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Mixed medullary-follicular carcinoma of the thyroid

A morphological, immunohistochemical and in situ hybridization analysis of 11 cases

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Abstract Mixed medullary-follicular carcinomas (MMFC) of the thyroid are rare tumours showing the morphological and immunochemical properties of both parafollicular and follicular cell lineages. Their recognition is based on a classical WHO definition, although several other patterns have been described in recent years. We investigated 11 cases of MMFC by immunohistochemistry and in situ hybridization (ISH) to analyse the structural features, the immunophenotypic profile and the calcitonin (CT) and thyroglobulin (TG) gene expression of the neoplasm. Histologically, 10 cases had mixed parafollicular and follicular cell populations in the primary tumour and 1 only in the lymph node metastasis. All cases were immunoreactive for CT (in medullary areas) and TG (in follicular areas and also in the solid component of 8/11 cases). These findings were confirmed by ISH analysis. Combined ISH and immunostaining showed that most cases had separate CT and TG gene expression, although rare cells with concurrent CT and TG gene expression were identified in 2 tumours. We conclude that (a) MMFC display heterogeneous morphological patterns and are a special type of thyroid tumour undergoing divergent differentiation; (b) in MMFC, CT and TG genes are generally not simultaneously expressed by the same cell, although dual expression of CT and TG was present in rare neoplastic elements; and (c) the origin of MMFC, whether they are derived from the ultimo-branchial body or result from neoplastic transformation of different cell populations following common oncogenic stimuli, is unclear.

Key words Thyroid · Carcinoma, mixed medullary-follicular · Thyroglobulin · In situ hybridization

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Introduction

Mixed follicular and medullary carcinomas (MMFC) [8, 14, 22, 30, 36, 43, 50, 51], show combined features of medullary (calcitonin immunoreactivity) and follicular (thyroglobulin immunoreactivity) carcinomas [17] and constitute a rare but challenging group of tumours of the thyroid. Some confusion regarding the definition of MMFC has arisen because of reports of a spectrum of thyroid tumours with characteristics similar to MMFC, including medullary carcinoma with thyroglobulin-immunoreactive cells [10, 18, 24, 27, 39, 47] on the one hand and carcinomas of follicular cell origin with neuroendocrine differentiated cells [7, 31, 35, 49], on the other. True medullary carcinomas or follicular/papillary carcinomas having scattered signs of differentiation towards follicular or parafollicular C-cell lineages do not belong to the MMFC group. For MMFC to be recognized as a distinct tumour entity, strict morphological and functional criteria are required.

We collected a series of 11 thyroid tumours with mixed medullary-follicular features on both light microscopy and immunocytochemistry. We checked for any morphological differences recognizable among the various types of MMFC and to see whether calcitonin and thyroglobulin genes were expressed simultaneously in the same cells in MMFC. We also investigated whether MMFC showed expression of dual (divergent) differentiation in a single tumour type or whether this was the result of collision tumours, and whether the prognosis of MMFC differs from that of other thyroid carcinomas.

Materials and methods

Eleven cases of MMFC were collected: 6 cases were from Mayo Clinic; 3 (5%) were selected from a series of 59 consecutive cases of medullary carcinoma of the thyroid (MCT) diagnosed at Mayo Clinic from 1985 to 1994, and 3 were seen in consultation by two of us (J.A.C., R.V.L.) in the same period. The remaining 5 cases were from the Department of Pathology of the University of Turin; 2 (3.2%) were selected from a series of 62 consecutive MCTs diagnosed from 1974 to 1995 and 3 were seen in consultation by one of us (G.B.) in the same period. One of the latter cases (no. 1) has already been reported [51]. The clinico-pathological and follow-up data of all 62 cases of MCT have been reported elsewhere [40]. No relevant differences in the clinical presentation of classical versus mixed cases were observed.

Nine cases met the histopathological criteria for diagnosis of MMFC proposed by the WHO classification [17]. In 1 case the "follicular" component was represented by papillary carcinoma in lymph node metastases, the primary tumour having been a mixed medullary-follicular carcinoma. In the final case, there were separate papillary and medullary carcinomas. This case was included in the study because of the mixed features in the metastatic deposits. Another 2 cases were eliminated because the different neoplastic cell populations were clearly separate and these were examples of collision tumours rather than MMFC.

One to seven H&E-stained slides from each primary tumour and from lymph node metastases in some cases were available for review. One or two representative blocks of the tumour and of the metastases were used for further immunohistochemical (IHC) and in situ hybridization (ISH) analyses.

Clinical data and follow up information were obtained from all but 1 patient.

For IHC, serial sections were collected onto poly-L-lysine-coated slides. Sections were processed for calcitonin (CT) and thyroglobulin (TG) immunostaining with the ABC method [20]. The reaction product was revealed by diaminobenzidine. The primary antibodies were a polyclonal serum to TG (Dako, Glostrup, Denmark) diluted 1/3000 and a polyclonal serum to CT (Ortho Diagnostic, Raritan, USA) diluted to one half the strength of the original kit solution.

