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Dedifferentiation of Neoplastic Cells in Medullary Thyroid Carcinoma: Report of a Case

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Abstract: We report herein the unusual case of a man who was diagnosed as having sporadic medullary thyroid carcinoma (MTC) at the age of 29 years, and subsequently followed up for a period of 18 years. A total thyroidectomy with radical neck dissection was initially performed, followed by a stable interval of 16 years with regional metastases. He then developed widely disseminated metastases resulting in death within 2 years at the age of 47 years. While the neoplastic tissue from localized metastases in the soft tissue of the neck expressed strong immunohistochemical positivity to calcitonin (CT), calcitonin gene-related peptide, carcinoembryonic antigen, neuron-specific enolase, and chromogranin A during the stable interval, extremely weakened immunoreactivity to those markers was observed in samples from the disseminated metastases in the subcutaneous tissue after his clinical deterioration. Furthermore, only a few neoplastic cells in specimens obtained at postmortem sampling exhibited a weak response to CT. Ultrastructurally, the characteristic secretory granules in the neoplastic cells decreased remarkably in number, consistent with the immunohistochemical findings. These granules also diminished in diameter and intracytoplasmic small lumina and intercellular clefts with microvilli, interpreted as an attribute of anaplastic thyroid carcinomas, were frequently observed in tissues obtained after his clinical deterioration or at postmortem sampling. These cytological changes might represent dedifferentiation of the neoplastic cells or the anaplastic transformation of MTC.

Key Words: medullary thyroid carcinoma, dedifferentiation, calcitonin, immunohistochemistry, ultrastructure

Introduction

Medullary thyroid carcinoma (MTC) is generally considered to be a low-grade malignant tumor associated with a fairly good prognosis, particularly in familiar cases.[1,2] However, rare cases of MTC with rapidly progressive behavior resulting in a poor prognosis have been reported as "anaplastic variants."[3–12] In 1973, Hill et al.[13] proposed that patients with MTC could be grouped into three categories according to their clinical course after the first treatment, namely: those having an indolent course with no symptoms and prolonged survival; those developing inexorable progression leading to rapid death within 2 years; and those having a latent, symptom-free interval followed by inexorable progression with recurrence and/or metastases and consequent death. We describe herein the case of a patient with sporadic MTC following an unusual clinical course, which might belong to the third category. Special reference is made to the immunohistochemical and ultrastructural features of the neoplastic cells examined.

Case Report

A 29-year-old man first presented to our hospital in October 1978 with a nodule in the right submandibular region and a 2-year history of dull pain in the neck. Histological examination of a biopsy specimen from the nodule confirmed a diagnosis of metastatic MTC. Although he was in euthyroidism, a high plasma calcitonin (CT) level of 1350 pg/ml was noted, the normal value being <100 pg/ml.

A subtotal thyroidectomy with modified right neck dissection was performed in December 1978, revealing a primary lesion, 15 × 15 mm in size, located in the right lobe of the thyroid with cervical node involvement, invading the surrounding muscular structures and right parathyroid glands. Additional excision of the remnant

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thyroid with radical neck dissection was performed 1 month later, as his plasma CT level increased to 2033 pg/ml after the first operation. Although no remaining lesion was found, subcutaneous nodules were observed on both sides of the neck and recurrence of the MTC was confirmed histologically in January 1980. The plasma CT level was also found to be exceedingly high at 45500 pg/ml. Similar nodules exhibiting tenderness grew one after another in the cervical region, and subsequent surgical excision was required eight times during a period of 14 years from 1980 to 1993. No other metastases were observed clinically or radiologically in that period. Although the patient often complained of general fatigue and pain in his hands, he was generally in good health. The plasma CT levels gradually increased after confirmation of the recurrence, maintaining persistently high values in the range of 40000–90000 pg/ml with marked fluctuation from 1984 onward. On the other hand, the plasma levels of carcinoembryonic antigen (CEA) were only slightly elevated at 6.73 ng/ml, the normal value being <2.5 ng/ml, when recurrence was first recognized, subsequently increasing slowly from 1981 to 1985. Thereafter, the plasma CEA levels revealed a steep rising slope throughout the period up to 1991, followed by less fluctuating high values of 200–250 ng/ml (Fig. 1).

