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Immunohistochemical Analysis of Thyroglobulin and Keratin in Benign and Malignant Thyroid Tumours

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Summary. 56 thyroid gland tumours and non neoplastic alterations were studied for keratin and thyroglobulin staining, using the indirect immunoperoxidase method on serial formalin fixed paraffin embedded sections. Papillary carcinomas showed a strong reaction with anti-keratin serum but a weak reaction with anti-thyroglobulin serum. Follicular adenomas and carcinomas showed virtually no reaction for keratin but a strong reaction for thyroglobulin. Undifferentiated and medullary carcinomas did not react with either antiserum, except for single cells in two undifferentiated carcinomas which reacted with anti-keratin serum. In nodular goiters, hyperplastic follicles showed little or no reaction with anti-keratin serum and strong reaction with anti-thyroglobulin serum.

It is suggested that this virtually type-specific staining for keratin or thyroglobulin may be related to different degrees of cellular differentiation and organelle content in the tumour cells.

Key words: Thyroid tumour – Immunohistochemistry – Keratin – Thyroglobulin – Differential diagnosis

Introduction

Keratin has been demonstrated as the protein component of cytoskeletal intermediate filaments in many epithelial tissues (Altmannsberger et al. 1981; Caselitz et al. 1981; Gabbiani et al. 1981; Franke et al. 1978; Lazarides 1980; Schlegel et al. 1980; Sun et al. 1979; Sun and Green 1978). So far, to our knowledge, the thyroid gland and particularly its tumours have not been examined for the presence of keratin. Different types of thyroid carcinoma exhibit distinct biological behaviour which is of prognostic importance

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(Bubenhofer and Hedinger 1977; Byar et al. 1979; Franssila 1971, 1975 and 1978; Georgii 1977; Löhrs et al. 1977 and 1980; Williams et al. 1977; Woolner et al. 1968). It has been shown that thyroglobulin is a reliable indicator of the metabolic capacity retained by thyroid tumours and that its presence is correlated with characteristic cytological and ultrastructural differentiation (Böcker 1979; Böcker et al. 1980; Dralle and Böcker 1977; Kawaoi et al. 1981; Johannessen et al. 1978; Wilson et al. 1978). In this study we have compared the localisation of thyroglobulin as a marker of differentiated, organ specific function with that of keratin, a cytostructural marker which is apparently unrelated to differentiated function, but which is expressed to a different extent by various thyroid tumours (Permanetter et al. 1982).

Material and Methods

56 formalin fixed, paraffin embedded thyroid surgical specimens from the files of the Munich Pathology Institute were studied. In addition, frozen sections or 95% alcohol – 1% acetic acid fixed (Sainte-Marie 1962) thyroid tissues were examined, when available. The cases were classified according to the WHO-classification (Hedinger and Sobin 1974) and comprised 14 follicular carcinomas, including 4 of oncocytic types, 13 papillary carcinomas, two with lymph node metastases, 9 undifferentiated carcinomas and 3 medullary carcinomas. In addition to these malignant tumours, 5 follicular adenomas, 5 diffuse toxic goiters, 5 nodular goiters, 1 thyroiditis de Quervain and 1 thyroiditis Hashimoto were studied.

Rabbit anti-thyroglobulin serum was kindly supplied by Dr. K. Gärtner (Medizinische Klinik Innenstadt Universität München) and had been prepared as described by Böcker (1980). Anti human epidermal keratin antiserum with "broad spectrum" distribution of reactivity was kindly supplied by Dr. L.K. Trejdosiewicz (Department of Cellular Pathology, Imperial Cancer Research Fund Laboratories, London). It had been prepared after immunization of rabbits with human 40–63,000 MW epidermal keratin, isolated by the procedure of Sun and Green (1978), alkylated with a two-fold molar excess of iodoacetamide, and purified by three cycles of polymerization and depolymerization. The antigen, prepared in this way gave 4 major bands and 3 minor bands in SDS-electrophoresis, all with molecular weight between 40,000 and 63,000 and could be assembled in vitro in 80 Å filaments. The rabbit antiserum was affinity-purified on a column of keratin bound to Affigel-10 (Bio Rad Laboratories, Watford, GB). Unbound proteins were removed by washing in 1 M NaCl and specific antibody was obtained by counterflow elution with 3 M KCNS.

