Virchows Arch A (1987) 410:455–460

Virchows Archiv A

Ultrastructural study of poorly differentiated medullary carcinoma of the thyroid

Kennichi Kakudo [1], Akira Miyauchi [2], Shoichi Katayama [3], and Keiichi Watanabe [1]

[1] Department of Pathology, Tokai University School of Medicine, Isehara,
[2] Department of Surgery, Kagawa Medical School, Kagawa, and
[3] Department of Pathology, Osaka University School of Medicine, Osaka, Japan

Summary. Five cases of sporadic medullary carcinoma of the thyroid (MTC) with rapidly progressive disease were studied ultrastructurally. The tumour cells had poorly differentiated C cell characteristics. They exhibited smaller secretory granules in their narrow cytoplasm. Morphometric analysis disclosed that the average diameter of the secretory granules of the cases with a poor prognosis was 173.0 nm in comparison with 254.2 nm of well differentiated cases. The granules were fewer in the poor prognostic group ($1.31/\mu^2$) than the well differentiated group ($2.75/\mu^2$). Increased free ribosomes and polysomes were noted in the cytoplasm and dispersed chromatin in the nuclei. These cases should be therefore classified as poorly differentiated MTC rather than atypical or anaplastic MTC.

Key words: Thyroid neoplasms – Ultrastructure prognosis – C cell – Calcitonin

Introduction

Medullary carcinoma of the thyroid is regarded as a low grade malignant tumour originating from the C cells of the thyroid (Hazard et al. 1959; Williams 1966). Recently some cases of atypical MTC or of an anaplastic variant with poor prognosis have been reported (Nishiyama et al. 1972; Kakudo et al. 1978; Zeman et al. 1978; Bussolati and Monga 1979; Medelsohn et al. 1980; Kruseman et al. 1982; Martinelli et al. 1983). Five cases of MTC with rapidly progressive disease (less than 3 years of survival period) are reported here to clarify the subcellular characteristics of this type of MTC.

Offprint requests to: K. Kakudo, Department of Pathology, Tokai University School of Medicine, Isehara, Bouseidai, Kanagawa, 259-11 Japan

Materials and methods

Five cases with virulent disease were selected from 64 cases of histologically proven cases of MTC. Table 1 shows the clinical summary of the 5 cases. None had a family history of thyroid disease. The patients presented at hospitals with thyroid tumours and neck nodal metastasis. Four cases (except for case No 4) were initially treated in the hope of cure, but later developed a tumour recurrence or metastasis clinically. Only incisional biopsy of the thyroid tumour was carried out in case No 4 and no further surgical treatment could be done. The average age at presentation was 53.2(41–65) years old. All the patients died with tumour recurrence and metastasis within 3 years of initial symptoms. Postmortem examination confirmed this in 2 cases (case No 1 and 2).

Plasma calcitonin levels were high in all patients. The doubling times of plasma calcitonin were calculated in the 4 cases (Table 1), and were found to be shorter than those of well differentiated MTCs (Miyauchi et al. 1984). Tumour tissue specimens were taken from surgery or biopsy and were processed for elec-

Table 1. Summary of the clinical data of 5 poorly differentiated medullary carcinomas of the thyroid. Doubling time: doubling time of plasma calcitonin

Case	Age	Sex	Treatments	Doubling time	Survival period	Metastasis
1.	46	f	Op + Rad	0.3 year	3 years	Liver + Lung + Bone
2.	52	m	Op + Chemo	0.25 year	2 years 2 months	Liver + Lung
3.	41	m	Op + Rad + Chemo	0.14 year	3 years	Bone + Liver
4.	65	f	Rad + Chemo	–	8 months	Liver + Bone
5.	62	f	Op + Rad	0.12 year	3 years	Bone + Brain + Skin

tron microscopic examination by standard methods and embedded in epoxy resin. Electron micrographs were taken at the magnification of 10,000 and were printed at a final magnification of 20,000.

