 10 min, washed in Tris-buffer pH 7.4, counterstained with haemalaun and mounted in glycerine gelatin.



d e

Substitution of antibodies by nonimmune rabbit serum or incubation with only the second antiserum on adjacent sections constituted our antibody controls.

Double immunoenzymatic labelling was performed in one case using a method described by Mason, Abdulaziz, Falini and Stein (1983).

Results

The cases will be described in the following groups:
Group I: anaplastic carcinoma of spindle cell type
Group II: anaplastic carcinoma of giant cell type
Group III: malignant haemangioendothelioma
Group IV: follicular and papillary carcinoma

Group I

Histological findings. Five tumours classified as anaplastic carcinomas of spindle cell type showed a striking sarcomatoid appearance.

With one exception all tumours showed focal necrosis. Two cases revealed accumulations of haemosiderin-laden macrophages lining areas of haemorrhage. Elements of differentiated carcinomas or areas of transitional changes between differentiated and anaplastic carcinoma could not be detected. The microscopic picture of one tumour was characterized by small areas of pseudovascular structures and clefts reminiscent of those seen in malignant haemangioendothelioma of the thyroid (Fig. 1). Erythrophagocytosis could not be observed. Haemosiderin deposits were present in the interstitium.

Immunohistological findings. None of the five tumours reacted with antibody against thyroglobulin. Tumour cell-staining yielded weak immunoreactivity for EMA in 2 cases and only single tumour cells were stained.

Three of the 5 tumours were reactive to mAB lu-5 showing an intense cytoplasmic staining. In one case tumour cells failed to stain with mAB lu-5 while nontumorous epithelial thyroid cells immediately adjacent to carcinoma gave a moderate to strong reaction. One tumour revealed scattered islands of mAB lu-5 – immunoreactive cells in vast areas of immunonegative tumour cells. No decision could be reached whether these islands represent highly differentiated areas of the carcinoma or tumour invaded thyroid tissue. The cytoplasm of the tumour cells was uniformly stained by Vimentin antibody in all cases. Vessels were clearly decorated by the three vascular markers. In general, antibody to UEA I and BMA 120 gave the most reliable results, while FVIII-RAG revealed a great variation in intensity of staining. For technical reasons staining for BMA 120 was not evaluable in 2 cases. The tumour cells gave negative reactions with all vascular markers. Neoplastic cells located in areas reminiscent of HAE were positive for mAB lu-5. In conclusion 4 tumours of the spindle cell type virtually showed a coexpression of mAB lu-5 and vimentin in their tumour cells. Table 1.

