Atypical Thymic Carcinoid Associated with Cushing's Syndrome

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(Received March 17, 2010; Accepted June 4, 2010)

A 56-year-old Japanese woman with adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome (CS) was admitted to hospital, where she was diagnosed as having a mediastinal tumor with ectopic ACTH production. The tumor and associated lymph node metastases were resected endoscopically, and the pathological diagnosis was atypical thymic carcinoid. Radiation therapy and administration of metyrapone, an inhibitor of 11β-hydroxylase to decrease the cortisol level, were attempted, but the levels of ACTH and cortisol were unresponsive. Bilateral adrenalectomy and hydrocortisone replacement were performed to ameliorate the patient's hypercortisolism. She subsequently developed multiple vertebral metastases, but was unwilling to undergo chemotherapy. Her condition deteriorated progressively, and she died of heart and respiratory failure 3 years and 6 months after the first admission. Immunostaining for ACTH, chromogranin A, synaptophysin, and neuron-specific enolase was positive in the carcinoid cells. Since somatostatin (SS) and SS analogues inhibit the growth of carcinoid via the SS receptor (SSTR) 2, we evaluated the expression of SSTR2 in the carcinoid cells using reverse transcription-polymerase chain reaction, and this confirmed the expression of SSTR2 in the carcinoid cells. Our experience of this patient with CS due to an ectopic ACTH-producing atypical thymic carcinoid suggests that SS analogues may be useful for treatment of carcinoid showing expression of SSTR2.

Key words: Atypical thymic carcinoid, Cushing's syndrome, Somatostatin receptor, Somatostatin analogue

INTRODUCTION

Thymic carcinoid, for which the term well differentiated neuroendocrine carcinoma (NEC) of the thymus has been proposed, accounts for less than 5% of all carcinoid tumors. Thymic carcinoid is classified into two types: atypical and typical. Atypical thymic carcinoid is more aggressive than the typical type, and shows areas of necrosis and/or two to 10 mitoses per 2 mm^2 [1, 2]. Thymic carcinoids are also associated with a number of endocrinological disorders, including Cushing's syndrome (CS) and multiple endocrine neoplasia (MEN) type 1 [3], and the diagnosis and treatment of CS is clinically challenging [4–6].

Somatostatin (SS) has a broad spectrum of biological actions, exerting suppressive effects on multiple organs, and appears to be an endogenous growth inhibitor [7]. SS and its analogues inhibit the growth of various kinds of neuroendocrine cells via the SS receptor (SSTR) [8]. Five different subtypes of human SSTR have been identified [9]. Based on binding studies of the cloned receptors, SSTR2, one type of SSTR, has been suggested to be the main target of SS analogues [10]. The use of one type of SS analogues, octreotide, has become a well established medical treatment for neuroendocrine tumors, e.g. pituitary adenomas [11, 12].

We describe a 56-year-old Japanese woman with ACTH-dependent CS due to atypical thymic carcinoid with ectopic ACTH production. In this case, we also evaluated the expression of SSTR2 using reverse transcription-polymerase chain reaction (RT-PCR) to assess the possibility of treatment with an SS analogue.

PATIENT AND METHODS

Case report

A 56-year-old Japanese woman was admitted to our hospital complaining of leg edema. On physical examination she was 145 cm in height and 55 kg in weight. Blood pressure was 192/110 mmHg and sinus tachycardia was evident with a pulse rate of 108 beats/min. She showed a moon face, central obesity, and buffalo hump, but there were no purple striae. Her legs showed pitting edema. She had a medical history of acute glomerulonephritis at the age of 26. Her father had died of myocardial infarction and her mother had bronchial asthma, but there was no medical or family history of CS or MEN type 1.

Complete blood cell counts revealed elevated numbers of white blood cell and neutrophils, and decreased numbers of lymphocytes and eosinophils. Blood chemistry confirmed hypokalemia and hyperlipidemia (Table 1).

Basal endocrinological examinations showed elevated levels of plasma ACTH, serum cortisol, urine free cortisol, and urine 17-hydroxycorticosteroid (17-OHCS) (Table 2). These data indicated ACTH-dependent hypercortisolism.