For ISH, 5-μm-thick sections were deparaffinized twice for 5 min in xylene, hydrated through a standard series of ethanols to PBS and digested with 0.2 mg proteinase K (Sigma)/ml in 0.1 M Tris-HCl, 50 mM EDTA, pH 8.0, for 15 min at 37°C. After blocking twice for 5 min in ice-cold 0.1 M glycine/PBS, sections were treated with 0.25% (v/v) acetic anhydride in 0.1 M triethanolamine, pH 8.0, for 10 min, rinsed for 3 min in 2× SSC and dehydrated. An RNA probe corresponding to bases 838–1245 of the TG complementary DNA (cDNA) [3, 4] was labelled with ^{33}P-UTP (Amersham International, Zurich, Switzerland) by transcription of a polymerase chain reaction (PCR)-generated template containing a suitable phage RNA polymerase promoter at each end [44]. Briefly, two primers (Table 1) were used to amplify the portion of TG cDNA encompassing positions 838–1245. As starting material, a small amount of plasmid #2 [3] (kindly provided by Dr. J.-L. Bergé-Lefranc) was used. The 457-base pair PCR product contains the amplified thyroglobulin sequence flanked by two unique restrictions sites (Pst I and Sst I) and two promoters specific for the T7 and the SP6 RNA polymerases, respectively. Cutting the PCR product with Pst I and transcribing with SP6 RNA polymerase yielded a 413-base RNA probe complementary to the TG mRNA ("antisense"). Cutting the same PCR product with Sst I and transcribing it with T7 RNA polymerase, a RNA probe of the same length as the first probe, but with a "sense" sequence, was obtained and used as negative control.

Table 1 Sequence of the primers used for generation of the thyroglobulin template by PCR. (The sequences of the promoters are in italics, the restriction sites are underlined, and the thyroglobulin proper sequence is in bold characters)

SP6-**SstIAS**
5′-*GAT-TTA-GGT-GAC-ACT-ATA-GAA-TAC*-**GAG-CTC-CTT-GAT-CGT-GGG-TG**-3′

T7-**PstIS**
5′-*TCT-AAT-ACG-ACT-CAC-TAT-AGG-GAG*-**ACT-GCA-GAG-ACG-GTT-CCT-CGC**-3′

For the hybridization, the labelled probe (final concentration 10^5 cpm/μl) was boiled for 3 min in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 500 μg/ml tRNA from *Escherichia coli* (Sigma), rapidly chilled on ice and added to a mixture containing 50% formamide, 0.3 M NaCl, 20 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1× Denhardt's solution, 10% dextran sulphate (Sigma) and 1 U/μl RNAse Inhibitor (Promega Biotech, Madison, Wis.). The hybridization mix was applied to sections and covered with silicon-treated coverslips. Slides were then incubated overnight at 42°C under prewarmed mineral oil. After hybridization, the oil was removed by chloroform washes and dried slides were washed for 1 h in 2× SSC at room temperature (RT) and then digested with 20 μg RNAse A/ml and 10 U RNAse T1/ml (Boehringer Mannheim, Rotkreuz, Switzerland) in 0.5 M NaCl, 10 mM Tris-HCl for 30 min at 37°C. After washing in 2× SSC for another 30 min, slides were washed in 0.1× SSC for 10 min at 12°C, then in 0.1× SSC for 30 min at RT and finally dehydrated. Slides were exposed for 2–3 weeks to Ilford K2 nuclear emulsion (Ilford, Fribourg, Switzerland), developed in D19 (Kodak), fixed in Unifix (Kodak) and mounted.

ISH for CT mRNA was performed with ^{35}S-labelled oligonucleotide probes and was done as previously described [15].

Control experiments included pretreatment with RNAse A before hybridization, as a negative control. The RNAse treatment abolished the hybridization signal. Parallel sections were also stained with an unrelated probe, which gave negative reaction.

Combined staining was performed to investigate TG immunoreactivity and CT mRNA expression, and also CT immunoreactivity and TG mRNA expression, in the same slide. In these test cases IHC staining preceded the ISH procedure.

Results

The patients (7 men and 4 women) had a median age of 58 years. In the 9 cases with known location, the tumour was in the left lobe (5 cases), isthmus (1 case) and right lobe (1 case). In the 2 remaining cases [multiple endocrine neoplasm (MEN) type 2A-affected patients], bilateral tumours were present (all had tubular areas within the medullary carcinoma component). The tumour size was 1–5.5 cm (mean 2.9). Only 1 patient (case 5) underwent radioiodine treatment at the time of first relapse (2 years after diagnosis). Radioiodine was administered in two sessions, but the uptake in both local recurrence and metastatic sites was very poor; the patient eventually died of widespread disease 6 years later, with high blood levels of CT. Six patients had either lymph node metastases at diagnosis (3 cases) or had developed lung, mediastinal and lymph node spread at the time of follow-up examination (3 cases). Follow-up (mean 3.2 years) of 10 patients showed that 5 of them were free of disease, while the other 5 were alive with disease or had died of their disease (Table 2). One patient was lost to follow-up.