Ten representative photographs were selected in 3 cases (cases 3,4 and 5 respectively) for morphometric analysis. The diameter of the secretory granules (SG) and number of SG in square micron of cytoplasm were calculated using a Nikon Magiscan. Two of the 6 well differentiated MTCs were examined in the same manner for comparison. The tumour tissues and non-tumourous thyroid tissues were also processed for light microscopic examination after fixation in 10% formalin solution and embedded in paraffin. The sections were stained with haematoxylin and eosin, Congo red for amyloid and Grimelius for argylophil granules. Immunohistochemical stain for calcitonin was applied on paraffin sections using an indirect method.

Results

Light microscopy

Histologically, the 5 cases with poor prognosis showed solid growth with fibrous stroma, and proved to be medullary carcinoma of the thyroid. The tumour cells exhibited alveolar, trabecular, rosette and spindle cell histological patterns and there were areas of small cells (increased nuclear/cytoplasmic ratio, Fig. 1). Necrosis and mitoses in the tumour cells were seen and only a little amyloid deposit was identified in the stroma. The findings were uniform in all the five cases; all disclosed positive immunoreactivity for calcitonin in the cytoplasm. The staining patterns of calcitonin were variable in the tumours, some areas showed only a few positive cells, while others demonstrated a majority of neoplastic cells positively stained for calcitonin (Fig. 2). There was uniform argyrophilia with Grimelius's silver impregnation. Histological examination of the non-tumourous thyroid tissue disclosed no C cell hyperplasia (Block et al. 1980; Emmertsen et al. 1983).

