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ORIGINAL ARTICLE

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"Atypical" medullary thyroid carcinoma with little or no calcitonin expression

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Abstract In a retrospective analysis of 142 medullary thyroid carcinomas, four sporadic cases with an unusual histological and immunohistochemical appearance were found. Three cases (two males, one female) had very few calcitonin-positive tumour cells, while the fourth case (male) completely lacked calcitonin immunoreactivity at both mRNA and protein levels, whereas a variety of neuroendocrine markers were positive in at least 50% of tumour cells. The four tumours were completely devoid of carcinoembryonic antigen expression and of amyloid. Differential diagnosis and histogenetic considerations are discussed.

Key words Neuroendocrine thyroid tumour · Medullary thyroid carcinoma · Lack of calcitonin

Introduction

Neuroendocrine tumours of the thyroid designated as medullary thyroid carcinoma (MTC) are defined as a malignant tumours composed of cells showing evidence of C cell differentiation and *usually* containing calcitonin [16]. Although calcitonin is present in the vast majority of MTC and many cases show extensive calcitonin expression immunohistochemically, calcitonin positivity is occasionally restricted to very few cells or small and focal areas [14, 32]. However, according to LiVolsi [24], it seems to be impossible to define a tumour as MTC if it does not produce calcitonin; the diagnosis of a *calcitonin-free* MTC should only be made within a familial setting or if the tumour occurs in a thyroid with unequivocal C cell hyperplasia [24].

The thyroid gland can be the site of other neuroendocrine tumours than MTC. Paraganglioma [3, 7, 21, 27], intrathyroidal parathyroid tumours [8, 34], primary oat-cell carcinoma of the thyroid [9], and metastatic neuroendocrine tumours of the thyroid [26] have repeatedly been described in the literature. Since MTC may be indistinguishable on morphological grounds, various methods, including immunohistochemistry, in situ hybridisation, and electron microscopy, have been applied to arrive at the final diagnosis.

We describe four cases of well-differentiated neuroendocrine tumours of the thyroid with strong expression of neuroendocrine markers (chromogranins, synaptophysin, NSE) but very little or complete lack of calcitonin. The differential diagnosis and possible origin of such tumours are discussed.

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Materials and methods

Routinely processed formalin-fixed and paraffin-embedded tissues from 142 MTC were retrieved from the files of the Department of Pathology, University of Munster/Westphalia, Germany, and the Department of Pathology, University of Innsbruck, Austria. The present series was made up of 34 familial MEN 2A- and 2B-associated cases (23 female, 11 male patients aged 6–47 years, mean 20.8 years) and 108 sporadic tumours (71 female, 37 male patients aged 22–86 years, mean 49.2 years). The tumour size ranged from 0.3 to 3.5 cm in the familial cases and from 0.8 to 6.3 cm in the sporadic cases.

In all cases immunohistochemical staining with antibodies against calcitonin, chromogranin A, carcinoembryonic antigen (CEA), and thyroglobulin were routinely performed (for antibody sources and dilutions see Table 1). These immunohistochemical stains were reassessed and cases were categorised according to the staining reaction patterns found. The four cases described in detail were further investigated with a variety of antibodies (see Table 1).

Human calcitonin mRNA was detected using a 28–29 mer 5' digoxigenin-labelled probe mixture (British Biotechnology Products, Oxford, UK). The in situ hybridisation protocol has been published elsewhere [39].

Results

Immunohistochemically, calcitonin, chromogranin A, and CEA were moderately to strongly expressed in the