In the spring of 1994, rapid progression of the disease suddenly developed. Numerous subcutaneous nodules erupted all over the patient's body and right pleural invasion was found. Furthermore, disseminated bone metastases were demonstrated by scintigraphy in October 1994. Watery diarrhea also developed which persisted for several weeks and was finally controlled with morphine. Chemotherapy consisting of adriamycin, 5-fluorouracil, and cyclophosphamide was commenced in December, but it did not have a marked effect. The plasma CT levels dropped to 52800 pg/ml in August 1995; however, the plasma CEA levels increased further to 310 ng/ml. The patient's general state of health deteriorated progressively due to disseminated metastases in the liver, lung, and brain, and he died, at the age of 47 years, in May 1996. The highest plasma levels of CT and CEA, being 86900 pg/ml and 310.8 ng/ml, respectively, were found 1 month prior to death. Permission for autopsy was not granted by the patient's family, but they gave consent for postmortem needle sampling of the metastatic subcutaneous nodules.

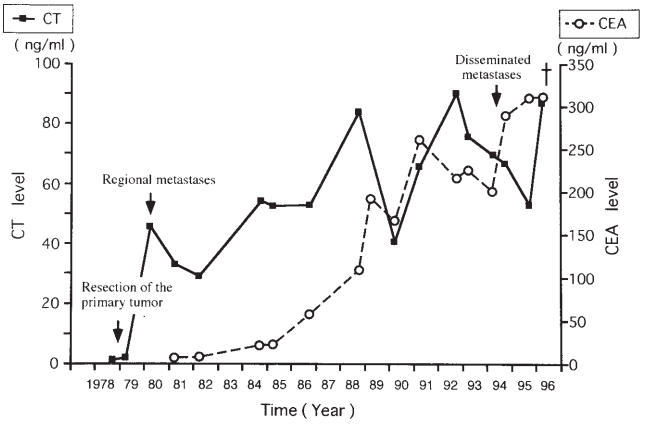

Fig. 1. Chronological changes in plasma calcitonin (CT) and carcinoembryonic antigen (CEA) levels. The plasma CEA levels showed a steep rising slope followed by less fluctuating high values, whereas the plasma CT levels showed high values with marked fluctuation after recurrence was first recognized. Both levels were highest 1 month prior to death

Tissue Analysis

Tissues surgically excised from the metastatic subcutaneous nodules in 1990, 1992, and 1994, and those obtained at postmortem sampling were available for histological examination. For light microscopy, each specimen was fixed with 10% formalin and embedded in paraffin. Sections were prepared for hematoxylin–eosin (H&E) stain, Grimelius' argyrophil stain, and immunohistochemistry.

Immunohistochemistry with antisera for CT (DAKO, Glostrup, Denmark), calcitonin gene-related peptide (CGRP) (Chemicon International, Temecula, CA, USA), CEA (Mochida Pharm., Tokyo, Japan), neuron-specific enolase (NSE) (IBL, Belmont, MA, USA), serotonin (5-hydroxytryptamine: 5-HT) (courtesy of Dr. R. Yui, Niigata University, Japan), and chromogranin A (CG) (Incstar, Stillwater, MN, USA) was performed using the avidin-biotin-peroxidase complex (ABC) method. For specimens obtained at postmortem sampling, immunohistochemistry with only antisera for CT was available.

For electron microscopy, each specimen was fixed in a phosphate-buffered solution of 2.5% gultaraldehyde, postfixed in 1% osmium tetroxide, then embedded in Epon 812. Ultrathin sections were cut on an Ultrotome V (LKB-Produkter, Bromma, Sweden) equipped with a diamond knife and stained doubly with uranyl acetate and lead citrate. The sections were examined under a JEM-100S electron microscopy (JEOL, Tokyo, Japan) at 80 kV.