Electron microscopy

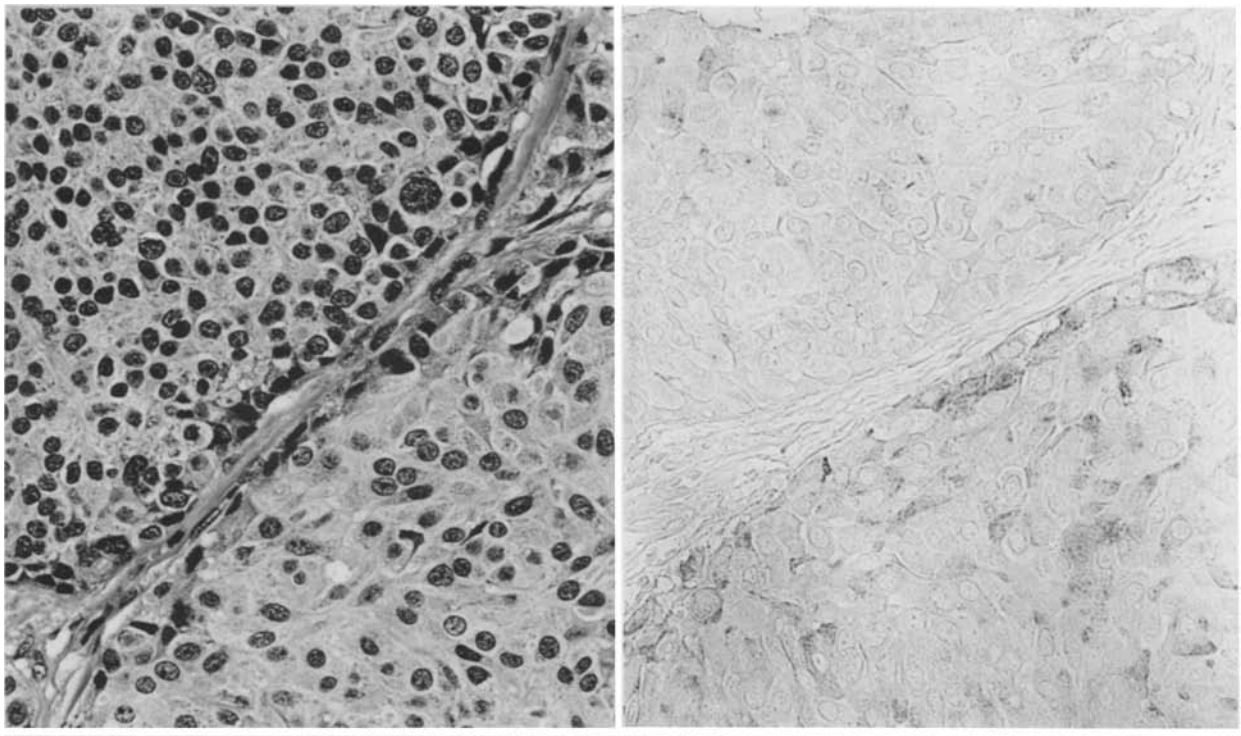
Electron microscopically, the tumour cells had a nucleus with wavy contour, dispersed chromatin and small nucleoli. The cell membrane was rather simple and equipped with a few cellular attachments. These tumour cells had predominantly small secretory granules and poorly developed cytoplasmic organelles in their relatively narrow cytoplasm (Figs. 3 and 4). There were increased free ribosomes and polysomes in the cytoplasm (Fig. 4). These findings, together with dispersed chromatin pattern of the nuclei, are often observed in immature cells. Well differentiated MTCs, in contrast, disclosed numerous large secretory granules, well developed rough endoplasmic reticulum and Golgi apparatus in their wide cytoplasm (Fig. 5). Tables 2 and 3 show the morphometric analysis of the 2 groups. The average diameters ± 1 standard deviation of the SG of 3 poorly differentiated MTCs were 162.3 ± 18.8 nm (case No 3), 223.7 ± 52.8 nm (case No 4) and 132.3 ± 18.8 nm (case No 5) and the average diameter of SG of the 3 MTCs was 172.8 nm. Those of 2 well differentiated MTCs were 224.3 ± 18.4 nm (case No 5) and 309.0 ± 46.3 nm (case No 6) respectively and the average of the 2 cases was 266.7 nm. The difference of the average diameters between the 2 groups of MTCs was statistically significant ($P < 0.001$). Although there was no significant difference between case No 4 of the poorly differentiated MTC and the case No 5 of the well differentiated MTC, the comparison of other cases each between the 2 groups was statistically significant ($P < 0.001$). The number of granules (average) per square micron was 1.31 for the 3 poorly differentiated MTCs and less than 2.75 for the 2 cases of well differentiated MTCs. There were a few small granule cells in well differentiated MTCs shown in the upper field of the Figure 5. However they were different from the tumour cells in poorly differentiated MTCs because they were only a minor component of the tumour in well differentiated MTCs

Table 2. Diameter and number of granules in poorly differentiated medullary thyroid carcinoma

Case	Diameter (nm)	Number of granules ($/\mu^2$)
1. SK	186.7	–
2. MS	160.2	–
3. NS	162.3	1.489
4. OK	223.7	0.769
5. CA	132.3	1.679
Ave	173.0	1.312

Table 3. Diameter and number of granules in well differentiated medullary thyroid carcinoma

Case	Diameter (nm)	Number of granules ($/\mu^2$)
1. SI	276.9	–
2. KI	216.1	–
3. HT	242.6	–
4. KT	256.2	–
5. MM	224.3	2.492
6. YK	309.0	3.001
Ave	254.2	2.747