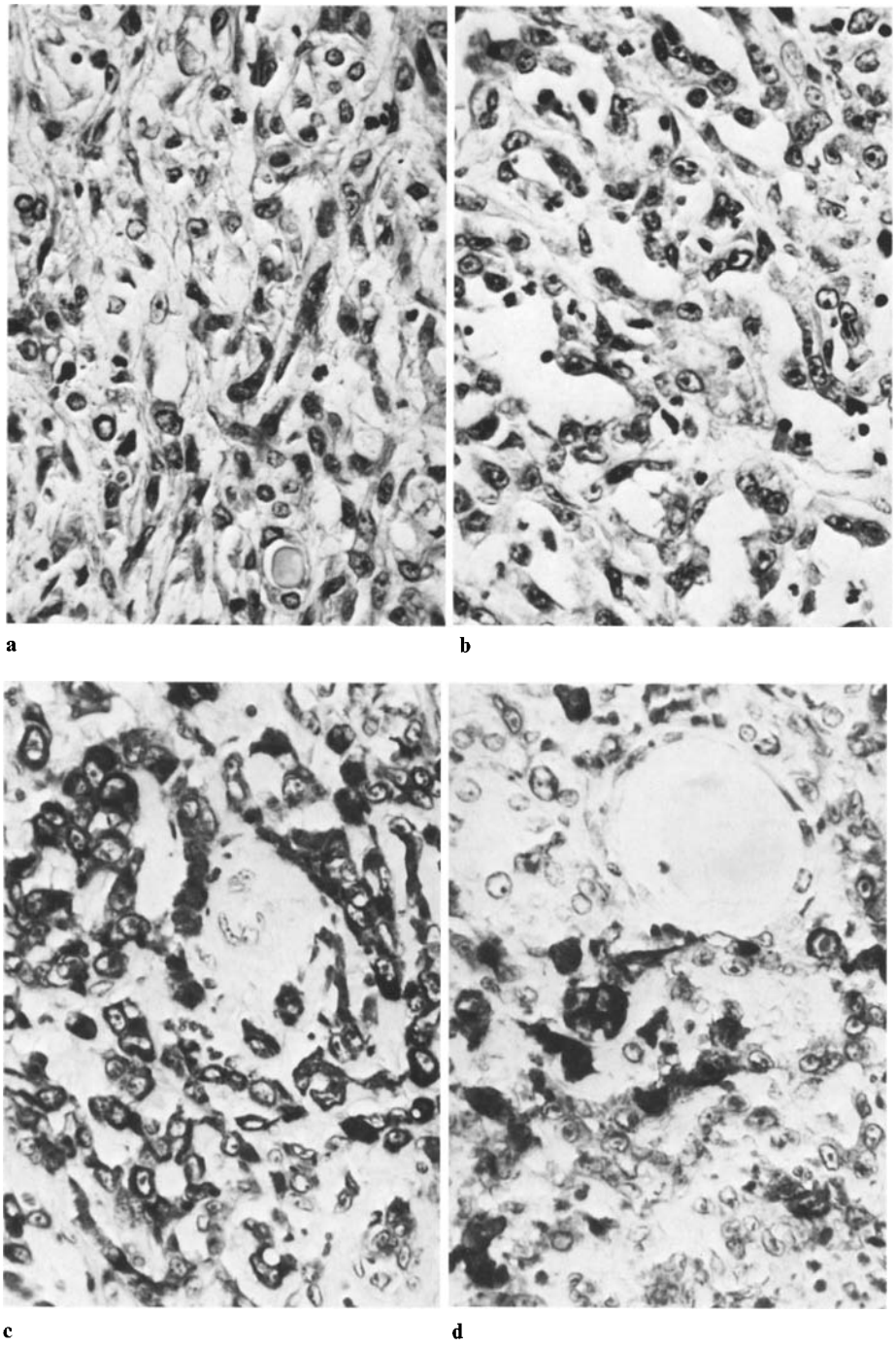


Fig. 4a–e. Anaplastic carcinoma of giant cell type with hemangioendotheliomatous features, showing coexpression. **a** Solid component of the tumour (H+E, ×350); **b** Angiosarcoma-like component of the tumour (H+E, ×350); **c** mAB lu-5 (PAP, ×350); **d** BMA 120 (IAP, ×350); **e** vimentin (IAP, ×350)

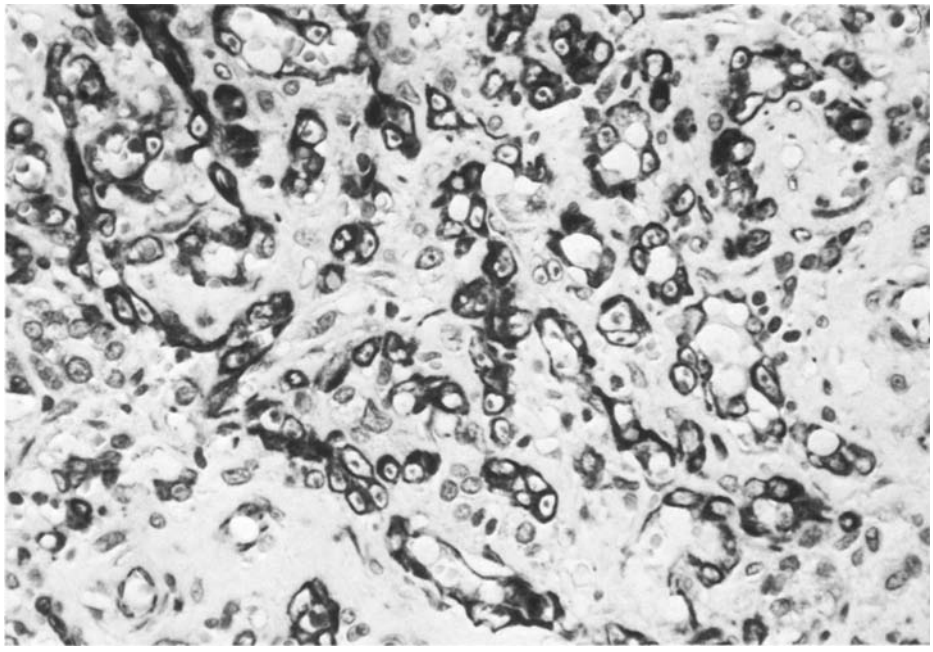
Group II

Histological findings. Anaplastic carcinoma of giant cell type was observed in 11 cases. Multinucleated cells were numerous, mitoses were frequent and often atypical. Admixtures of spindle cells and smaller mononuclear cells could be found in all cases. In 7 tumours areas of focal or extensive necrosis were seen. Haemorrhage leading to haemosiderin deposits was a feature common to all cases.

Three cases presented with foci of follicular carcinoma. Isolated thyroid follicles were entrapped among the tumour tissue in 2 cases but it was difficult to decide whether the nodules were tumorous or not.

According to their pattern of growth the tumours could be distributed in two categories: Solid portions predominated in 6 tumours resulting in an epithelial-like appearance (Fig. 2a; Fig. 3a+b). Squamoid features could not be detected. Erythrophagocytosis was observed in one case.

Five tumours demonstrated haemangioendo-



e

theliomatous features and epithelial-like structures in combination (Fig. 4a+b). Three of these tumours exhibited erythrophagocytosis in atypical cells lining pseudovascular, cleft-like spaces. A polymorphonuclear leucocyte infiltration was usually present among the haemangiomatous areas.

Immunohistological findings. Areas of differentiated follicular carcinoma turned out to be positive for thyroglobulin in the 3 cases already mentioned. Giant and anaplastic tumour cells were consistently negative. In 2 cases artefactual spread of thyroglobulin from normal follicles to adjacent tumour cells resulted in a weak cytoplasmic staining.

Staining for EMA gave similar results. Cells of the follicular carcinoma component were positive while anaplastic cells were negative. In contrast, one case revealed strong EMA-positivity in anaplastic tumour cells. Areas of follicular or papillary carcinoma could not be detected in this tumour.