Results

The primary tumor exhibited findings of a typical MTC with a solid growth pattern highly invasive to the surrounding structures and parathyroid glands. A small amount of amyloid deposition was also found in the stroma. Specimens from the metastatic subcutaneous nodules surgically resected in 1990 and 1992 showed

identical cytological features. The tumor cells were of a small round to polygonal shape and often formed nests of various sizes with a tendency toward organization scattered throughout a thick fibrous stroma (Fig. 2a). Mitotic figures were infrequently seen. In the specimens obtained in 1994, after rapid clinical deterioration had begun, moderate pleomorphism of neoplastic cells exhibiting a loss of polarity was observed and mitotic figures were sparsely encountered (Fig. 2b). On the other hand, the tissue taken at postmortem sampling was composed cytologically of undifferentiated cells with one or more bizarre nuclei. Although the majority of these cells were small, there was considerable variation in the cell size, and mitotic figures were more frequently seen. The tumor cells from each tissue sample examined exhibited a negative reaction to Grimelius' argyrophilia. Most neoplastic cells from the tissues obtained in 1990 and 1992 while the patient exhibited a stable clinical course showed strongly positive immunoreaction to CT and CGRP (Fig. 3a). Numerous cells reacted positively to CEA and NSE, whereas occasional scattered cells showed a positive reaction to CG, but not to 5-HT. In contrast, very faint reactions to CT, CGRP, and NSE were observed in the tissue excised in 1994 when clinical deterioration had begun (Fig. 3b); however, the immunoreactivity to CEA, CG, and 5-HT exhibited no significant difference compared with that observed in the tissues formerly obtained. Furthermore, in specimens taken at postmortem sampling, only a few neoplastic cells exhibited a weak response to CT (Fig. 3c).

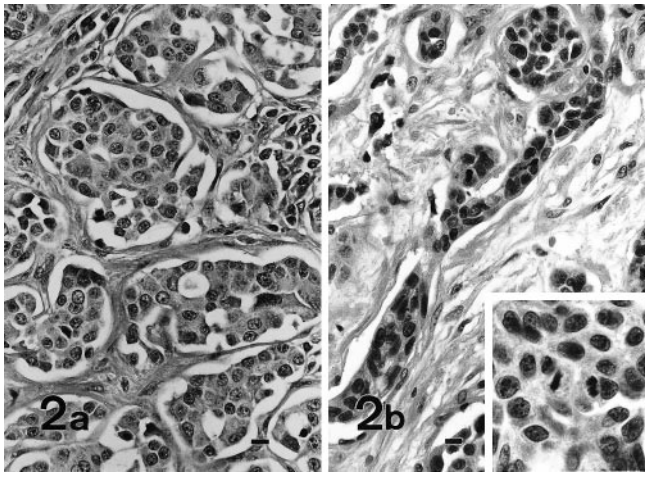

Fig. 2a,b. Changes in the neoplastic cells (H&E stain). **a** A specimen taken in 1990 showed a tendency toward organization of the neoplastic cells with the formation of occasional pseudoglandular structures. **b** A specimen taken in 1994 exhibited a loss of polarity of neoplastic cells with nuclear atypia and hyperchromatism along with an increased numer of mitoses (*inset*). *Bar* = 10 μm (*inset bar* = 20 μm)

Under the electron microscope, neoplastic cells in specimens obtained before the manifestation of clinical deterioration consisted of clear cells with round or oval nuclei and dark cells with pycnotic nuclei. Both cell types possessed numerous round secretory granules (100–350 nm) characteristic of neoplastic cells in MTC (Fig. 4a). The most notable ultrastructural changes observed in the neoplastic cells obtained during the clinical deterioration were an extreme decrease in the number of secretory granules and a remarkable increase of glycogen particles in the cytoplasm. No secretory granules were found in the majority of neoplastic cells, although careful observation revealed a small number of secretory granules of small size (50–150 nm) at the periphery of some neoplastic cells (Fig. 4b). Additional ultrastructural changes were seen at the neoplastic cell boundaries. Narrow clefts, into which small microvilli extended, were frequently seen be-