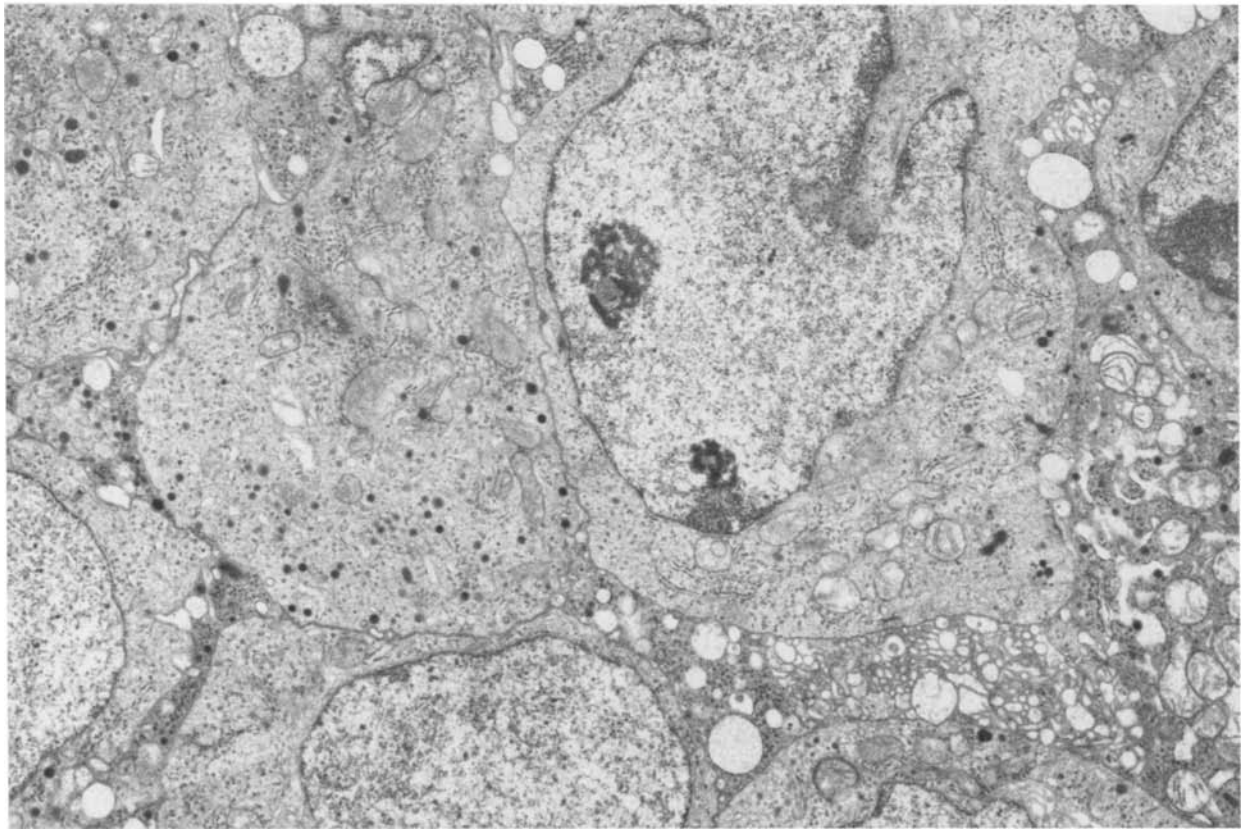


Fig. 1. Lymph node metastasis from case 2 showing solid growth of small cell type of tumour cells in left upper field. Note right lower area contains larger tumour cells with wide granular cytoplasm common in typical MTC. Haematoxylin and eosin, ×400

Fig. 2. Calcitonin immunoreactivity is shown in the tumour cytoplasm (case 2). Note only a few positive cells in the upper left field which corresponds to small cell type tumour cells, in comparison with more positive cells in the lower right. Immunoperoxidase for calcitonin, ×400

Fig. 3. Electron micrograph of case 2. The tumour cells are arranged compactly with simple cellular membrane. The cytoplasmic organelles are poorly developed and few small secretory granules are observed. Uranyl acetate and lead, ×5,800