MMFCs had a heterogeneous pattern of growth. The relative proportions of the two components varied widely, from 10% of the medullary carcinoma area in case 6 to approximately 80% in other cases having a minor tubular/follicular component. For descriptive purposes only, the two tumour cell populations will be reported separately.

Table 2 Mixed follicular/medullary carcinomas of the thyroid (*L* left, *R* right, *n.a.* not available, *MCT* medullary carcinoma, *NED* no evidence of disease, *AWD* alive with disease, *DOD* died of disease, *m.foc* multifocal, *pap ca* papillary carcinoma, *foll.ca* follicular caracinoma, *TG* thyroglobulin, *CT* calcitonin, *-ve* negative)

No.	Sex/age	Location/size (cm)	Pathology	Metastases/Recurrences	Follow up (years)	Comments
1	M/68	L/4	Mixed MCT+solid foll. ca	Lymph nodes	NED 1	Medullary areas TG mRNA -ve
2	F/53	L/5	Separate pap ca & MCT	Mixed lymph node mets	DOD 8	Medullary areas (of lymph node) TG mRNA -ve
3	M/55	n.a.	MCT with tubules	None	NED 5	Separate cell populations express CT or TG
4	M/74	R/2	MCT with tubules	None	DOD 1	Separate cell populations express CT or TG
5	M/68	Isthmic/3	MCT+follicles (pap ca?)	Lymphnodes Local recurrence/bone metastases[a]	DOD 8	Separate cell populations express CT or TG
6	M/68	n.a.	Oxyphilic ca+micro-MCT	Lung	DOD 1	Medullary areas TG mRNA -ve
7	F/26	L/2.5	MCT with tubules and papillae	None	NED 0.4	Separate cell populations express CT or TG
8	F/49	R/1+L/0.2 m.foc/MEN2a	MCT with tubules	None	NED 3.2	Separate cell populations express CT or TG
9	F/42	L/5.5	MCT with tubules	Mediastinum	lost	Separate cell populations express CT or TG Rare cells with double CT & TG staining
10	M/58	L/n.a.	MCT with tubules	None	NED 1	Separate cell populations express CT or TG
11	M/69	R/1.7+L/1.1 m.foc/MEN2a	MCT with tubules	Lymph nodes	AWD 3	Separate cell populations express CT or TG Rare cells with double CT & TG staining

[a] Patient had local recurrence and bone metastases 2 and 3 years respectively after diagnosis

In the tumour, the medullary carcinoma component was either separate from (3 cases) or intermingled with (8 cases) the follicular component. Structurally, the tumours were composed predominantly of trabecular (4 cases), spindle (4), alveolar (2) and pseudo-papillary (1) types of medullary carcinoma. Amyloid was present in 5 of the 11 cases, necrosis in only 1 case (no. 9) and focal atypia in 5 cases. Cells were medium sized to large, only 1 case (no. 11) being made up of relatively small cells. C-cell hyperplasia (defined as presence of more than 20 C cells per low-power field arranged in the form of either diffuse or nodular hyperplasia) was found in the peritumour thyroid tissue in 2 cases (nos. 8, 11), both in known MEN2A-affected families.