Table 1 Source, pre-treatment, and dilution of antibodies applied

Antibody	Source	Antigen retrieval	Dilution
Calcitonin	Immunotech	–	1:15
Carcinoembryonic	Dako	–	1:400
Thyroglobulin	Immuntech	–	1:10
Chromogranin A	Immunotech	–	1:10
Chromogranin B	R. Fischer-Colbrie, Innsbruck, Austria	–	1:4000
Secretogranin II	R. Fischer-Colbrie, Innsbruck, Austria	–	1:500
Calciton in gene-related paptide	Amersham	–	1:3200
Neurone-specific enolase	Dako	–	1:1500
Synaptophysin	Boehringer	–	1:20
Somatostatin	Dako	–	1:1600
Adrenocorticortropic hormone	Dako	–	1:600
Parathyroid hormone	B. Jasani, Cardiff, UK	–	1:1600
S-100 protein	Dako	–	1:6000
Vimentin	Imunotech	–	1:2
Cytokeratin 5/6	Medac	Wet autoclaving [2]	1:1000
Cytokeratin 7	Medac	Wet autoclaving	1:10
Cytokeratin 8	Medac	Wet autoclaving	1:20
Cytokeratin 13	Progen	Wet autoclaving	1:1500
Cytokeratin 14	Medac	Wet autoclaving	1:300
Cytokeratin 18	Sigma	Wet autoclaving	1:800
Cytokeratin 19	Medac	Wet autoclaving	1:800
Cytokeratin 20	Progen	Protease	1:50
p53 (CM-1)	Medac	Wet autoclaving	1:10,000
p53 (DO-7)	Medac	Wet autoclaving	1:250
p53 (Ab 1801)	Medac	Wet autoclaving	1:400
p53 (Ab 240)	Medac	Wet autoclaving	1:50

majority of tumour cells in all familial cases (see Table 2). In sporadic cases the proportion of cells with expression of these three markers ranged from almost all tumour cells to very few. In some cases calcitonin-immunoreactive cells were substantially outnumbered by chromogranin A-immunoreactive cells. In less well-differentiated cases CEA immunoreactivity was usually stronger than calcitonin and/or chromogranin A stains. These findings were independent of the histological subtype of MTC. With the exception of entrapped follicles, all cases investigated lacked convincing thyroglobulin immunoreactivity.

Three cases (two men and one woman, aged 28, 46, and 45 years, respectively) showed very few calcitonin-positive cells (Fig. 1); a fourth case (37-year-old man) lacked calcitonin immunoreactivity completely. The immunohistochemical calcitonin pattern observed was confirmed in these four cases by a similar mRNA pattern in in situ hybridisation. All four cases showed diffuse chromogranin A positivity in more than 50% of tumour cells (Fig. 2) and a complete lack of CEA and thyroglobulin immunoreactivity (Fig. 3). All tumours were grossly encapsulated but showed vascular and/or capsular invasion and an admixture of organoid clusters or trabecular structures histologically. These consisted of rather uniform cells (Fig. 4) and areas with some enlarged, hyperchromatic nuclei (Fig. 5). In the latter areas a somewhat greater mitotic activity was observed. Amyloid was not detectable by means of Congo red staining, although three tumours showed a slightly hyalinised stroma. Normal C cells without evidence of C cell hyperplasia were demonstrated in the adjacent thyroid parenchyma. Additional immunohistochemical stains revealed strong positivity for neurone-specific enolase (Fig. 6). Broad-spectrum cytokeratins (antibody Kl-1), cytokeratin 7, 8 and cytokeratin 19 (Fig. 7) were found in the majority of tumour cells, whereas cytokeratin 18 was detectable in some tumour areas in two cases. Calcitonin-gene-related peptide (CGRP) was found in a few cells in two cases, whereas the remaining two cases lacked CGRP completely.

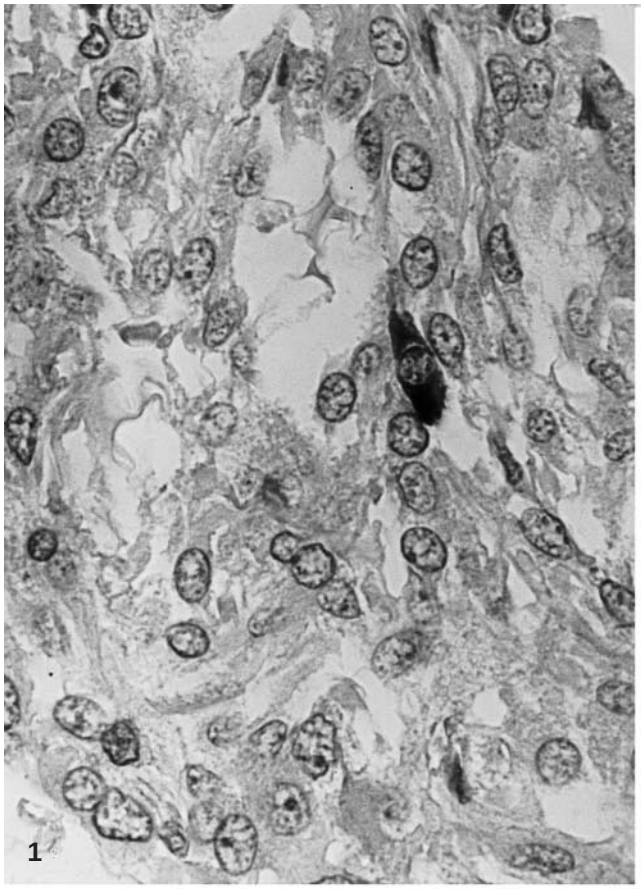
Focal vimentin positivity was observed, predominantly in spindle cell areas. A few somatostatin- (Fig. 8) and ACTH-immunoreactive cells and focal chromogranin B and secretogranin II positivity (the latter two mainly in the same areas as chromogranin A) were additional immunohistochemical findings. Negative immunoreactivity was found with antibodies against serotonin, parathyroid hormone (PTH), S-100 protein, p53 (antibodies CM-1, DO7, PAb240, PAb1801), and cytokeratins 5, 6, 13, 14, and 20.