Regardless of their cytological and histological features all but one of the carcinomas showed immunoreactivity for mAB lu-5 (Figs. 2b, 3c, 4c). The reaction for this antibody was stronger in nontumorous thyroid tissue or in the follicular components of ACA than in the anaplastic tumour cells. Intensity of staining tended to be heterogenous and differed from case to case. Tumours with areas of follicular carcinoma showed a striking contrast between mAB lu-5-positive cells of follicular carcinoma and the surrounding focally reacting (1 case) or nonreacting (2 cases) anaplastic tissue. One case revealed strong background staining and could not be evaluated.

Table 2. Immunohistological results in anaplastic carcinoma of giant cell type

case	THY	EMA	LU-5	VIM	FVIII	UEA I	BMA
1	+[1a]	(+)[1]	+[1]	+++	–	–	–
2	++[1]	++[1]	++[1]	++	–	–	–
3	–	(+)	0	+	–	–	–
4	–	++	+++	++	(+)	+	+
5	–	–	+++	+++	(+)	+++	+++
6	–	0	+++	+++	0	–	0
7[b]	–	–	++	++	+	+++	+
8[b]	–	–	+++	+++	++	+++	0
9[b]	–	–	+++	+++	++	+++	0
10[b]	+[1]	+[1]	++[2]	++	+	+++	0
11[b]	–	–	++	+++	++	0	+++

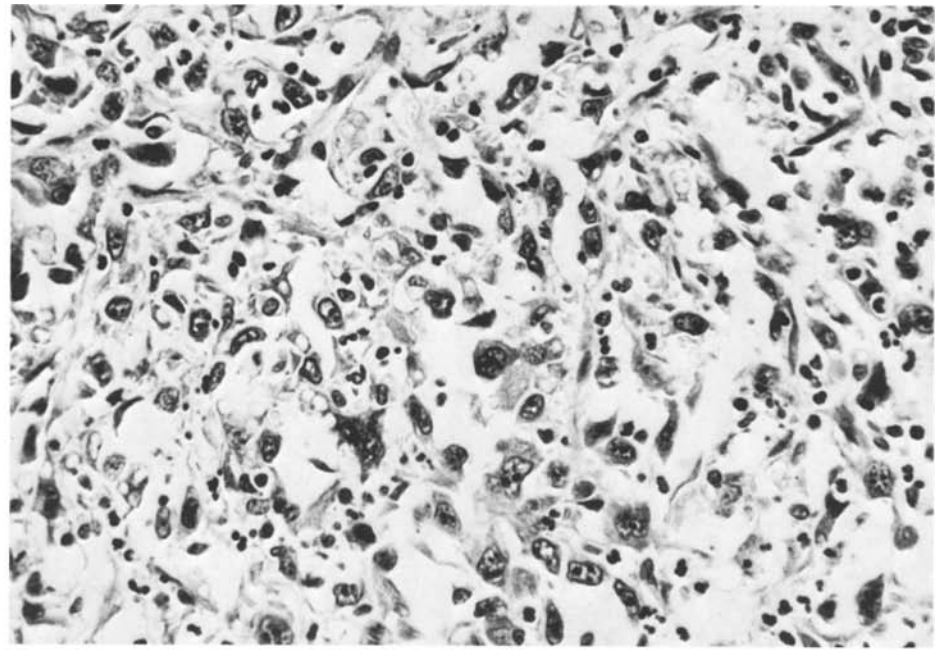
[a] symbols explained in Table 1

[b] revealing angiosarcoma-like features

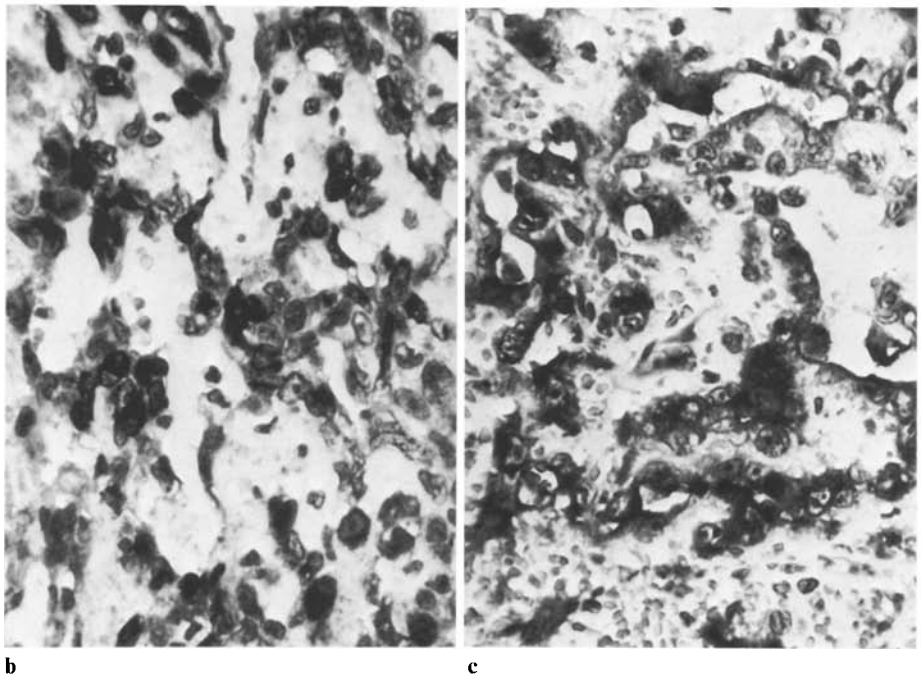
1 tumor cells of follicular carcinoma component; 2 tumor cells of follicular carcinoma component and few anaplastic tumour cells; 0 no evaluation possible

Vimentin was consistently positive in all tumours (Figs. 2c, 3e and 4e). Cells of the follicular carcinoma component gave a cap-like positive reaction in their basal areas.