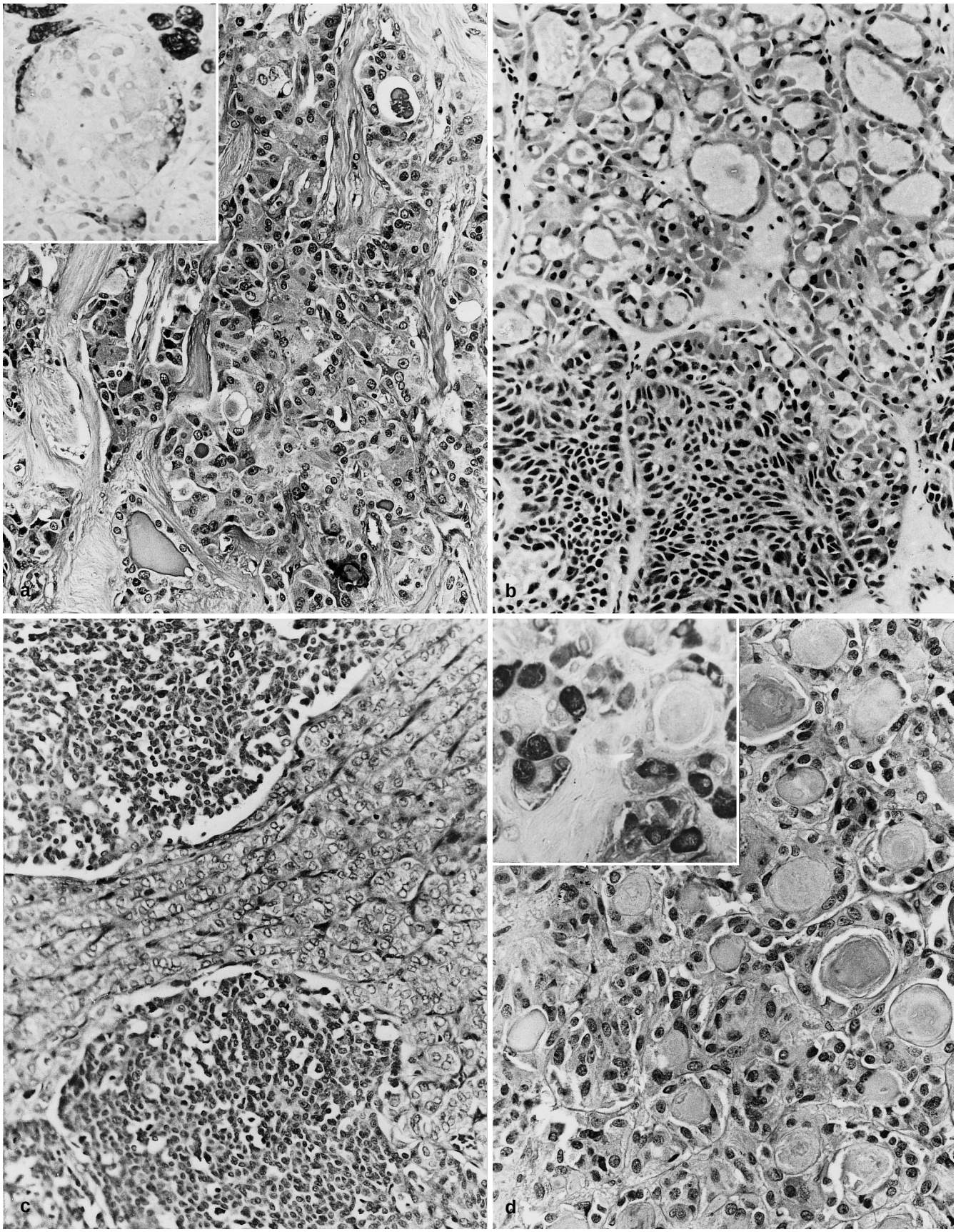
The follicular component displayed several different structural patterns, which reproduced all the known features of follicle-derived tumours except for anaplastic carcinoma. In 8 cases, single well-differentiated small follicles (CT negative, but TG immunoreactive) containing colloid were present throughout the tumour, admixed with the "medullary" component (Fig. 1a, d). This pattern was identical to that recognized in cases of so-called medullary carcinoma with TG immunoreactivity. In one case (no. 2) a papillary carcinoma (classical type) was present. Thin fibrovascular cores were lined by cubic cells having a clear nucleus with pseudoinclusions and grooves. This pattern was also present in a lymph node metastasis (see below). In another case (no. 6), the follicular component was entirely made up of oxyphilic cells arranged in small follicles containing scant colloid. Similar cells also invaded peritumour vascular spaces. In this particular case the "medullary" component was restricted to microscopic foci of spindled CT-positive cells (Fig. 1b). Finally, case 1 had small islands (1–2 mm in size) of medullary and follicular differentiated cells, variously intermingled. The follicular component lacked follicles and was mainly represented by solid nests or cords of uniform cells (Fig. 1c), as observed in poorly differentiated (solid) carcinomas.

Lymph node metastases had features similar to the primary tumour in cases 1 and 11. In case 2 (Fig. 2a, b), a mixed papillary-medullary carcinoma was found in the metastasis, although the primary tumours were topographically clearly separate. In case 5, metastases of a cystic papillary carcinoma, associated with MCT in the cyst wall (Fig. 2c), developed from an MCT with prominent follicular features in the primary tumour.

CT immunostaining was positive in the medullary carcinoma areas (11/11 cases), whereas TG immunostaining was observed both in follicular (11/11 cases) and in solid areas (7/11 cases).

ISH for CT mRNA gave a strong signal in medullary carcinoma zones, but not in areas with follicular differentiation. TG mRNA was expressed in follicular area in all cases and in individual cells of solid areas in 8 cases. The signal intensity surrounding the tumours was lower than that of normal thyroid parenchyma.

By combined labelling, CT mRNA was examined in parallel with TG immunoreactivity. TG mRNA expres-