Fig. 1 Atypical medullary thyroid carcinoma with a single calcitonin-positive tumour cell. APAAP, ×250 ▶

Fig. 2 The same tumour as shown in Fig. 1, with numerous chromogranin A-positive tumour cells. APAAP, ×100

Fig. 3 Grossly encapsulated thyroglobulin-negative tumour with some entrapped thyroglobulin-positive nonneoplastic follicles. APAAP, ×40

Fig. 4 Tumour area with organoid clusters and trabecular structures. Some cells show enlarged nuclei. H&E, ×40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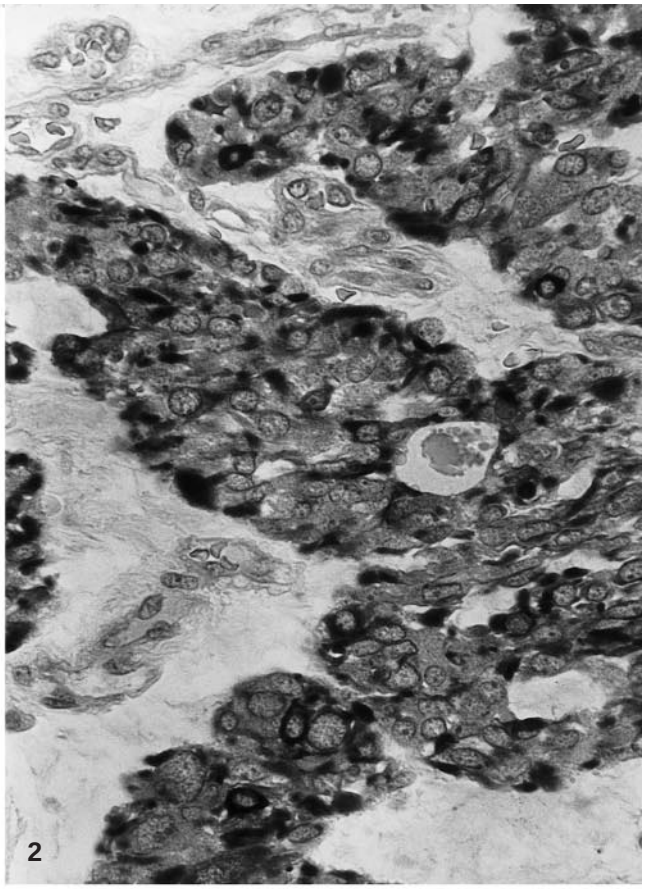