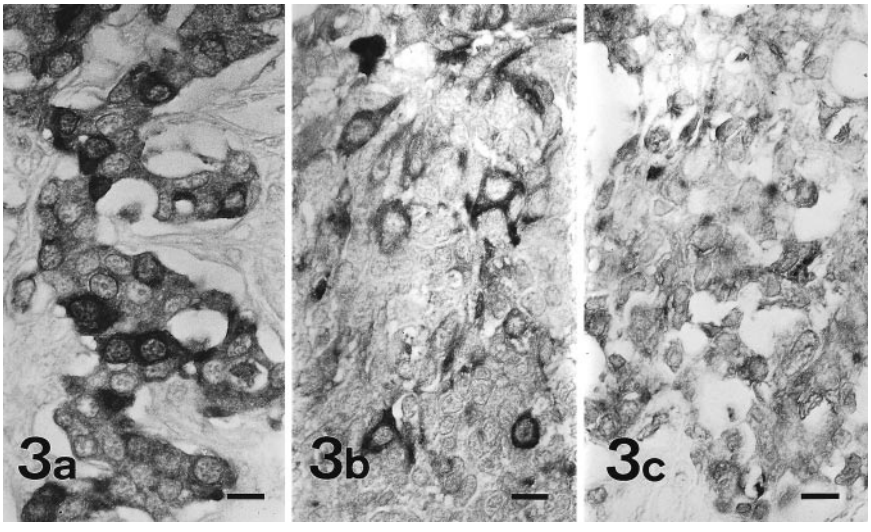

Fig. 3a–c. Changes in the immunoreactivity of neoplastic cells to CT (CT immunostaining). **a** Most neoplastic cells in a specimen taken in 1990 exhibited a strongly positive reaction to CT. **b** Some of the neoplastic cells in a specimen taken in 1994 revealed weak immunoreactivity to CT. **c** A few neoplastic cells in a specimen taken at postmortem sampling in 1996 reacted faintly to CT. *Bar* = 10 μm

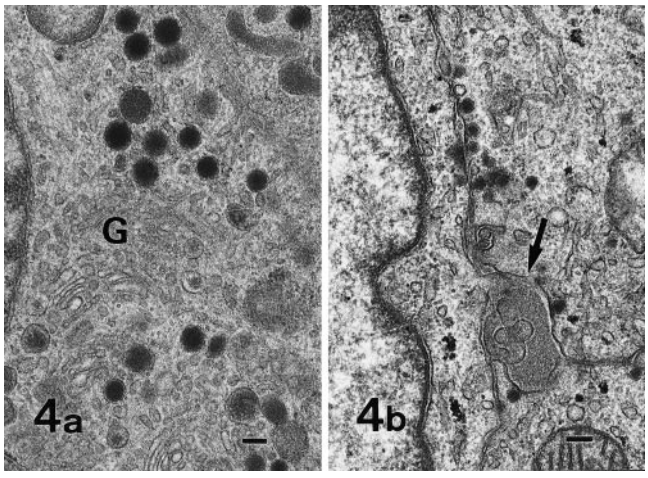

Fig. 4. a Typical secretory granules in a neoplastic cell obtained in 1992. *G*, Golgi apparatus. **b** Small round granules were observed in the vicinity of the plasma membrane in a specimen obtained in 1994. Note the narrow cleft with short microvilli containing floccular material (*arrow*). *Bar* = 200 nm