The vascular markers (FVIII-RAG, UEA I and BMA 120) regularly stained the vessels in most cases. Seven cases revealed results: at least two of the vascular markers were positive in the tumour cells of each case showing strong cytoplasmic staining. This finding predominated in tumours with



a



b

c

Fig. 5a–c. Malignant hemangioendothelioma of the thyroid. **a** H+E, ×350; **b** Demonstration of mAB lu-5 in tumour cells lining cleft-like structures (PAP, ×350); **c** Demonstration of BMA 120 in tumour cells lining cleft-like structures (PAP, ×350)

haemangioendotheliomatous features but was also found in two cases with solid tumour growth. Immunoperoxidase staining was established in atypical cells lining the vascular spaces and in the more solid, epithelial-like areas of the tumours (Figs. 3d and 4d).

As a result solid and angiomatous regions of anaplastic carcinoma showed epithelial as well as vascular endothelial features of the tumour cells (Table 2).

Double immunoenzymatic labelling was performed in one case which revealed angiosarcoma-like features (case 11, Table 2). At least part of the tumour cells coexpressed FVIII-RAG and mAB lu-5 while the largest population of tumour cells expressed either FVIII-RAG or mAB lu-5.

Group III

Histological findings. Four tumours showed the typical angiomatous pattern of malignant haeman-

gioendothelioma (Fig. 5a): cleft-like structures containing erythrocytes were lined by flat or pleomorphic cells. These cells showed erythrophagocytosis in all tumours investigated. In some areas neoplastic cells were found in an epithelial arrangement forming solid sheets. Haemorrhage with haemosiderin deposits and extensive areas of necrosis were a constant finding as well as a marked inflammatory component.

In 3 cases thyroid parenchyma adjacent to the tumour tissue showed adenomatous goitrous changes. Two of them revealed remnants of thyroid follicles interspersed with tumorous areas.

Immunohistological findings. Immunoperoxidase staining for thyroglobulin and EMA was consistently negative in all tumours. Remnants of thyroid follicle epithelium could be easily recognized and distinguished from the neoplastic cells by thyroglobulin positivity.

In all evaluable cases tumour cells were well stained with mAB lu-5 regardless of their cytological features (Fig. 5b). Staining was similar in areas with pseudovascular features and in the more solid portions of the tumours. One case revealed high background staining and could not be evaluated.

Staining for Vimentin yielded similar results. Three cases turned out to be immunopositive, while one case could not be assessed definitely.

All cases exhibited strong cytoplasmic tumour cell-staining with at least two of the vascular markers (Table 3). Immunoreactivity was demonstrated in malignant cells lining the vascular spaces but also in isolated cells within the more epithelial-like areas (Fig. 5c). The markers gave a granular or both granular and diffuse cytoplasmic staining in positive cells. In addition BMA 120 revealed cell membrane-staining of the neoplastic cells.

In two cases BMA 120-positivity was confined to a restricted number of tumour cells, while a larger population of neoplastic cells contained FVIII-RAG and/or UEA I. Unstained tumour cells were interspersed in all cases.

No staining was observed when primary antiserum was replaced by normal rabbit serum or Albumin.

The localization of cells positive for vascular markers corresponded with the localization of mAB lu-5 positive cells. However, in some parts of the tumour mAB lu-5 positive cells predominated in the solid foci while vascular markers were especially expressed in the angiomatous areas. Immunohistological results are given in Table 3.

Based upon these findings, coexpression of epithelial and vascular markers was present in 3 cases of haemangioendothelioma indicating a close relationship to those tumours of giant cell type which showed the same feature (Tab. 2).

Table 3. Immunohistological results in malignant haemangioendothelioma

case	THY	EMA	LU-5	VIM	FVIII	UEA I	BMA
1	–[a]	–	0	0	+	++	++
2	–	–	++	+++	++	0	++
3	–	–	++	++	++	++	+
4	–	–	++	++	++	++	+

[a] symbols explained in Table 1
0 no evaluation possible

Group IV

Three follicular and two papillary carcinomas investigated as controls yielded the following immunohistological findings: All cases uniformly stained for thyroglobulin. Reactions were stronger and diffuse in follicular carcinomas than in papillary carcinomas. The intensity of staining varied greatly within each tumour. In papillary carcinomas cytoplasmic immunoreactivity was often restricted to the apical parts of the tumour cells.

EMA-positive malignant cells were found in one follicular and one papillary carcinoma. MAB lu-5 gave a strong reaction in all tumours.

Staining for vimentin revealed: in follicular carcinoma a weak cytoplasmic reaction and a marked, predominantly granular reaction in the basal areas of the tumour cells was observed.

In papillary carcinoma a finely granular or spot-like reaction was seen in the basal perinuclear areas of the cells.

Malignant cells were consistently negative for all vascular markers while vascular endothelial cells were clearly decorated.