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Table 1 Complete blood cell counts and blood chemistry

	values	normal ranges
WBC (/μl)	8900	4000-8000
neutrophils (%)	83.5	40-70
lymphocytes (%)	13.6	20-40
eosinophils (%)	0.2	1-4
basophils (%)	0.1	0-1
monocytes (%)	3.6	3-8
RBC ($\times 10^4$/μl)	491	380-480
hemoglobin (g/dl)	12.3	11.5-15.5
hematocrit (%)	34.8	34.0-42.0
platelets ($\times 10^4$/μl)	17.4	14.0-40.0
glucose (mg/dl)	99	70-109
urea nitrogen (mg/dl)	12	8-20
creatinine (mg/dl)	0.5	0.5-0.8
sodium (mEq/l)	145	136-145
potassium (mEq/l)	2.6	3.5-4.8
chloride (mEq/l)	99	98-108
Albumin (g/dl)	4.0	3.9-4.8
AST (IU/l)	24	<30
ALT (IU/l)	28	<35
T-Chol (mg/dl)	308	140-220
TG (mg/dl)	200	50-150

WBC, white blood cells; RBC, red blood cells; AST, aspartate aminotransferase; ALT alanine aminotransferase; T-Chol, Total cholesterol; TG, triglyceride.

Table 2 Basal endocrinological examinations

	values	normal ranges
plasma ACTH (pg/ml)	258	7.4-55.7
serum cortisol (μg/dl)	29.0	4.0-18.3
urine free cortisol (μg/day)	1070	11.2-80.3
urine 17-OHCS (mg/day)	20.3	2.2-7.3
urine 17-KS (mg/day)	9.6	2.4-11.0
plasma renin activity (ng/ml/h)	0.5	0.3-5.4
plasma aldosterone (pg/ml)	40	39-159
plasma epinephrine (pg/ml)	33	<100
plasma norepinephrine (pg/ml)	323	100-450
plasma dopamine (pg/ml)	<10	<20

ACTH, adrenocorticotropic hormone; 17-OHCS, 17-hydroxycorticosteroid; 17-KS, 17-ketosteroids.

Overnight 1-mg dexamethasone suppression test (DST) was unable to suppress serum cortisol (Table 3-1). Liddle's original DST (administration of 2 mg dexamethasone for 2 days, and 8 mg dexamethasone for 2 days) was unable to suppress urinary 17-OHCS (Table 3-2). Circadian rhythms of plasma ACTH and serum cortisol were not evident, because levels of plasma ACTH and serum cortisol are highest early in the morning (Table 3-3). A corticotropin releasing hormone (CRH) stimulation test indicated that ACTH did not respond to CRH stimulation (Table 3-4). These results of endocrinological examinations indicated that an ectopic ACTH-producing tumor had caused the CS in this case.

Magnetic resonance imaging (MRI) detected no pituitary adenoma. Cavernous sinus sampling was performed, and the central-to-peripheral (C/P) ACTH ratio was calculated. The right central and peripheral ACTH values were both 280 pg/ml, and the left central and peripheral ACTH values were both 320 pg/ml (Table 3-5). Thus, the right and left C/P ACTH ratios were both 1, suggesting absence of any pituitary adenoma producing ACTH.

Thoracic computed tomography (CT) revealed a mediastinal tumor (Fig 1). Abdominal CT showed a slightly enlarged bilateral adrenal gland, but the liver, pancreas, and other intra-abdominal organs were normal. The ultrasonographic appearance of the thyroid was normal, and endoscopic examinations of the esophagus, stomach, duodenum, and large intestine revealed no abnormality.

The mediastinal tumor and lymph node metastases were resected endoscopically. Radiation therapy with a total dose of 50 Gy was delivered using a linear accelerator. However, after these treatments, the levels of plasma ACTH and serum cortisol did not decrease to the normal ranges. Administration of metyrapone, an inhibitor of 11β-hydroxylase, was attempted to decrease

Table 3-1 1-mg dexamethasone suppression test

	1 mg DST	normal response
serum cortisol (μg/dl)	26.5	<5

Table 3-2 Liddle's original dexamethasone suppression test

	basal	2 mg DST	8 mg DST	normal response
urine 17-OHCS (mg/day)	20.3	17.0	21.8	<50% of the base line

Table 3-3 Circadian rhythms of ACTH and cortisol

	6:00 a.m.	0:00 a.m.
Plasma ACTH (pg/ml)	254	346
Serum cortisol (μg/dl)	26.8	32.1

Table 3-4 Corticotropin releasing hormone stimulation test

	basal	30 min	60 min	120 min
Plasma ACTH (pg/ml)	292	319	319	271

Table 3-5 Cavernous sinus sampling of ACTH

	right	left
Central plasma ACTH (pg/ml)	280	320
Peripheral plasma ACTH (pg/ml)	280	320
C/P ratio	1.0	1.0

ACTH, adrenocorticotropic hormone; 17-OHCS, 17-hydroxycorticosteroid; DST, dexamethasone suppression test; C, central; P, peripheral.