a
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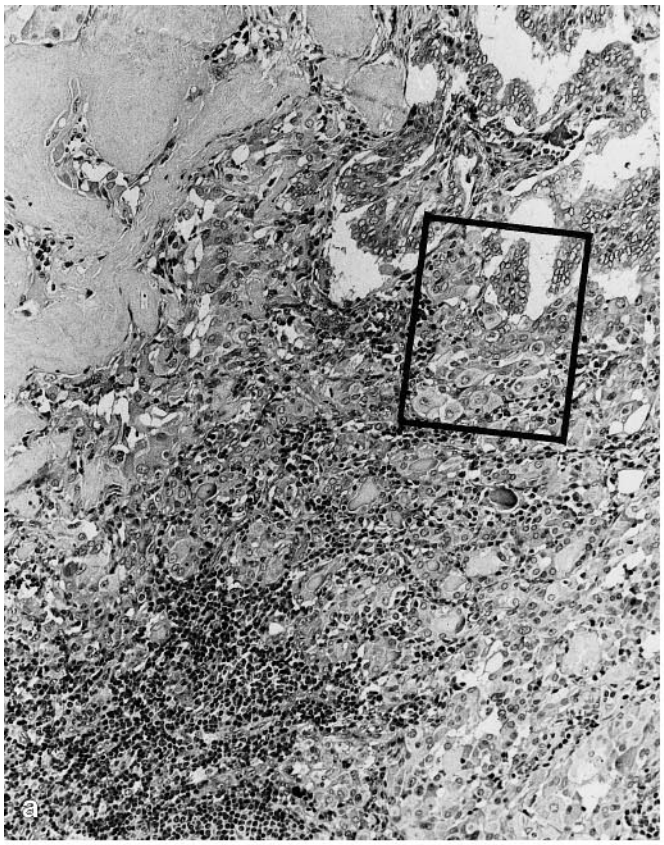


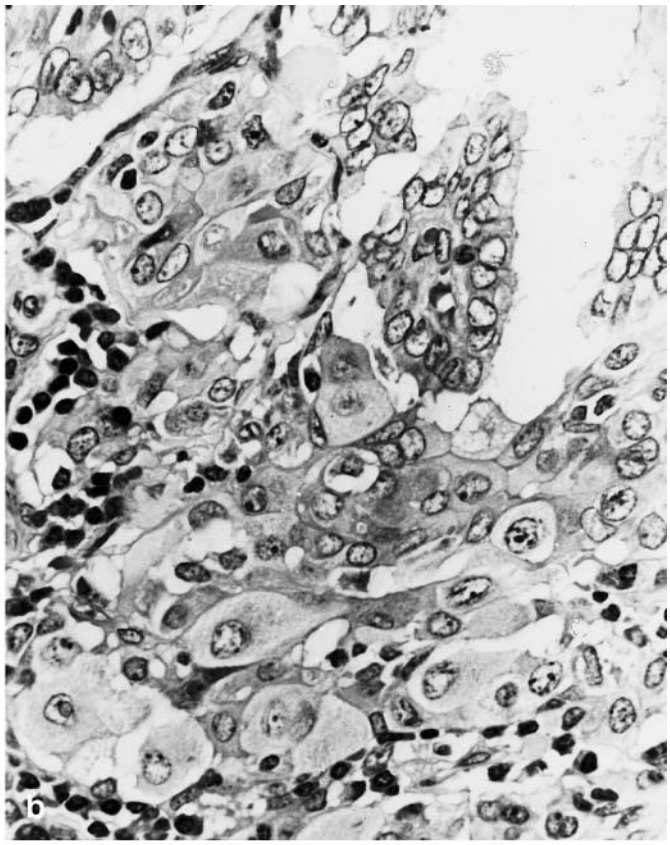
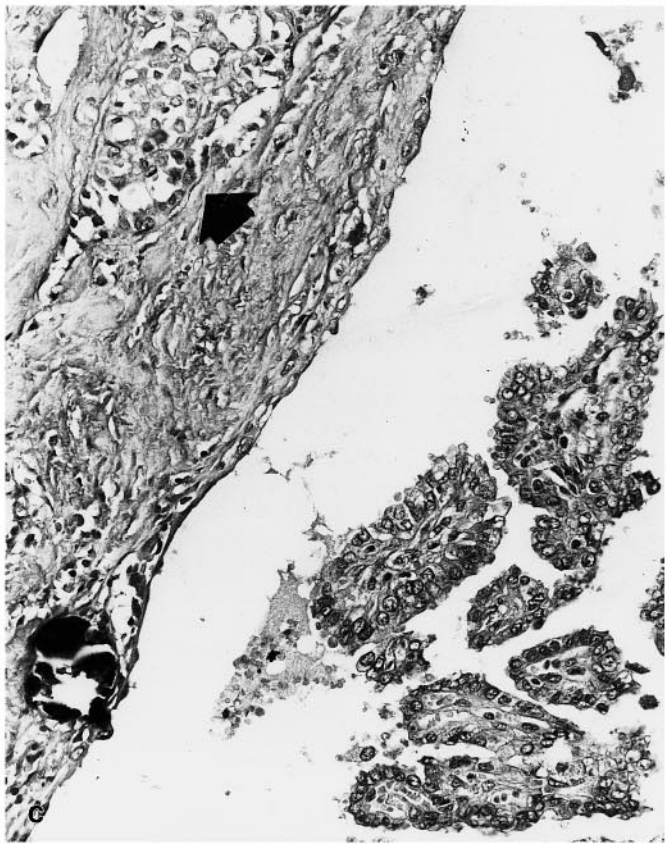


Fig. 2a Case 2. Lymph node metastasis of mixed medullary-papillary carcinoma. Topographically separate primary tumours in the same lobe developed a mixed lymph node metastasis having papillae merged with cords and nests of medullary carcinoma H&E, ×140. **b** A higher magnification of the framed area in **a** better outlines the classical features of papillary carcinoma. H&E, ×400. **c** Case 5. Lymph node cystic metastasis containing papillary fronds in the lumen and clusters of medullary carcinoma cells in the wall (*arrow*) H&E, ×160