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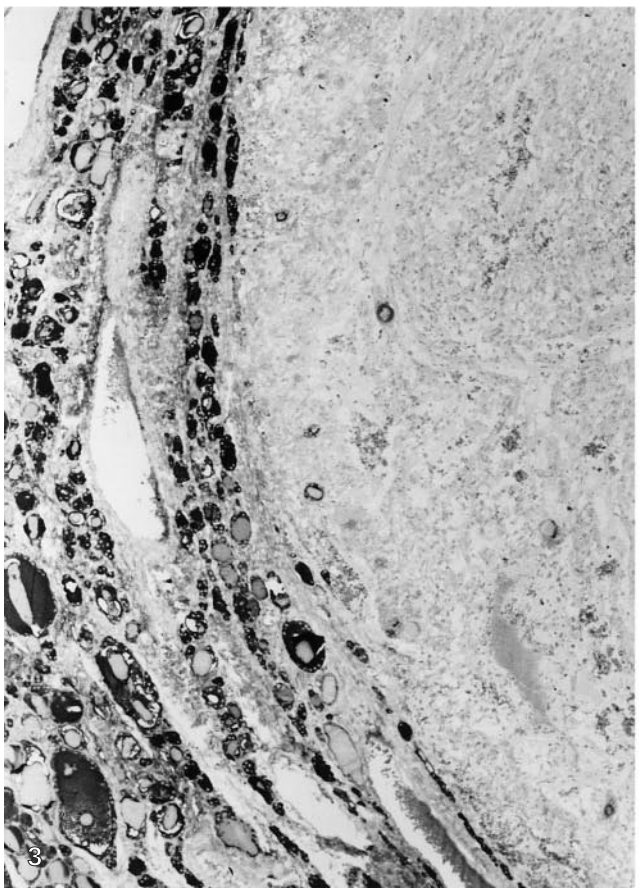
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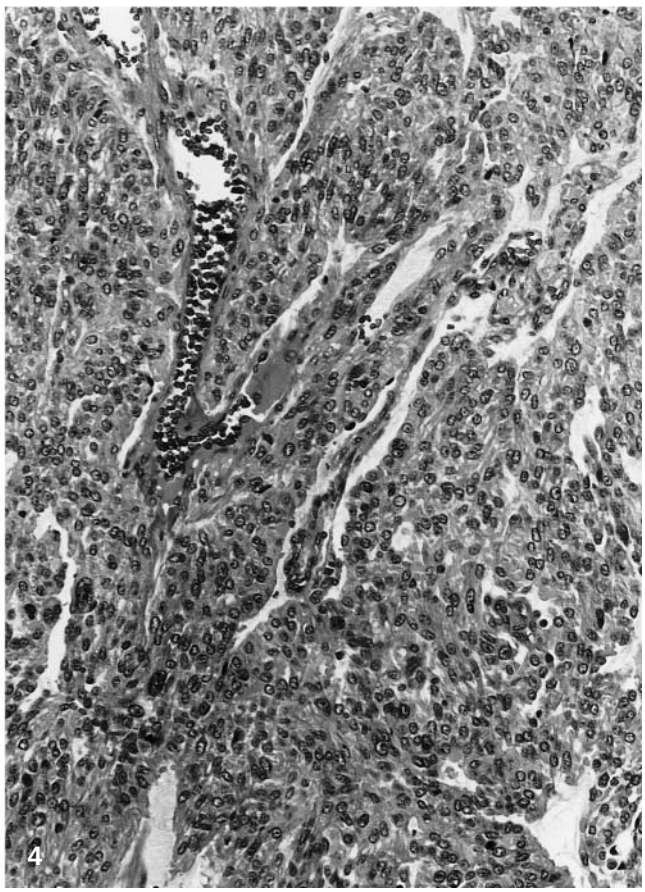
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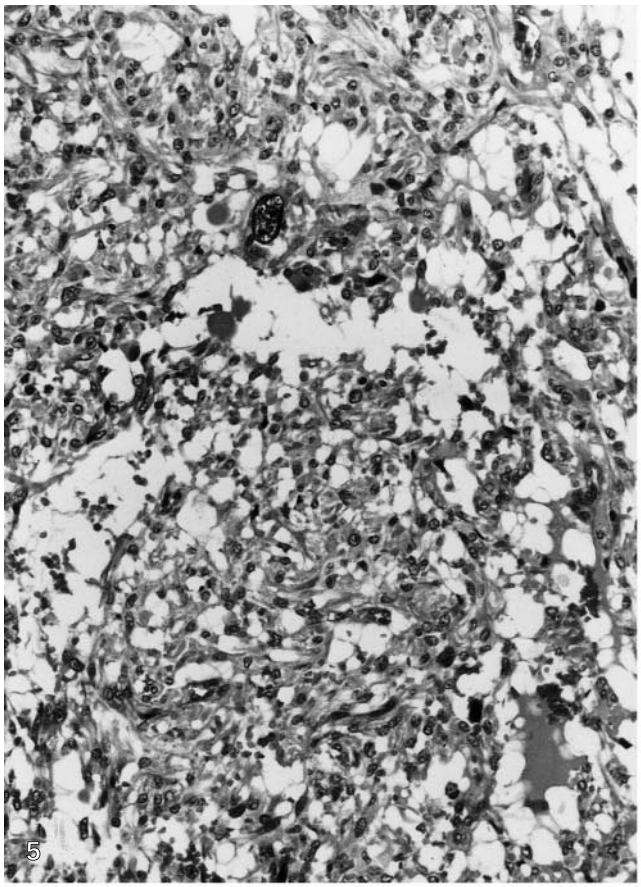
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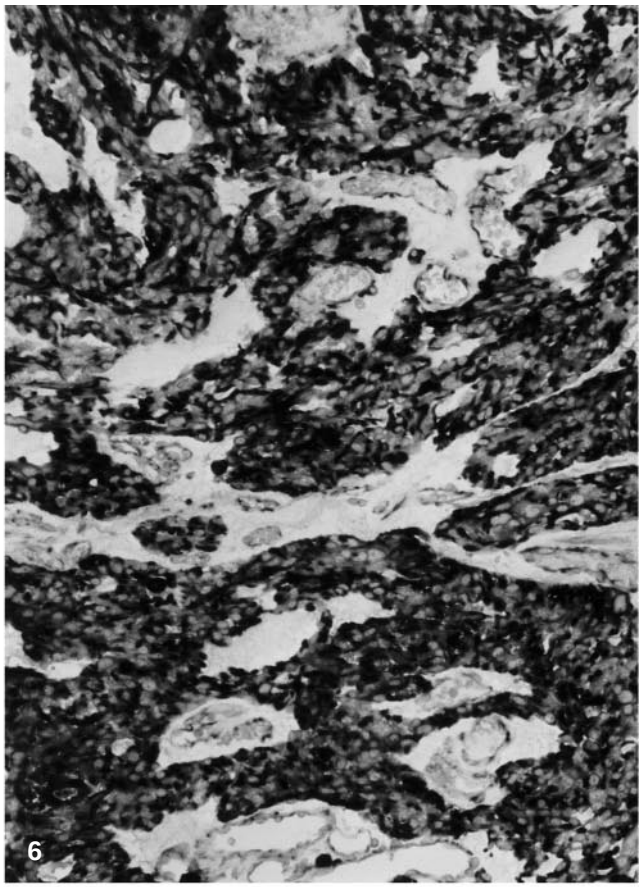
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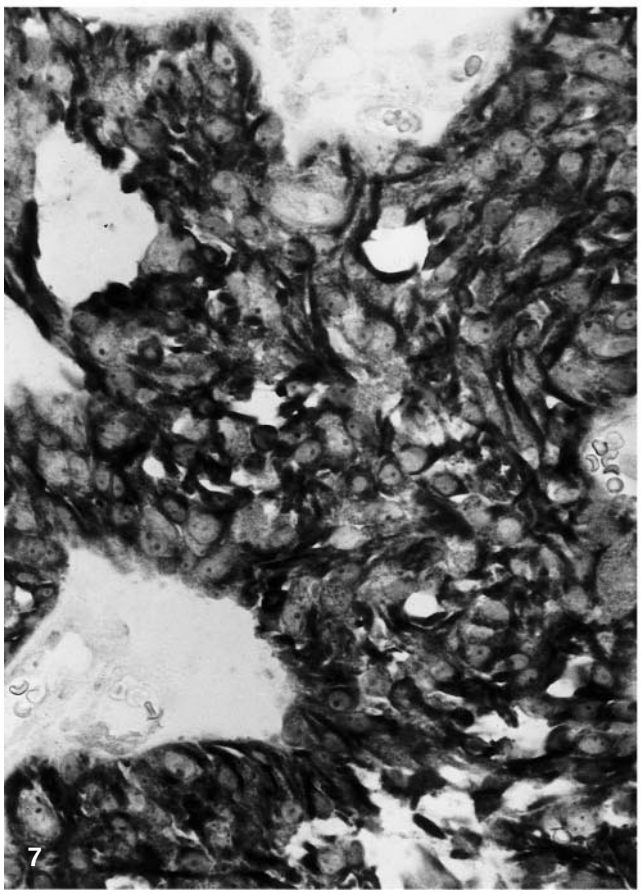
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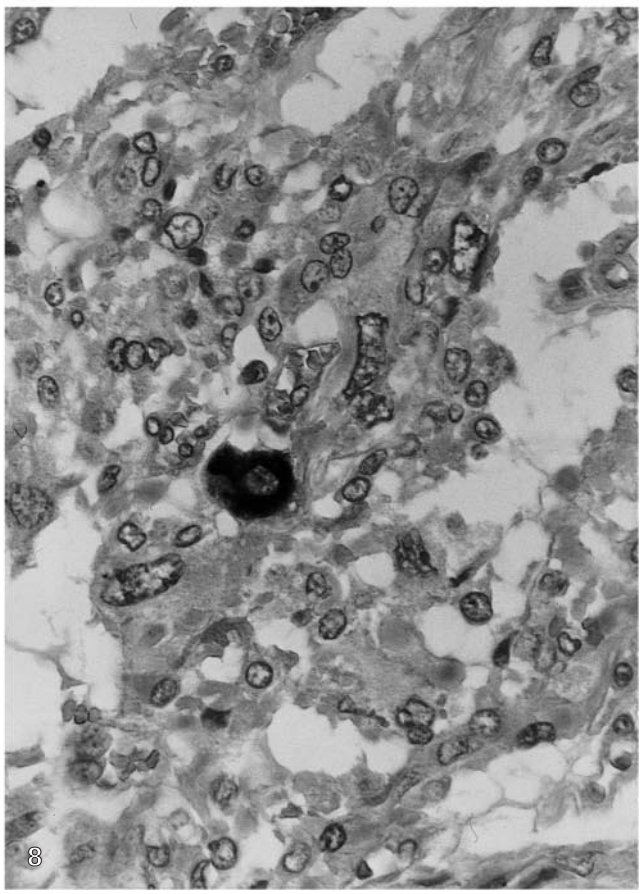
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Three patients are tumour free after a mean observation time of 47.4 months; one male patient developed lymph node metastases 19 months after the operation. These were not available for immunohistochemical investigation. Although there was a clinical suspicion of malignancy in three cases, all patients lacked clinical symptoms of MTC. Postoperative MEN family screening was negative in all four cases. None of the patients has any signs of a neuroendocrine carcinoma that might have metastasised to the thyroid.