The indirect immunoperoxidase method (IPO) was carried out as described previously (Nathrath et al. 1982). 5 µm sections from formalin fixed, paraffin embedded tissue blocks were dewaxed in xylene, rehydrated through graded alcohols and incubated with 0,1% protease type VII (Sigma, München, FRG) for 30 min (Huang S.N. 1975). Endogenous peroxidase activity was quenched by sequential treatment with 7,5% hydrogen peroxide (5 min), periodic acid (5 min) and 2.28% sodium borohydride (2 min). After overlaying for 5 min in normal sheep serum, rabbit antiserum (anti-keratin serum diluted 1:40, anti-thyroglobulin 1:100) in Dulbecco's phosphate buffered saline A (PBS) was applied for 15 min. After washing in PBS, peroxidase conjugated sheep antirabbit IgG antiserum (Miles-Yeda GmbH, Frankfurt/Main, FRG) diluted 1:500 was applied for 15 min. Controls included substitution of first and/or second antiserum by PBS and preimmune serum. After further washing the sections were stained with 0.01% 3-amino-9-ethylcarbazole (Sigma, München, FRG) freshly prepared in Michaelis buffer pH 7.4 containing 0.0015% H_2O_2 and 6% dimethylsulfoxide (Marck, Darmstadt, FRG) (Graham et al. 1965; Schaefer and Fischer 1968). Sections were counterstained in Mayer's hemalum, blued in tap water and mounted in Kaiser's glycerol gelatine (Merck, Darmstadt, FRG).

Results

The results obtained with the anti-thyroglobulin and anti-keratin serum, using the indirect immunoperoxidase method on formalin fixed paraffin embedded human thyroid neoplastic and non-neoplastic alterations are summarised in Table 1.

Nodular Goiters

The distribution of thyroglobulin was inhomogenous within the tissue. The anti-thyroglobulin serum gave a strong reaction with both the colloid and the cytoplasm of follicular epithelial cells, and did not react with nuclei and interstitial tissue. The strongest reaction was seen in areas of tall hyperplastic epithelial cells. The antikeratin serum, in general, did not react with any tissue component, except flat inactive follicular epithelial cells in involuted areas.

Toxic Goiter

Anti-thyroglobulin serum gave a very strong and regularly distributed reaction in follicular epithelial cells of hyperplastic thyroid glands. This reaction was the strongest of all thyroid alterations examined.

Anti-keratin serum showed virtually no reaction; however, occasionally a few follicular epithelial cells were weakly stained, mainly in their outer cytoplasm.

Table 1. Immunohistochemical analysis of 56 neoplastic and nonneoplastic thyroid alterations. The grading of reaction includes the staining intensity and the number of cells stained.

Microscopic Diagnosis	Number of cases reacting for					
	Keratin			Thyroglobulin		
	a	b	c	a	b	c
Follicular carcinoma		3	7	6	4	
Oncocytic carcinoma		1	3		4	
Papillary carcinoma	12	1		1	11	1
Follicular adenoma		1	4	5		
Toxic goiter		2	3	5		
Nodular goiter		1	4	4	1	
Undifferentiated carcinoma		2 (only single cells)	7			9
Medullary carcinoma			3			3
Th. Hasthimoto			1		1	
Th. de Quervain			1		1	

[a] almost all cells stained intensely
[b] less than 50% of cells staining, generally weak
[c] generally minimal or no reaction

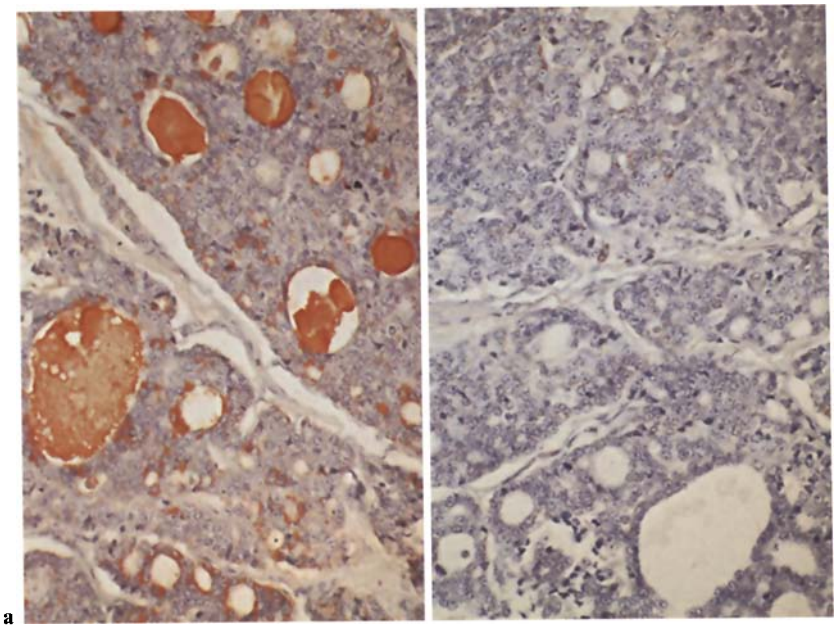

Fig. 1a, b. Moderately differentiated follicular carcinoma. **a** Reaction in epithelium and colloid with antithyroglobulin serum (×200). **b** Virtually no reaction with anti-keratin serum (×200)

Thyroiditis

The two cases of thyroiditis (1 Hashimotos's, 1 de Quervain's) showed irregular staining for thyroglobulin but none for keratin.