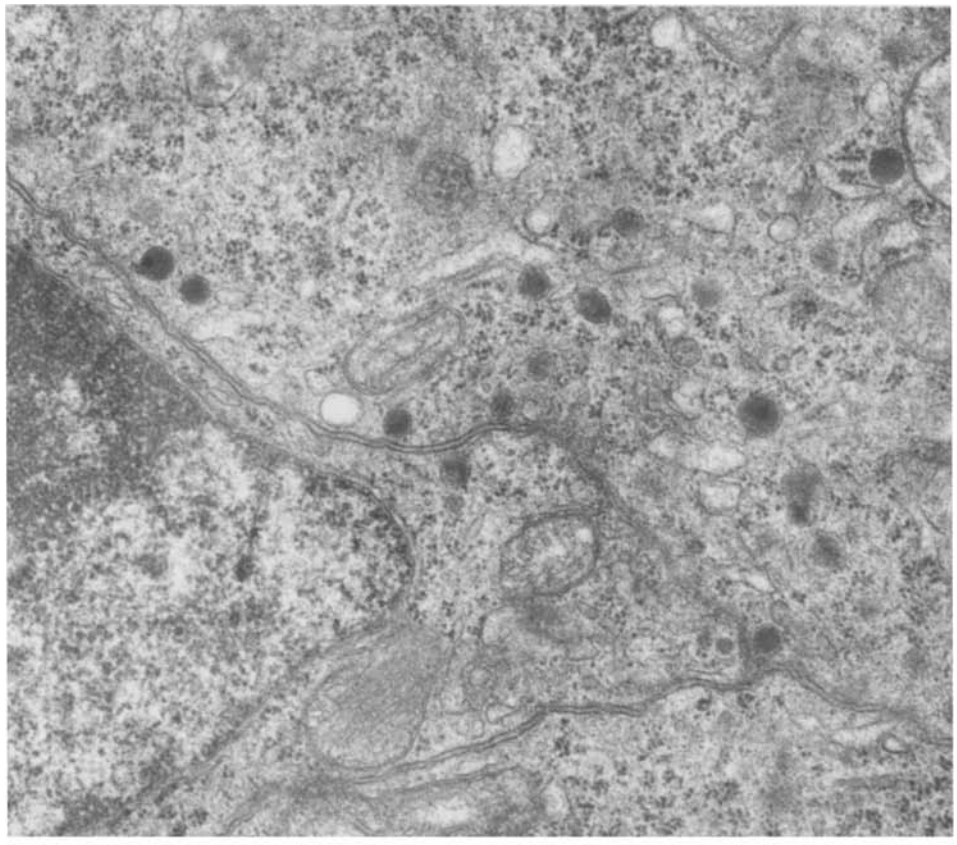


Fig. 4. Electron micrograph of case 5. Few small secretory granules and increased free ribosomes and polysomes are seen in the cytoplasm. Uranyl acetate and lead, ×25,000