Discussion

Anaplastic carcinomas (ACA) of the thyroid display a large morphological spectrum and variability (Hedinger 1969; Noser 1985). They may show giant cell and/or spindle cell pattern or even display sarcomatoid features. This may lead to potential confusion with sarcomas. The question of whether ACA mostly represents anaplastic transformation of better differentiated neoplasms (follicular or papillary carcinoma) is controversial (Carcangiu et al. 1985).

Stains for thyroglobulin were always negative in the anaplastic tumour cells reflecting a loss of

thyroglobulin synthesis. (see also Böcker et al. 1980; Permanetter et al. 1982; Carcangiu et al. 1985.) Thyroglobulin-positivity was found exclusively in non-neoplastic follicles entrapped in the tumour tissue or in foci of follicular carcinoma. In contrast, Logmans and Jöbsis (1984) reported a focal and weak reaction for thyroglobulin in 14 of 23 morphologically undifferentiated thyroid carcinomas.

EMA, which was first detected on human milk fat globule membranes (Heyderman et al.1979; Sloane and Ormerod 1981), turned out to be a disappointing marker for ACA. Our data extend results obtained by Pinkus and Kurtin (1985). One tumour reacted strongly, while three stained only weakly and focally. Seven were consistently negative and three showed positivity restricted to the follicular carcinoma components of ACA.

Several studies have demonstrated immunostaining for cytokeratin in the anaplastic cells regardless of shape and size (Miettinen et al. 1984; Carcangiu et al. 1985). This finding lends strong support to the notion that ACA is of predominantly epithelial nature. In our study 14 out of 16 ACA turned out to be positive for the panepithelial marker mAB lu-5. This antibody seems to detect a cytoskeleton-associated antigen (von Overbeck et al. 1985). Five cases revealed either foci of follicular carcinoma or remnants of thyroid follicles with questionable status.

As a result these data are in line with the suggestion that at least a majority of ACA is epithelial in nature and may represent anaplastic transformation of pre-existing follicular or papillary tumours.

As reported by Miettinen et al. (1984) and Droese et al. (1984) all ACAs investigated expressed vimentin in their tumour cells. Vimentin is the major protein of the intermediate-sized filaments predominant in mesenchymal cells (Franke et al. 1978; Franke et al. 1979). Vimentin-positive cells were seen both in solid parts of the tumours and in areas with "sarcomatous" pattern. Their localization corresponded widely with the localization of mAB lu-5-positive cells. This phenomenon of coexpression, already described by Miettinen et al. (1984), may occur in certain other instances. A coexpression of cytokeratin type and vimentin type intermediate filaments was also observed in pleomorphic adenomas of the human parotid gland (Caselitz et al. 1981; Krepler et al. 1982) and in renal carcinoma cells (Holthöfer et al. 1983).

A decisive interpretation of this phenomenon is not possible. The first possibility, that tumour cells might originate from nonmalignant cells which also coexpress mAB lu-5 and vimentin is contradicted by the findings of Miettinen et al. (1984): in normal thyroids and nodular goiters follicular epithelial cells were negative for vimentin.

Secondly, tumour cells might originate from mAB lu-5-positive cells which acquire the ability to produce vimentin filaments after malignant transformation. This interpretation may be in line with the fact that several cultured epithelial cells acquire the vimentin-system in addition to their intermediate filament-type specific for the differentiated cell of origin (Franke et al. 1979).

As shown by Miettinen et al. (1984) cytokeratin and vimentin are present in the same cells. This finding seems to rule out the possibility that ACA might be a mixed tumour composed of both epithelial and mesenchymal cells.

The complexity of ACA is extended by our results obtained with FVIII-RAG, UEA I and BMA 120. FVIII-RAG has been shown to be present in endothelial cells of blood vessels (Hoyer et al. 1973; Jaffe et al. 1973; Jaffe 1977; Mukai et al. 1980; Burgdorf et al. 1981), megakaryocytes and platelets (Piovella et al. 1978) and mast cells (Kindblom 1982). In contrast FVIII-RAG seems to be undetectable in some benign and malignant endothelial proliferations (Nadji et al. 1981; Sehested et al. 1981). UEA I and BMA 120 are reliable markers for vascular endothelium (Holthöfer et al. 1982; Miettinen et al. 1983; Borisch et al. 1983; Alles and Bosslet 1985).

Foci with the cytological and histological features recquired for the diagnosis of malignant haemangioendothelioma were present in 1 of our cases classified as ACA of spindle cell type and in 5 of our cases of giant cell type. Interestingly 5 of the 6 cases showed tumour cells positive for at least two vascular markers. Tumour cells decorated by the vascular markers were found both in the angiomatous and the solid parts of the tumours. In several cases FVIII-RAG-staining was weak and only focally expressed. UEA I gave the most reliable results with strong cytoplasmic staining in 5 cases. It is well known that a few tumours unrelated to endothelial cells show positive reaction for UEA I (Brabec et al. 1980; Holthöfer et al. 1981). In addition Gonzalez-Campora et al. (1986) reported a localized and granular supranuclear staining in some cells of follicular carcinoma of the thyroid. However, confusion with follicular epithelial cells could be avoided in our study because the endothelial nature of tumour cells was confirmed by immunoreaction with at least one other marker (FVIII-RAG or BMA 120).