Follicular Tumours

In general, adenomas and carcinomas, including the oncocytic carcinomas, showed identical staining patterns with both antisera. While in adenomas the staining pattern was regular, that in carcinomas tended to be more heterogenous, and this was particularly marked in poorly differentiated tumours. The anti-thyroglobulin serum gave a very strong cytoplasmic reaction, particularly in the apical areas of cells and in colloid (Fig. 1a). In oncocytic carcinomas, there was only focal reaction in the cytoplasm of some tumour cells, and in the small amounts of colloid.

The anti-keratin serum showed virtually no reaction (Fig. 1b). However, one follicular carcinoma with large fibrotic areas showed a reaction with anti-keratin serum in some isolated tumour cells.

Papillary Carcinomas

The pattern of reaction was completely different from that in follicular tumours.

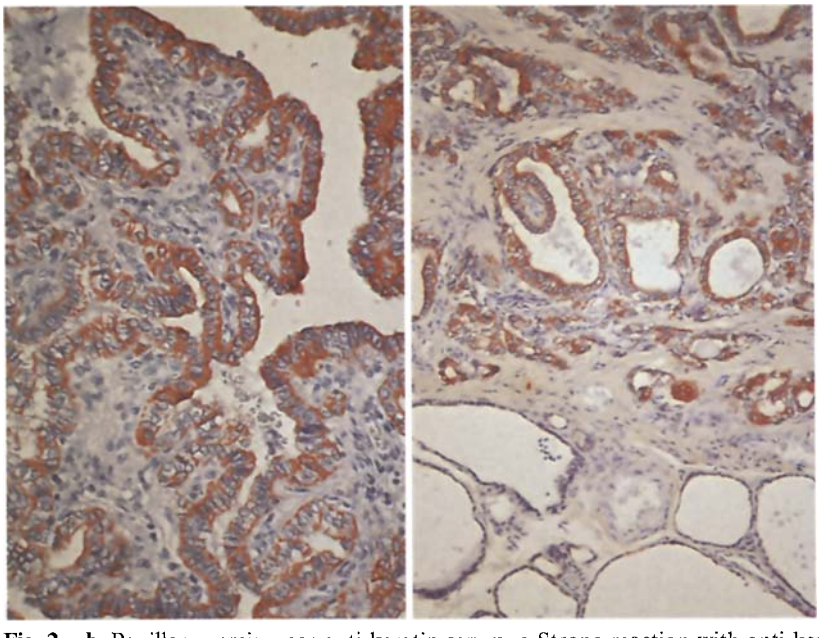

Fig. 2a, b. Papillary carcinoma; anti-keratin serum. **a** Strong reaction with anti-keratin serum in the whole cytoplasm of almost all carcinoma cells (×200). **b** At the border, a striking contrast between the reactive infiltrating carcinoma and the nonreactive surrounding normal thyroid tissue (×100)

By contrast to follicular tumours, in papillary carcinomas there was a consistent and very strong reaction of the whole cytoplasm with the anti-keratin serum (Fig. 2a). At the border of the tumour there was a striking contrast between the keratin reactive infiltrating papillary carcinoma and the surrounding non-reactive normal thyroid tissue (Fig. 2b). Papillary carcinomas showed only an irregular and relatively weak reaction for thyroglobulin (Fig. 3). In mixed papillary-follicular areas, the reaction for keratin was much stronger in papillary or solid portions than in the follicular components. These areas showed an almost opposite staining pattern for thyroglobulin, which was strong in the follicular components, and keratin, which was strong in the papillary portions. Metastases showed the same pattern of staining as the primary carcinomas.

Undifferentiated Carcinomas

No reaction for thyroglobulin was seen, except in some remnant thyroid follicles.

Similarly, keratin was not demonstrable, except in two of the nine cases, which contained a few solitary keratin reactive carcinoma cells.

Medullary Carcinomas

No reaction was seen with either anti-thyroglobulin or with anti-keratin serum.