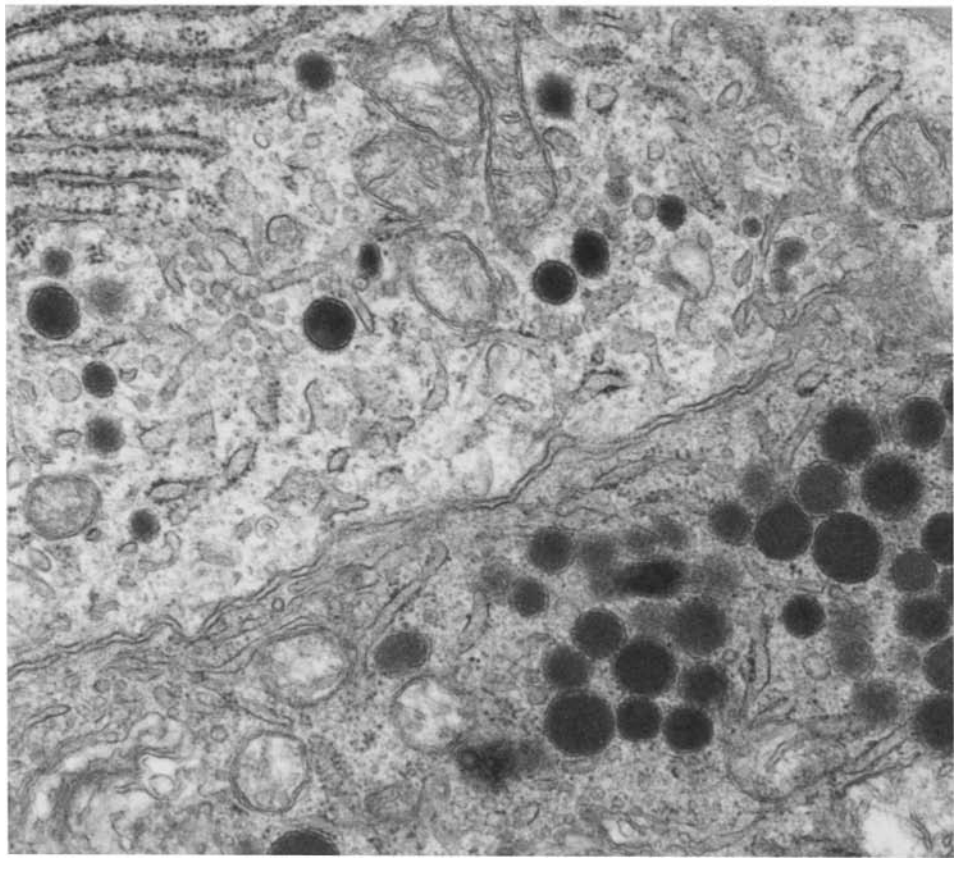


Fig. 5. Well developed r-ER, Golgi apparatus and many large secretory granules are observed in well differentiated MTC case 6. Uranyl acetate and lead, ×25,000

and had well developed Golgi apparatus and rough endoplasmic reticulum. These 3 electron microscopic features; namely the size of SG, the number of SG and the development of cytoplasmic organelles, make it possible to differentiate immature C cells of poorly differentiated MTCs from well developed C cells of well differentiated MTCs.

Discussion

Recently several useful prognostic factors have been reported for MTC. They include: – histological characteristics reported as the atypical or anaplastic variant, seen in MTC with poor prognosis (Nishiyama et al. 1972; Kakudo et al. 1978; Zeman et al. 1978; Bussolati and Monga 1979; Mendelsohn et al. 1980; Kruseman et al. 1982; Martinelli et al. 1983), calcitonin immunostaining patterns that were less intensively stained in virulent cases in comparison with the diffuse intensive reaction in well differentiated cases (Kakudo et al. 1978; Lippman et al. 1982; Saad et al. 1984b; Mendelsohn et al. 1984), doubling times of plasma calcitonin which reflected tumour growth rate and plasma CEA (Miyauchi et al. 1984; Saad et al. 1984a; Busnard et al. 1984), and staging of the tumour and clinical settings of MTC and other endocrine abnormalities defined by autosomal dominant inheritance (Rossi et al. 1980; Saad et al. 1984c; Kakudo et al. 1985). Although all of these characteristics are useful to predict the patient's prognosis, the histological characteristics are the most important and reliable factors obtainable from primary surgical treatment of the patient.

We reported 5 cases of MTC with atypical histological patterns, so-called small cell variation, of which the cytological immaturity has been confirmed ultrastructurally. They had features of immature and poorly differentiated cells such as abundant ribosomes and polysomes in the cytoplasm as well as a dispersed chromatin pattern in the nucleus. The cytoplasmic organelles which indicate well differentiated endocrine cells and are closely related with protein synthesis, like Golgi apparatus and r-ER, were poorly developed. The tumour cells which are rich in secretory granules and cytoplasmic organelles related to protein synthesis are more differentiated and, therefore, grow slowly and have a better prognosis. Small granule tumour cells have poorly developed cytoplasmic organellae, are less differentiated C cells and subsequently more aggressive.