◀ **Fig. 1a** Case 5. Classical nests of medullary carcinoma cells are admixed with tubules and follicles. The latter are positive for thyroglobulin (*inset*), but not for calcitonin. H&E, ×260; *inset*: immunoperoxidase, ×200. **b** Case 6. The tumour has two components of oxyphilic (Hurthle) cells and spindled medullary carcinoma cells. H&E, ×200. **c** Case 1. The tumour has admixed nests of solid poorly differentiated follicular carcinoma and splindle cell medullary carcinoma. H&E, ×200. **d** Case 9. In this field a prominent follicular cell population is intermingled with individual or clustered medullary carcinoma cells. The latter react for calcitonin (*inset*), the follicles being negative. H&E, ×320; *inset*: immunoperoxidase, ×400

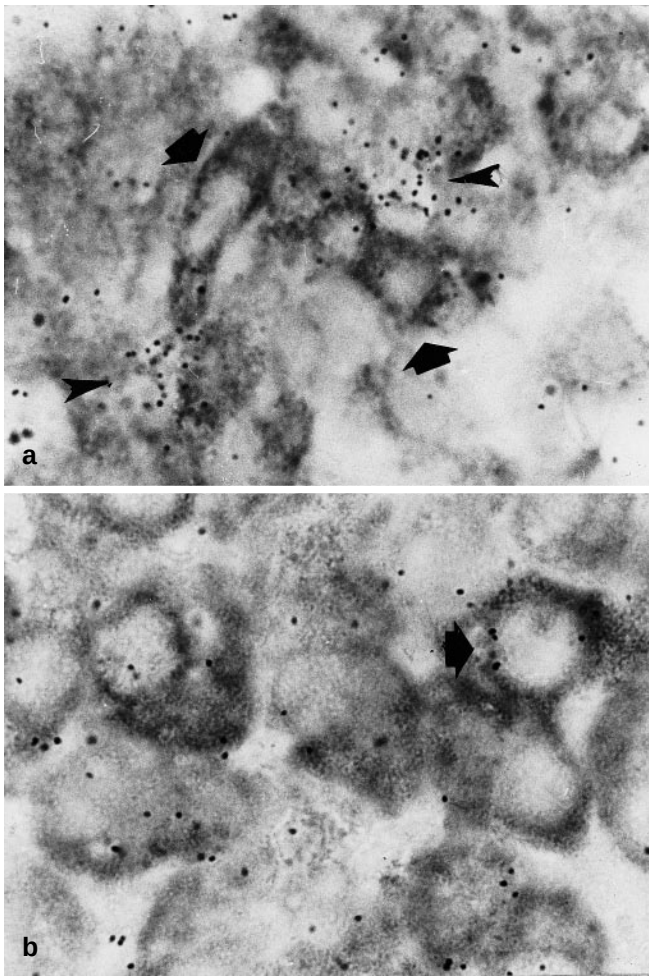


Fig. 3a Case 7. Two separate cell populations express immunoreactive calcitonin (*arrows*) or thyroglobulin mRNA (*arrowheads*). **b** Case 9. The same combined staining shows rare cells co-expressing both calcitonin and thyroglobulin (*arrows*). Combined staining with immunoperoxidase for CT and in situ hybridization for TG, **a** ×850, **b** ×1000

sion was also investigated in association with CT immunoreactivity. This staining allowed us to exclude the possibility of passive absorption of TG by medullary carcinoma cells. In most cases CT-immunoreactive cells were different from those containing TG mRNA (Fig. 3a). These same findings were observed in lymph node metastases of the 2 cases in which material was available for parallel studies. In 2 cases (nos. 9 and 11) rare cells seemed to be positive for both CT and TG (Fig. 3b), but this was uncommon.

Discussion

The clinico-pathological features and the phenotypic profile of a series of MMFC of the thyroid gland examined in this study led to various observations.