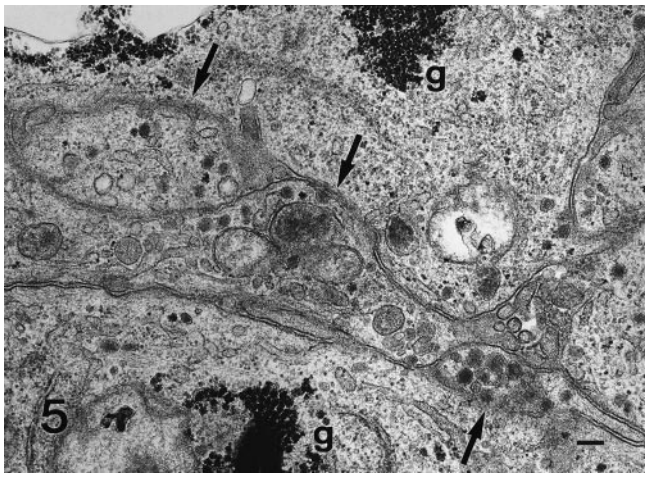

Fig. 5. The intercellular region of neoplastic cells in a specimen excised in 1994. Some cytoplasmic fragments (*arrows*) containing small granules as well as small mitochondria and vesicles were seen. *g*, glycogen particles. *Bar* = 200 nm

tween neighboring neoplastic cells (Fig. 4b). Additionally, numerous small cytoplasmic fragments containing many secretory granules and small mitochondria were observed in the intercellular spaces (Fig. 5). Those structures were interpreted as profiles of cytoplasmic processes protruding from neighboring neoplastic cells.

In the tissue obtained at postmortem sampling, the neoplastic cells possessed frequently plural bizarre nuclei. In addition to lysosome-like granules, typical secretory granules (80–150 nm) were infrequently found. In the cytoplasm of some neoplastic cells a few small round lumina with microvilli were observed. These lumina contained floccular substances and were surrounded by electron-dense granules (80–150 nm) (Fig. 6). Such peculiar structures were not observed in the other specimens taken prior to the patient's death.

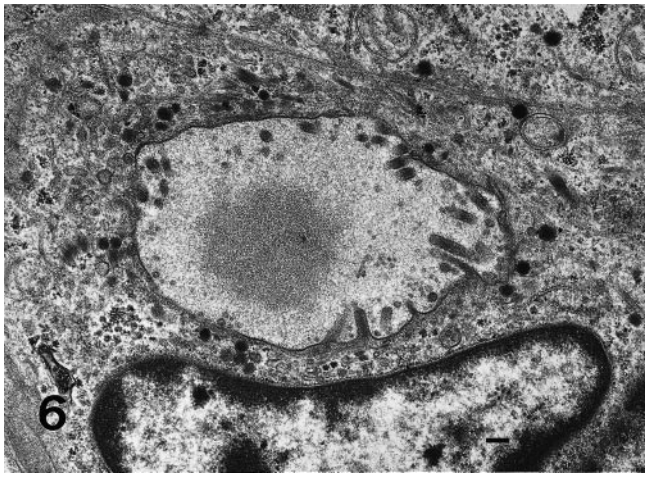

Fig. 6. An intracytoplasmic microlumen with microvilli containing floccular material surrounded by electron-dense granules seen in a specimen obtained at postmortem sampling in 1996. *Bar* = 200 nm

Discussion

MTC is a malignant tumor originating from the parafollicular cells (C-cells) of the thyroid[14] and is considered to exhibit nonaggressive clinical behavior even though metastases exist.[1,2] However, unusual cases of MTC exhibiting anaplastic cytological features resulting in a poor prognosis have been reported.[3–12] These tumors were diagnosed as MTC by the positivity of CT immunoreaction and/or the ultrastructural evidence of typical secretory granules in the neoplastic cells, differentiating them from anaplastic carcinomas of the thyroid. Most authors interpreted such tumors as variants of MTC subsequent to anaplastic transformation.