In general, it is recommended that carcinoma with endocrine differentiation should be subdivided into small cell type and intermediate cell type for pathological and prognostic reasons (DelJunco and Silvia 1984). Both the small cell type and intermediate cell type correspond to poorly differentiated and well differentiated MTCs in this study. This small cell pattern has been reported in MTC as a histological variant (Szijji et al. 1969; Kakudo et al. 1982). As noted in other endocrine tumours, poorly differentiated MTC with a small cell pattern is more aggressive than the intermediate MTCs.

MTCs have been regarded as of low grade malignancy. However, in clinical behavior, some are quite aggressive, developing distant metastasis and resulting in death within 3 years. These cases are histologically distinguishable from typical well differentiated MTCs because of their cytological characteristics. They exhibit histologically a small cell pattern with an increased N/C ratio, increased mitoses and presence of tumour necrosis. The tumour cells have few small secretory granules in their cytoplasm which may reflect the weak immunostaining of calcitonin (Saad et al. 1984b) or presence of other hormones (Bordi et al. 1972; Capella et al. 1975). In addition, the size of the secretory granules in MTCs have been described as heterogenous in several reports (Bordi et al. 1972; Capella et al. 1975; Kakudo et al. 1977; Kameya et al. 1977; DeLellis et al. 1978). There are at least 2 different size groups of secretory granules in MTCs. However, DeLellis et al. (1978) disclosed both large and small granules contained calcitonin by immunoelectron microscopy. The small granules observed predominantly in poorly differentiated MTCs may be immature secretory granules which also contain calcitonin.

Acknowledgements. The authors wish to express their thanks to Mr. J Itoh and H Suemizu for their excellent photographic works and to Ms. Y Inagaki for secretary work.

References

Block MA, Jackson CE, Greenwald KA, Yott JB and Tashjian AH Jr (1980) Clinical characteristics distinguishing hereditary from sporadic medullary thyroid carcinoma. Arch Surg 115:142–148

Bordi C, Amersa P, Vitali-Mazza L (1972) Ultrastructural study of calcitonin secreting tumor. Typology of the tumor cells and origin of amyloid. Virch Arch [Pathol Anat] 357:145–161

Busnard B, Girelli ME, Simioni N, Nacamulli D, Busetto E (1984) Nonparallel patterns of calcitonin and carcinoembryonic antigen levels in the follow up of medullary thyroid carcinoma. Cancer 53:278–285

Bussolati G and Monga G (1979) Medullary carcinoma of the thyroid with atypical patterns. Cancer 44:1769–1777

Capella C, Bordi C, Monga G, Buffa R, Fontana P, Bonfanti S, Busolati G, Solcia E (1975) Multiple endocrine cell types in thyroid medullary carcinoma. Evidence for calcitonin,

somatostatin, ACTH, 5-HT and small granule cells. Virch Arch [Pathol Anat] 377:111–128

DelJunco GW Jr and Silvia EG (1984) The terminology of microscopic tumor pattern: Clarification in pursuit of standardization. Pathology Annual, Sommers SC and Rosen PP (ed) Appleton-Century-Crofts, Norwalk, Connecticut, vol 19, Part 1, pp 93–123

DeLellis RA, May L, Tashjian AH Jr, Wolfe HJ (1978) C cell granule heterogeneity in man, an ultrastructural immunocytochemical study. Lab Invest 38:263–269

Emmertsen K, Erno H, Henriques U and Schroder HD (1983) C-cells for differentiation between familial and sporadic medullary thyroid carcinoma. Danish Med Bull 30:353–356

Hazard JB, Hawk WA, Crile G Jr (1959) Medullary (solid) carcinoma of the thyroid. – A clinicopathologic entity. – J Clin Endocrinol Metab 19:152–161

Kakudo K, Miyauchi A, Katayama S (1977) Ultrastructural study of thyroid medullary carcinoma. Acta Pathol Jpn 27:605–622