A major matter of concern was the identification of selection criteria for the study. MMFC is defined by the WHO classification [17] as a "tumour showing both the morphologic features of medullary carcinoma together with immunoreactive calcitonin and the morphologic features of follicular carcinomas with immunoreactive thyroglobulin." This definition does not differentiate between the tumours of follicular cell lineage (follicular vs papillary vs poorly differentiated or anaplastic). In recent years, several tumours exhibiting concurrent expression of medullary and follicular morpho-functional features have been described [1, 2, 6, 13, 21, 25, 26, 34, 41, 42]. In some cases, CT and TG immunoreactivity were not necessarily restricted to areas with clear-cut morphological features of medullary and follicular carcinoma, respectively [12, 45]. In the present series of MMFC, a wide range of morphological patterns was recognized. The medullary component was always well represented, although amyloid deposits were present in less than half the cases. Classical alveolar, spindle, and trabecular patterns of the neoplasm were present, and even pseudopapillary features were recognized. In the follicular component, all known patterns of tumours of follicular cell origin except anaplastic carcinoma were found. Since MMFC probably has a different histogenesis, further subtyping of the follicular component has been regarded as unnecessary by some authors [12, 46]. Nevertheless, we believe that the knowledge of all the possible tumour type combinations is useful from a practical standpoint for correct identification of MMFC cases. The relative proportion of follicular and parafollicular components was extremely variable in our cases. At one end of the spectrum, an oxyphilic (Hurthle cell) carcinoma admixed with scattered microscopic foci of medullary carcinoma of spindle-cell type (accounting for approximately 10% of the tumour area) was found. At the other end, medullary carcinoma cells were admixed with neoplastic tubules (CT negative/TG positive); the latter accounted for 10–20% of the tumour area. This latter pattern recalled that described by Ljungberg and coworkers [29, 31] in their cases of "intermediate thyroid carcinomas". The occurrence of TG-positive cells in otherwise ordinary medullary carcinomas was reported by some authors [10, 12, 19, 24], who preferred to label these neoplasms as "medullary carcinoma with TG immunoreactivity". The distinction of these latter tumours from MMFC is currently poorly characterized. MMFC probably includes those cases described as medullary carcinoma with TG immunoreactivity, but also encompasses other morphological variants, as described above.

To clarify whether the tumours we studied were undergoing dual differentiation (with variable degrees of phenotypic expression), immunochemistry was not sufficient, since TG can easily be passively absorbed by neighbouring cells [28]. ISH techniques allow TG mRNA to be selectively demonstrated in TG-producing cells [4]. In MMFCs, we observed that a proportion of neoplastic cells expressed TG mRNA irrespective of their morphology (whether arranged in solid clusters or in small tubules). Solid areas may express TG, as also revealed by Sobrinho-Simoes and Fonseca [48] by means

of IHC. In the majority of the present cases, TG mRNA positive cells were not co-expressing CT in double IHC/ISH-stained slides. In no case did areas of follicular differentiation express CT mRNA, a differential criterion from the tubular/follicular variant of medullary carcinoma [16].

Co-expression of CT and TG by the same cell was found by Ljiungberg and Nilsson [29] by double immunostaining of cases of intermediate carcinoma. Because of the possibility of passive absorption, TG mRNA detection provided a more specific demonstration of follicular differentiation [37]. However these authors did not investigate the expression of both CT and TG in the same neoplastic cell. In only 2 cases in the present series were both CT and TG present concurrently but overall, our findings indicated that the expression of TG and CT genes in separate cells is much more common than simultaneous gene expression in the same cell.

The existence of multidirectional divergent differentiation in both endocrine and nonendocrine tumours [9] is a well-known phenomenon. The thyroid, however, contains two different cell populations of presumably separate embryological origin, and it is difficult to explain the simultaneous occurrence of two neoplastic cell populations (of apparently different derivation) in the same tumour. MMFC may develop from multipotent stem cells of unknown derivation with ultimo-branchial body remnants as possible candidates. In such cells the potential of activating both CT and TG genes is retained [29, 52] and this may explain the dual cell population found in MMFC. Alternatively, it is possible that common oncogenic stimuli may favour neoplastic transformation of both follicular and parafollicular cells, or that currently unknown growth factors may reciprocally modulate cell growth. For instance, *MET* oncogene-encoded receptor and its ligand hepatocyte growth factor (HGF) have been demonstrated in carcinomas of follicular cell origin [11], but have also been traced in a few medullary carcinomas (M. Papotti et al., unpublished observation). *RET* proto-oncogene alterations are also implicated in both papillary and medullary carcinoma. *RET* is rearranged in a percentage of papillary carcinomas [5]. The role of point mutation, deletion or inversion of *RET* is well known in classical medullary carcinoma, where point mutations have been identified in both familial medullary carcinoma, in MEN types 2A and 2B, and also in a percentage of sporadic medullary carcinomas [18, 23]. A genetic analysis of MMFC searching for mutations in various

Table 3 Literature review of reported cases of mixed/concurrent medullary-follicular carcinomas of the thyroid (*LN* lymph node/s, *DOC* died of other cause, *TG IR* thyroglobulin immunoreactivity, *ca* carcinoma, *var* variant)