In our hands BMA 120 gave widely differing

results in reliability of staining. It seems possible that the epitope detected by BMA 120 was not so well preserved as are lectin binding sites during tissue processing and storing.

Pfaltz et al. (1983) described FVIII-RAG-positive tumour cells in 3 borderline cases between anaplastic carcinoma and haemangioendothelioma. Two cases of anaplastic carcinoma with haemangioendotheliomatous features were FVIII-RAG-negative. Similar results are reported by Carcangiu et al. (1985). However, as mentioned above FVIII-RAG may yield inconsistent results and therefore absence of staining does not preclude the endothelial nature of the tumour cells investigated. Negative or weak staining may be due to lack of differentiation, rapid turnover or leakage (Hosaka et al. 1985).

As shown by double immunoenzymatic labelling in one case, at least a part of the tumour cells seemed to coexpress mAB lu-5 and FVIII-RAG. Interestingly endothelial cells express the vimentin type of intermediate filament proteins (Franke et al. 1979). If we interpret mAB lu-5 expression as a hint of epithelial differentiation and FVIII-RAG expression as one of endothelial differentiation, vimentin represents the common feature of a tumour cell showing potential epithelial and mesenchymal differentiation. In fact, vimentin was found in all our tumours investigated.

With regard to the finding that the expression of numerous markers may not be immutable in any cell line (Gould 1986) it is easy to understand that a lot of anaplastic tumours of the thyroid do not express vascular features. The question must be raised whether tumours of the thyroid exist with only mesenchymal features. Malignant haemangioendothelioma has been supposed to be such a true sarcoma of endothelial origin but is still a subject of controversy. Ultrastructural studies have yielded contrary results (Egloff 1977; Egloff 1983; Ruchti et al. 1984). The lack of blood group isoantigens known to be cellular wall antigens of endothelial cells has been reported as an argument against the true mesenchymal nature of HAE (Feigl et al. 1978). Recent studies could demonstrate FVIII-RAG in some of these tumours (Schäffer and Ormanns 1983; Pfaltz et al. 1983; Ruchti et al. 1984). This has been suggested as proof of the endothelial nature of HAE. However, all studies failed to use epithelial markers to rule out a possible epithelial nature of part of the tumour cells. In our study, 3 of 4 cases classified as HAE showed a coexpression of epithelial and vascular markers indicating a close relationship to anaplastic carcinoma with haemangioendotheliomatous features. It may be difficult to draw a clear-cut line between HAE and ACA mimicking HAE morphologically.

As a result it seems to be a distinctive attribute of malignant cells of the thyroid to show transitions from epithelial to mesenchymal differentiation. This has already been suggested by ultrastructural and lightmicroscopical findings (Egloff 1977). Whether this coexpression can be caused by the variability in expression of a single malignant population or by two different neoplastic populations with features of epithelial and vascular differentiation has to be clarified by further studies. The first possibility is favored by our results obtained with double immunoenzymatic labelling. Neoplastic populations are capable of multidirectional differentiation regardless of their presumed histogenesis (Gould et al. 1981). Our findings of coexpression may be interpreted by the assumption that many anaplastic tumours of the thyroid are essentially epithelial and endothelial in nature.

Although our findings await independent confirmation we suggest a close histogenetic relationship between anaplastic carcinoma and malignant haemangioendothelioma of the thyroid.

Acknowledgements. The excellent technical assistance of Mrs. E. Gschwend is gratefully acknowledged. We thank Mrs. E.-M. Eusebio for preparing the photographs.

References

Albores-Saavedra J, Nadji M, Civantos F, Morales AR (1983) Thyroglobulin in carcinoma of the thyroid: An immunohistochemical study. Hum Pathol 14:62–66

Aldinger KA, Samaan NA, Ibanez M, Hill CS (1978) Anaplastic carcinoma of the thyroid – A review of 84 cases of spindle and giant cell carcinoma of the thyroid. Cancer 41:2267–2275

Alles JU, Bosslet K (1985) Immunohistochemical and immunochemical characterization of a new endothelial cell specific antigen. J Histochem Cytochem (in press)

Böcker W, Dralle H, Hüsselmann H, Bay V, Brassow M (1980) Immunohistochemical analysis of thyroglobulin synthesis in thyroid carcinomas. Virchows Arch [Pathol Anat]: 385:187–200

Borisch B, Möller P, Harms D (1983) Lektin Ulex Europaeus I als Marker in der Differentialdiagnose von Gefäßtumoren. Pathologe 4:241–243

Brabec RK, Peters BP, Bernstein IA, Gray RH, Goldstein IJ (1980) Differential lectin binding to cellular membranes in the epidermis of the newborn rat. Proc Natl Acad Sci USA 77:477–479

Burgdorf WHC, Mukai K, Rosai J (1981) Immunohistochemical identification of Factor VIII-Related Antigen in endothelial cells of cutaneous lesions of alleged vascular nature. Am J Clin Pathol 75:167–171