Discussion

All unusual tumours of the thyroid should be investigated immunohistochemically [28, 46] with antibodies against thyroglobulin, calcitonin, CEA and chromogranin A [38]. However, defining a thyroid tumour as MTC on the grounds of very small numbers of calcitonin-positive cells remains a matter of subjective judgement. Nevertheless, we suggest that the term MTC is appropriate, despite lack of calcitonin production, which was clearly not originally described by Hazard et al. [15] as a diagnostic criterion. Any attempt to evaluate a calcitonin-free, immunohistochemically proven neuroendocrine tumour of the thyroid involves an obvious dilemma. If we deny the existence of calcitonin-negative MTC, we also have to reject S-100 protein-negative paragangliomas or parathyroid hormone-negative parathyroid tumours. Parathyroid tumours can occur without hyperparathyroidism, and paragangliomas can be S-100 protein negative [19]. Accordingly, it seems to be conceivable that expression of calcitonin may be lost during MTC progression.

The four cases presented (originally diagnosed as MTC on the grounds of their expression of neuroendocrine markers such as chromogranins) exhibit immunohistochemical patterns already observed in other series of MTC (Table 2). Calcitonin may be present only in a few tumour cells [14, 20, 47] or may be completely lacking [14, 41]. Usually the pattern of chromogranin A immunoreactivity resembles that of calcitonin reactivity [14, 37]; however, some MTC show a greater proportion of chromogranin-A immunoreactive than of calcitonin-immunoreactive tumour cells [14]. Rare cases of chromogranin A-negative MTC have been described [29], but chromogranin A was detectable in all 142 MTC of the present series. Completely CEA-negative MTC have been described by Krisch et al. [20]. Interestingly in their series of 30 MTC, 3 out of 4 MTC without CEA contained only a few tumour cells with calcitonin immunoreactivity. A completely calcitonin-negative MTC described by Sobol et al. [41] showed only weak to moderate CEA positivity.