Kakudo K, Miyauchi A, Ogihara T, Takai S, Kitamura H, Kosaki G, Kumahara Y (1978) Medullary carcinoma of the thyroid. Giant cell type. Arch Pathol Lab Med 102:445–447

Kakudo K, Miyauchi A, Ogihara T, Takai S, Kitamura H, Kumahara Y, Kawaoi A (1982) Medullary carcinoma of the thyroid with ectopic ACTH syndrome. Acta Pathol Jpn 32:793–800

Kakudo K, Carney JA, Sizemore GW (1985) Medullary carcinoma of thyroid. Biologic behavior of the sporadic and familial neoplasms. Cancer 55:2818–2821

Kameya T, Shimosato Y, Adachi I, Abe K, Kasai N, Kimura K, Baba K (1977) Immunohistochemical and ultrastructural analysis of medullary carcinoma of the thyroid. Am J Pathol 89:555–574

Kruseman ACN, Bosman FT, Henegouw JCVB, Cramer-Knijnenberg G, Riviere GB (1982) Medullary differentiation of anaplastic thyroid carcinoma. Am J Clin Pathol 77:541–547

Lippman SM, Mendelsohn G, Trump DL, Wells SA Jr, Baylin SB (1982) The prognostic and biological significance of cellular heterogeneity in medullary thyroid carcinoma: A study of calcitonin, L-DOPA decarboxylase and histaminase. J Clin Endocrinol Metab 54:233–240

Martinelli G, Bazzocchi F, Govoni E, Santini D (1983) Anaplastic type of medullary thyroid carcinoma. Virch Arch [Pathol Anat] 400:61–67

Mendelsohn G, Baylin SB, Bigner SH, Wells SA Jr, Eggleston JC (1980) Anaplastic variants of medullary thyroid carcinoma. A light-microscopic and immunohistochemical study. Am J Surg Pathol 4:333–341

Mendelsohn G, Wells SA Jr, Baylin SB (1984) Relationship of tissue carcinoembryonic antigen and calcitonin to tumor virulence in medullary thyroid carcinoma. An immunohistochemical study in early, localized and virulent disseminated stage of disease. Cancer 54:657–662

Miyauchi A, Onishi T, Morimoto S, Takai S, Matsuzuka F, Kuma K, Maeda M, Kumahara Y (1984) Relation of doubling time of plasma calcitonin levels to prognosis and recurrence of medullary thyroid carcinoma. Ann Surg 199:461–466

Nishiyama RH, Dunn EL, Thompson NW (1972) Anaplastic spindle cell and giant cell tumors of thyroid gland. Cancer 30:113–127

Rossi RL, Cady B, Meissner WA, Wool MS, Sedgwick CE, Werber J (1980) Nonfamilial medullary thyroid carcinoma. Am J Surg 139:544–560

Szijji I, Csapo Z, Laszlo FA, Kovacs K (1969) Medullary cancer of the thyroid gland associated with hypercorticism. Cancer 24:167–173

Saad MF, Fritsche HA Jr, Samaan NA (1984a) Diagnostic and prognostic values of carcinoembryonic antigen in medullary carcinoma of the thyroid. J Clin Endocrinol Metab 58:889–894

Saad MF, Ordonez NG, Guido JJ, Samaan NA (1984b) The prognostic value of calcitonin immunostaining in medullary carcinoma of the thyroid. J Clin Endocrinol Metab 59:850–856

Saad MF, Ordonez NG, Bashid RK, Guido JJ, Hill CS Jr, Hickey RC, Samaan NA (1984c) Medullary carcinoma of the thyroid. A study of the clinical features and prognostic factors in 161 patients. Medicine 63:319–342

Williams ED (1966) Histogenesis of medullary carcinoma of the thyroid. J Clin Pathol 19:114–118

Zeman V, Nemec J, Platil A, Pohunkova D, Neradilova M (1978) Anaplastic transformation of medullary thyroid cancer. Neoplasma 25:249–255

Accepted November 14, 1986