Saad et al.[15] reported that the numer of cells in primary tumors or metastases stained with CT immunostaining correlated well with survival, patients with CT-rich tumors surviving longer than those with CT-poor tumors. They emphasized the prognostic value of CT immunostaining for patients with MTC. Lippman et al.[16] showed a similar correlation, but later reported[17] that a patient who initially had a CT-rich primary tumor revealed a sudden rapid dissemination of CT-poor metastases subsequent to an indolent clinical course of 7 years. Saad et al.[15] described an identical case in their

report. Both these patients developed multiple endocrine neoplasia (MEN) IIa and died within 2 years. Our patient demonstrated a similar course, and his metastatic tumors showed the same changes in CT immunostaining 16 years after his first operation. This demonstrates that CT immunostaining is a valid marker for predicting the prognosis of patients with MTC, although CT-rich tumors might dedifferentiate to CT-poor tumors during long-term follow-up. Hill et al.[13] divided the clinical course of 72 patients with MTC after treatment into three groups, namely: the majority of patients who followed a favorable course; a considerable number of patients who showed inexorable progression resulting in death within 2 years; and 11 (11/72; 15.3%) patients who experienced a latent, symptom-free interval for approximately 7 years, followed by reactivation with inexorable progression and consequent rapid progression resulting in death. Based on the clinical course after the first operation, our patient might belong to the third group. Such cases appear to be very rare, because we were able to find only 13 documentations in the literature.[13,15,17] In addition to CT, many peptides or amines such as adrenocorticotropic hormone, somatostatin, CGRP, CEA, NSE, and 5-HT have been demonstrated in the neoplastic cells of MTC.[18,19] Among these, CT, CGRP, CEA, and NSE were most frequently expressed, in accordance with our results. The plasma levels of these tumor markers have been proposed to be of significant diagnostic and prognostic value.[2,20–22] Saad et al.[20] reported that the rapid increase in plasma CEA levels correlated with the progression of the disease, whereas the plasma CT levels were of no prognostic value due to their marked fluctuations. The alteration of plasma CEA levels in our patient, as shown in Fig. 1, revealed a steep rising slope over 9 years prior to clinical deterioration. These findings appear to be in accordance with the results of Saad et al.[20] when the CEA time curve is verified retrospectively. However, the time of clinical deterioration could not be predicted in our patient as the plasma CEA levels did not increase until 3 years before his deterioration. Thus, it is possible that patients with MTC showing a rapid and continuous increase in plasma CEA levels during the follow-up period would develop sudden deterioration leading to death within several years.

Regarding the ultrastructural changes, Kakudo et al.[5,11] reported in detail the cytological features of anaplastic variants, described as "poorly differentiated" MTC. They observed that the neoplastic cells of these variants possessed fewer and smaller secretory granules (173.0 nm in mean diameter) with poorly developed cytoplasmic organelles, than those observed in tumors from well-differentiated MTC. The ultrastructural findings of the specimen obtained from our patient in 1994, when clinical deterioration begun, coincided with their results. Besides the changes in secretory granules, we observed intercellular cytoplasmic fragments interpreted as the profiles of cytoplasmic processes of the neoplastic cells. Similar cytoplasmic processes are generally observed in the normal human neonatal or fetal thyroid C cells.[23,24] Such ultrastructural findings might represent the immaturity or dedifferentiation of these neoplastic cells. The intercellular clefts and intracytoplasmic small lumina with microvilli we described in this report have rarely been found in MTC tumors.[25] Instead, such structures are frequently encountered in anaplastic or undifferentiated carcinomas of the thyroid with or without obvious follicular formation, and are considered to be characteristic of tumors of follicular cell origin.[26] It remains uncertain whether the electron-dense granules observed in the vicinity of the intracytoplasmic lumina contained thyroglobulin or calcitonin.

It is generally accepted that anaplastic carcinomas of the thyroid arise from preexisting differentiated carcinomas of follicular or papillary cell origin; however, whether or not medullary carcinomas can transform to become anaplastic carcinomas remains a subject of debate.[27] The classical concept that a lack of papillary and follicular differentiation is one of the histological features of MTC has become doubtful, since a number of cases of mixed medullary and follicular carcinoma have been reported.[28] Thus, electron microscopy as well as immunohistochemistry could provide information to aid in establishing a precise diagnosis or in predicting the prognosis of patients with this disease during their follow-up period, especially in those with metastases.

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