Table 2 Immunohistochemical patterns of classic and "atypical" (medullary thyroid carcinoma, – negative; + few positive cells; ++ focal positivity; +++ majority of tumour cells positive)

Antibody	Classic MTC	Atypical MTC
Calcitonin	++/+++	–/+
Carcinoembryonic antigen	++/+++	–
Thyroglobulin	–	–
Granins (chromogranins A and B, secretogranin II)	++/+++	++/+++
Calcitonin gene related peptide	++/+++	–/+
Neurone specific enolase	++/+++	++/+++
Synaptophysin	++/+++	++/+++
Somatostatin	–/+	–/+
ACTH	–/+	–/+
Parathyroid hormone	–	–
S-100 protein	–	–
Vimentin	+/+++	++/+++
Cytokeratin	++/+++	++/+++
Cytokeratin 7	++/+++	++/+++
Cytokeratin 8	++/+++	++/+++
Cytokeratin 18	++/+++	–/++
Cytokeratin 19	–/+	++/+++
Cytokeratins 5, 6, 13, 14, 20	–	–
p53 (CM-1, DO7, Ab1801, Ab240)	–	–

Immunoreactivity for NSE has been detected in different thyroid tumours [10]. In most of these, tumours NSE positivity seems to reflect an unusual metabolic state of the tumour cells rather than neuroendocrine differentiation [4, 33]. The demonstration of other neuroendocrine markers (chromogranins A and B, secretogranin II, synaptophysin) in the four tumours of this series confirmed their true neuroendocrine nature. These markers also exclude mucoepidermoid carcinoma of the thyroid [10, 33, 43]. As stated above, the lack of S-100 protein and parathyroid hormone does not entirely exclude the rare paraganglioma of the thyroid [3, 21] or intrathyroidal neoplastic or hyperplastic parathyroid tissue. In addition, we have recently shown that hyperplastic parathyroid tissues can express calcitonin both at the mRNA and at the protein level [39].

The differential diagnosis also includes hyalinising trabecular tumours [5]. This distinct type of thyroid neoplasm can have a similar histological appearance, as well as immunohistochemical expression of chromogranin A, somatostatin, and NSE [18, 40]. However, a constant hallmark of hyalinising trabecular tumours is their thyroglobulin expression [5, 18, 40]. An encapsulated variant of MTC resembling hyalinising trabecular adenoma has also been described; both tumours, however, showed strong calcitonin immmunoreactivity in the majority of

◀ **Fig. 5** Tumour areas with loose stroma consisting of polygonal and spindle cells. Note some enlarged hyperchromatic nuclei. H&E, ×40

Fig. 6 Strong NSE positivity in almost all tumour cells. APAAP, ×40)

Fig. 7 Tumour area with pronounced positivity for cytokeratin 19. APAAP after wet autoclave pretreatment for antigen retrieval, ×250

Fig. 8 Somatostatin-positive tumour cell. APAAP, ×250

tumour cells [17]. The four tumours in our series were well-differentiated tumours, virtually excluding primary oat-cell carcinoma of the thyroid [9] and metastatic deposits of neuroendocrine carcinomas to the thyroid [26].

We have recently suggested a possible thymic origin for neuroendocrine thyroid tumours with very little or no calcitonin expression [35]. Thymic rests or remnants of branchial pouches that retain the potential to differentiate along the thymic line may give rise to thyroid tumours [6, 13]; these tumours are ectopic cervical thymoma, ectopic hamartomatous thymoma, spindle epithelial tumour with thymus-like differentiation (SETTLE), or carcinoma showing thymus-like differentiation (CASTLE). (For an overview see [31].) However, neuroendocrine differentiation has not so far been attributed to thyroid tumours of putative thymic origin.

Morphologically, MTC show a broad variety of patterns, including a spindle cell form, a pigmented form [25] a small cell form [1, 11], and a carcinoid-like variant [12]. These histological patterns can also be observed in carcinoid tumours of the thymus: a spindle cell type [22, 42], a pigmented form [45], and even a subtype strongly resembling MTC with an amyloid-containing stroma [30, 44, 45]. Virtually all immunohistochemical markers found in thymic carcinoids, such as NSE, Leu 7, synaptophysin, chromogranin A, protein gene product 9.5, epithelial membrane antigen, neurofilament, somatostatin, serotonin, met-enkephalin, leu-enkephalin, and beta-endorphin have also been demonstrated in MTC (for reviews see [24, 36]). Nevertheless, calcitonin can be regularly demonstrated in carcinoid tumours of the thymus [23, 44]. However, there is currently no specific marker available to prove the putative thymic origin of those tumours.

With no specific markers available, it may be less important to argue about the histogenetical background of (almost) calcitonin-free neuroendocrine tumours of the thyroid than to document the existence and biological behaviour of these obviously rare “atypical” MTC. Molecular pathological methods may offer further clues for the better characterisation of primary neuroendocrine carcinomas of the thyroid with little or no calcitonin expression.

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