Reference	Sex/age	Location/size (cm)	Metastases/Recurrence	Follow-up (years)	Designation	Structure
[25]	F/66	R/1.5+0.3	n.a.	DOC 1.5	Concurrent	Separate MCT and follicular var of papillary microca
[14]	M/44	R/4	LN (bilateral)	AWD 2	Mixed	MCT (amyloid-rich)+ follicular var, papillary. ca
[30]	F/45	L/6	LN (after 7 months)	DOD 7	Compound	MCT+follicular ca
[43]	M/35	R/5	LN, mediastinum	n.a.	Mixed	MCT+follicular ca
[21]	F/31	R/2+1.5	?[a]	?[a]	Concurrent	Separate MCT+papillary carcinoma
[41]	M/51	R/2.5	LN	n.a.	Mixed	MCT+ follicular +papillary +anaplastic ca
[38]	F/42	R/1.7+0.2	None	n.a.	Mixed	MCT+follicles (+ papillary microcarcinoma)
[50]	F/51	L/3+0.8	LN	n.a.	Simultaneous	Separate MCT+follicular carcinoma
[1]	M/29	R/6	LN (bilateral)	NED 1	Mixed	MCT+follicular var of papillary ca
[1]	M/36	L/4	LN	NED 2	Mixed	MCT+follicular var of papillary ca
[51]	M/68	L/4	LN, mediastinum	NED 1	Mixed	Medullary+solid follicular foci
[37]	F/44	n.a.	LN	n.a.	Mixed	MCT+follicles (familial MCT)
[37]	M/48	n.a.	LN+liver	n.a.	Mixed	MCT+follicles (familial MCT)
[8]	M/56	R/n.a.	LN, mediastinum	DOD 10	Biphasic	MCT+follicles
[13]	F/27	R/3+0.4	None	NED 2	Concurrent	Separate MCT+ follicular ca (R) and papillary ca (L)
[22]	M/37	R/3	LN	n.a.	Mixed	Alveolar/trabecular structure (no follicles)
[22]	F/55	L/2	LN	n.a.	Mixed	Alveolar/trabecular/follicular structure
[33]	M/n.a.	n.a.	LN	n.a.	Mixed	MCT+follicles
[36]	M/27	R/3	LN	AWD 5	Mixed	MCT+follicles
[2]	M/48	R/5	LN	AWD 1.5	Composite	Alveolar/trabecular/follicular/papillary structure
[26]	F/49	L/5	Lung	AWD 3	Mixed/collision?	MCT+papillary ca (classic and follicular variant)
[26]	F/49	R/10	LN, lung, bone	DOD	Mixed/collision?	Separate MCT+papillary ca (mixed metastases)
[26]	M/28	R/2.6	LN	NED 1.5	Mixed/collision?	Papillary ca+foci of MCT
[34]	F/67	L/7	LN	n.a.	Compound	Trabecular MCT+papillary ca
[12]	F/26	L/6	None	NED 2	MCT with TG IR/mixed	MCT+follicles in a cellular stroma
[42]	M/41	L/3	LN	AWD 6	Concurrent	Separate MCT and papillary ca (mixed metastases)

[a] Article in Japanese and no translation is available

parts of the tumour might provide further insight in the pathogenesis of MMFC.

An additional problem is the interpretation of mixed features in metastatic deposits (lymph nodes), as opposed to the primary tumour. In 2 of our cases the lymph nodes contained a mixture of medullary and papillary carcinoma cell populations in the same node. The primary tumour had medullary and follicular features in 1 case (medullary carcinoma associated with CT-negative/TG-positive tubules), whereas in the other a papillary carcinoma was growing adjacent to a medullary carcinoma. Although the latter case may be an example of a collision tumour, it had an intermingling cell populations in the metastases. Common oncogenic stimuli and growth factors may well act on both primary tumours and secondary deposits. If a role of identical oncogenes is proven in the development of mixed tumours (follicular or papillary and medullary), it would become even harder to draw a sharp distinction between true MMFC and collision tumours. The latter develop as separate entities, while the former grow as mixed cell populations, but this difference may simply be a topographical one.

It is difficult to draw a definite conclusion on the biological behaviour of MMFC on the basis of experience. In a literature review of MMFC cases (Table 3), follow-up information was available for 15 patients: 8 of them (53.3%) had aggressive disease and had died or were alive with tumour progression. In the present series, 5 of 10 (50%) patients had died of their tumour or were alive with disease. In 4 of the 5 cases, elevated serum CT levels were documented at the time of relapse, indicating that the tumour was probably behaving as a medullary carcinoma. Cases of MMFC in the setting of MEN syndromes have been reported [36], and 2 additional cases are included in the present series.

Regarding the possibility of adjuvant treatment, since MMFC contain TG, they might theoretically respond to radioiodine treatment. One single case in our series was treated with radioiodine at the time of recurrence (but not at diagnosis) and had a very poor iodine uptake. Because of the limited number of cases reported to date, it is difficult to assess the responsiveness of MMFC to iodo-metabolic therapy. In practical terms, treatment of MMFC as medullary carcinoma together with additional iodine-131 therapy appears justified.

In summary, we have found that MMFC is a rare but heterogeneous group of tumours with dual differentiation along follicular and parafollicular C cell lineages, including all patterns recognized in classical parafollicular and follicular cell-derived tumours. MMFC does not seem to be a variant of MCT (although some MMFC develop in the setting of familial syndromes, such as MEN2A), as two separate cell populations are intermingled in this tumour. Concurrent expression of CT and TG mRNA by the same tumour cell has rarely been demonstrated. The origin of the tumour is unknown, but deregulation of neoplastic parafollicular cells or simultaneous neoplastic transformation of both follicular and C cells in response to common oncogenic stimuli may occur. Alternatively, dual differentiation in a single tumour derived from a totipotent cell possibly involving ultimobranchial rests in histogenesis must be considered.

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