Caselitz J, Osborn M, Seifert G, Weber K (1981) Intermediate-sized filament proteins (prekeratin, vimentin, desmin) in the normal parotid gland and parotid gland tumours. Immuno-

fluorescence study. Virchows Arch [Pathol Anat] 393:273–286
Carcangiu ML, Steeper TH, Zampi G, Rosai J (1985) Anaplastic thyroid carcinoma – A study of 70 cases. Am J Clin Pathol 83:135–158
Droese M, Altmannsberger M, Dralle H (1984) Verteilung der Intermediärfilamente in Schilddrüsencarcinomen. Verh Dtsch Ges Path 68:498
Egloff B (1977) Nicht-epitheliale Geschwülste der Schilddrüse. Verh Dtsch Ges Pathol 61:209–224
Egloff B (1983) The hemangioendothelioma of the thyroid. Virchows Arch [Pathol Anat] 400:119–142
Feigl W, Denk H, Davidovits A, Holzner JH (1976) Blood group isoantigens in human benign and malignant vascular tumors. Virchows Arch [Pathol Anat] 370:323–332
Feller AC, Parwaresch MR (1983) Simultaneous Enzyme-Immunocytochemical detection of antigens in monocellular specimens with monoclonal antibodies. J Immunol Methods 63:273–279
Franke WW, Schmid E, Osborn M, Weber K (1978) Different intermediate sized filaments distinguished by immunofluorescence microscopy. Proc Natl Acad Sci USA 75:5034–5038
Franke WW, Schmid E, Winter S, Osborn M, Weber K (1979) Widespread occurrence of intermediate sized filaments of the vimentin-type in cultured cells from diverse vertebrates. Exp Cell Res 123:25–46
Franke WW, Schmid E, Osborn M, Weber K (1979) Intermediate-sized filaments of human endothelial cells. J Cell Biol 81:570–580
Gonzalez-Campora R, Montero C, Martin-Lacave I, Galera H (1986) Demonstration of vascular endothelium in thyroid carcinomas using Ulex europaeus I agglutinin. Histopathology 10:261–266
Gould VE, Memoli VA, Dardi LE (1981) Multidirectional differentiation in human epithelial cancers. J Submicrosc Cytol 13:97–102
Gould VE (1986) Histogenesis and differentiation: A re-evaluation of these concepts as criteria for the classification of tumors. Hum Pathol 17:212–215
Guarda LA, Ordonez NG, Smith JL Jr, Hanssen G (1982) Immunoperoxidase localization of factor VIII in angiosarcomas. Arch Pathol Lab Med 106:515–516
Hedinger Chr (1969) Sarcomas of the thyroid. In: Hedinger CE (ed) Thyroid cancer. UICC monograph series, vol 12. Berlin, Springer Verlag, p 47
Hedinger Chr, Sobin LH (1974) Histological typing of thyroid tumours. International Histological Classification of Tumours Nr. 11. World Health Organization, Geneva
Hedinger E (1909) Zur Lehre der Struma sarcomatosa. 1. Die Blutgefäßendotheliome der Struma. Frankfurt Z Pathol 76:487–540
Heitz P, Moser H, Staub JJ (1976) Thyroid cancer. A study of 573 thyroid tumors and 161 autopsy cases observed over a thirty-year period. Cancer 37:2329–2337
Heyderman E, Steele K, Ormerod MG (1979) A new antigen on the epithelial membrane: its immunoperoxidase localisation in normal and neoplastic tissues. J Clin Pathol 32:35–39
Holthöfer H, Virtanen I, Petterson E, Törnroth T, Alfthan O, Linder E, Miettinen A (1981) Lectins as fluorescence microscopic markers for saccharides in human kidney. Lab Invest 45:391–399
Holthöfer H, Virtanen I, Kariniemi AL, Hormia M, Linder E, Miettinen A (1982) Ulex europaeus I lectin as a marker for vascular endothelium in human tissues. Lab Invest 47:60–66
Holthöfer H, Miettinen A, Paasivuo R, Lehto VP, Linder E, Alfthan O, Virtanen I (1983) Cellular origin and differentiation of renal carcinomas: a fluorescence microscopic study with kidney-specific antibodies, antiintermediate filament antibodies, and lectins. Lab Invest 49:317–326
Hosaka M, Murase N, Orito T, Mori M (1985) Immunohistochemical evaluation of factor VIII related antigen, filament proteins and lectin binding in haemangiomas. Virchows Arch [Pathol Anat] 407:237–247
Hoyer LW, de los Santos RP, Hoyer JR (1973) Antihemophilic factor antigen: Localization in endothelial cells by immunofluorescence. J Clin Invest 52:2737–2744
Jaffe EA, Hoyer LW, Nachman RL (1973) Synthesis of antihemophilic factor antigen by cultured human endothelial cells. J Clin Invest 52:2757–2764
Jaffe EA (1977) Endothelial cells and the biology of factor VIII. N Engl J Med 296:377–383
Jao W, Gould VE (1975) Ultrastructure of anaplastic (spindle and giant cell) carcinoma of the thyroid. Cancer 35:1280–1292
Kindblom LG (1982) Factor VIII related antigen and mast cells. Acta Pathol Microbiol Immunol Scand A90:437–439
Krepler D, Denk H, Artlieb U, Moll R (1982) Immunocytochemistry of intermediate filament proteins present in pleomorphic adenomas of the human parotid gland: characterization of different cell types in the same tumor. Differentiation 21:191–197
Krisch K, Holzner JH, Kokoschka R, Jakesz R, Niederle B, Roka R (1980) Hemangioendothelioma of the thyroid gland – true endothelioma or anaplastic carcinoma? Pathol Res Pract 170:230–242
Logmans SC, Jöbsis AC (1984) Thyroid-associated antigens in routinely embedded carcinomas: Possibilities and limitations in 116 cases. Cancer 54:274–279
Mambo NC, Miac ChB, Irwin SM (1984) Anaplastic small cell neoplasms of the thyroid: An immunoperoxidase study. Hum Pathol 15:55–60
Mason DY, Abdulaziz Z, Falini B, Stein H (1983) In: Polak JM, Van Noorden S (eds) Immunocytochemistry. Practical applications in Pathology and Biology. Wright. PSG. Bristol, London, Boston, pp 113–127
Meissner WA, Warren S (1969) Tumors of the thyroid gland. Atlas of tumor pathology. Sec Ser Fasc 4. Armed Forces Institute of Pathology, pp 123–126
Mendelsohn G, Brigner SH, Eggleston JC, Baylin SB, Wells SA Jr (1980) Anaplastic variants of medullary thyroid carcinoma. A light microscopic and immunohistochemical study. Am J Surg Pathol 4:333–341
Mepham BL, Frater W, Mitchell BS (1979) The use of proteolytic enzymes to improve immunoglobulin staining by the PAP technique. Histochem J 11:345–357
Miettinen M, Holthöfer H, Lehto VP, Miettinen A, Virtanen I (1983) Ulex europaeus I lectin as a marker for tumors derived from endothelial cells. Am J Clin Pathol 79:32–36
Miettinen M, Franssila K, Lehto VP, Paasivuo R, Virtanen I (1984) Expression of intermediate filament proteins in thyroid gland and thyroid tumors. Lab Invest 50:262–270
Mukai K, Rosai J, Burgdorf WHC (1980) Localization of factor VIII-related antigen in vascular endothelial cells using an immunoperoxidase method. Am J Surg Pathol 4:273–276
Nadji M, Morales AR, Ziegles-Weissman J, Penneys NS (1981) Kaposi's sarcoma. Immunohistologic evidence for an endothelial origin. Arch Pathol Lab Med 105:274–275
Noser A (1985) Anaplastische Schilddrüsenkarzinome. Frage ihrer Abstammung, Untersuchung mit immunhistochem-