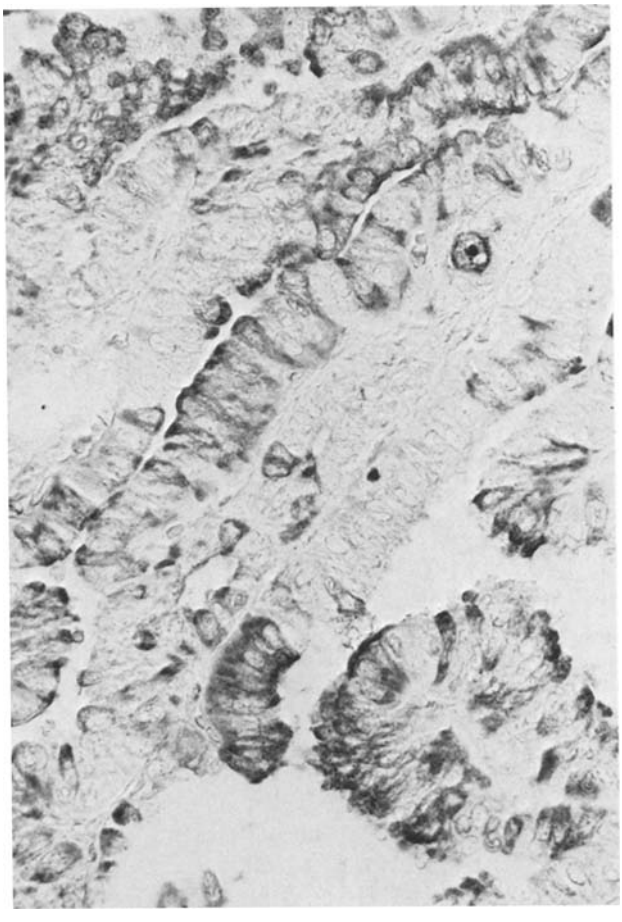

Fig. 3. Papillary carcinoma reactive with anti-thyroglobulin serum (×300); without nuclear counterstaining

Discussion

Keratin is a cytoskeletal protein associated with the maintenance of the structural integrity of epithelial cells (Franke et al. 1978; Lazarides 1980; Schlegel et al. 1980; Sun et al. 1979; Sun and Green 1978). It would not be expected to have any particular relation to the specific function of thyroid follicular epithelium. Surprisingly, however, our results showed a striking contrast between the presence of keratin and thyroglobulin. In normal thyroid glands, as expected, a strong reaction for thyroglobulin was seen while anti-keratin serum generally did not react.

Previously, the residual capacity of thyroid tumours to produce thyroglobulin had been proposed as the criterion for functional classification of thyroid tumours (Böcker et al. 1980; Dralle and Böcker 1977; Kawaoi et al. 1981). There is also a good correlation between the degree of residual functional capacity and the ultrastructural classification of these differentiated tumours (Böcker 1979; Dralle and Böcker 1977; Johannessen et al.

1978) which is based mainly on differences in content of cellular organelles. Typically, most cells of a papillary carcinoma contain only few organelles in a poorly differentiated cytoplasm (Böcker 1979). By contrast, typical cells of follicular adenomas and carcinomas are rich in organelles in a highly differentiated cytoplasm, thus resembling normal thyroid epithelial cells. Our results show that it is these tumours, known to maintain highly differentiated metabolic capacities, that virtually do not react for keratin. By contrast, papillary carcinomas, which are poor in organelles and do not produce thyroglobulin react very strongly for keratin. Other known differences between these types of tumour include occasional development of squamous metaplasia in papillary carcinomas and characteristically different patterns of growth and routes of metastases (Byar et al. 1979; Bubenhofer and Hedinger 1977; Franssilla 1975 and 1978; Georgii 1977; Heitz et al. 1976; Hubert et al. 1980; Krisch et al. 1977; Löhrs et al. 1977 und 1980; Williams et al. 1977; Wollner et al. 1968). All these differences are of prognostic importance. It is intriguing to speculate that the cytoplasmic presence of keratin correlates with differences in biological behaviour with regard to the invasive growth and route of metastases in papillary and follicular carcinomas. Differences in degrees of keratin staining may be due to the difference in absolute contents of intermediate filaments or to differences in their stainability dependent on the density of cellular organelles. The good staining of papillary carcinomas for keratin may be due to the presence of few cellular organelles, while the virtual lack of keratin reaction in follicular carcinomas may be explained by a masking effect of large numbers of cytoplasmic organelles. Further studies at the ultrastructural level, using sera to different keratin types, should help to clarify this point. It is concluded that keratin may help as an additional marker for the prognostically important distinction between follicular and papillary carcinoma of the thyroid gland.

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