ischen Methoden. Inaugural-Dissertation, Medizinische Fakultät der Universität Zürich

Ordonez NG, Batsakis JG (1984) Comparison of ulex europaeus I lectin and factor VIII-related antigen in vascular lesions. Arch Pathol Lab Med 108:129–132

Permanetter W, Nathrarth WBJ, Löhrs U (1982) Immunohistochemical analysis of thyroglobulin and keratin in benign and malignant thyroid tumors. Virchows Arch [Pathol Anat] 398:221–228

Pfaltz M, Hedinger Chr, Saremaslani P, Egloff B (1983) Malignant hemangioendothelioma of the thyroid and factor VIII-related antigen. Virchows Arch [Pathol Anat] 401:177–184

Pinkus GS, Kurtin PJ (1985) Epithelial membrane antigen – a diagnostic discriminant in surgical pathology: Immunohistochemical profile in epithelial, mesenchymal, and hematopoietic neoplasms using paraffin sections and monoclonal antibodies. Hum Pathol 16:929–940

Piovella F, Sitor GM, Ruggeri A, Dell'Orbo C, Ascari E (1978) The ultrastructural localization of Factor VIII-antigen in human platelets, megakaryocytes and endothelial cells using a ferritin labelled antibody. Br J Haematol 39:209–213

Rosai J, Carcangiu ML (1984) Pathology of thyroid tumors: Some recent and old questions. Hum Pathol 15:1008–1012

Ruchti Ch, Gerber HA, Schaffner Th (1984) Factor VIII-related antigen in malignant hemangioendothelioma of the thyroid: Additional evidence for the endothelial origin of this tumor. Am J Clin Pathol 82:474–480

Schäffer R, Ormanns W (1983) Immunhistochemischer Nachweis von Faktor-VIII-Antigen in malignen Hämangioendotheliomen der Schilddrüse. Ein Beitrag zur Histogenese. Schweiz Med Wochenschr 113:601–605

Sehested M, Hou-Jensen K (1981) Factor VIII related antigen as an endothelial cell marker in benign and malignant diseases. Virchows Arch [Pathol Anat] 391:217–225

Sloane JP, Ormerod MG (1981) Distribution of epithelial membrane antigen in normal and neoplastic tissues and its value in diagnostic tumor pathology. Cancer 47:1786–1795

Stephenson TJ, Griffiths DWR, Mills PM (1986) Comparison of ulex europaeus I lectin binding and factor VIII-related antigen as markers of vascular endothelium in follicular carcinoma of the thyroid. Histopathology 10:251–260

Sternberger LA, Hardy PH Jr, Cuculis JJ, Meyer HG (1970) The unlabeled antibody enzyme method of immuno-histochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–333

von Overbeck J, Stähli C, Gudat F, Carmann H, Lautenschlager C, Dürmüller U, Takacs B, Miggiano V, Staehelin Th, Heitz PhU (1985) Immunohistochemical characterization of an anti-epithelial monoclonal antibody (mAB lu-5). Virchows Arch [Pathol Anat] 407:1–12

Wegelin C (1926) Das Haemangioendotheliom der Schilddrüse. In: Henke F, Lubarsch O (eds) Handbuch der speziellen pathologischen Anatomie und Histologie, Band 8. Springer, Berlin, pp 297–302

Accepted August 28, 1986