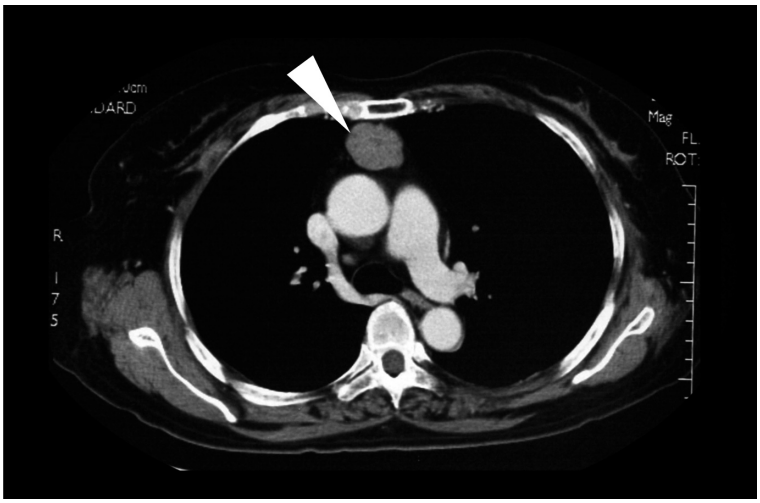

Fig. 1 Thoracic CT shows a mediastinal tumor (white arrowhead).

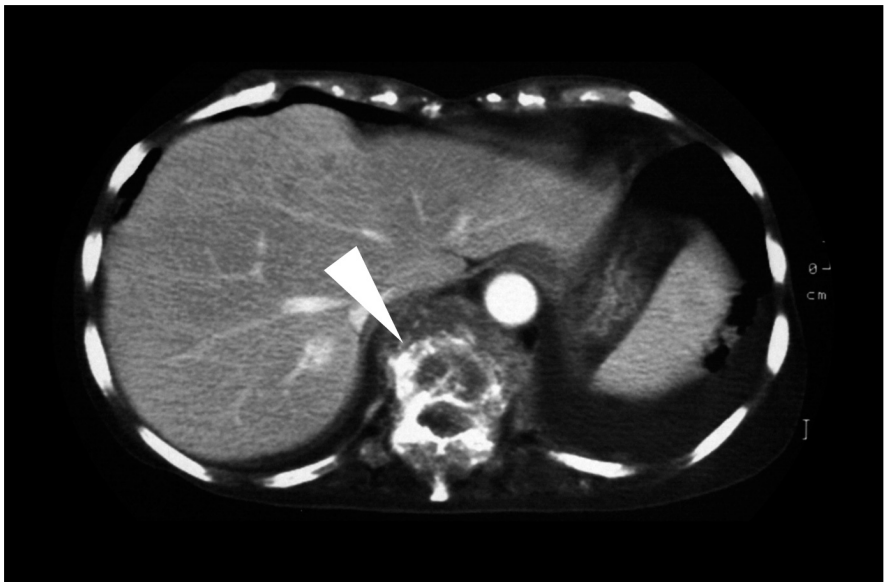

Fig. 2 Abdominal CT shows vertebral bone metastasis and destruction (white arrowhead).

the serum cortisol level, but the hypercortisolism was unresponsive. Therefore, bilateral adrenalectomy and replacement with hydrocortisone, 20 mg/day, were performed to ameliorate the hypercortisolism 60 days after resection of the mediastinal tumor. Later, the patient was unwilling to undergo any chemotherapy including octreotide therapy, because of concern about side effects.

Subsequently, she developed vertebral bone metastases, which showed gradual progression on spinal CT scanning (Fig 2). Her condition deteriorated progressively, and she died of heart and respiratory failure 3 years 6 months after the first admission. Autopsy was not performed.

MATERIALS AND METHODS

Immunohistochemistry and electron microscopy

The specimens were fixed in 10% buffered formalin, processed and embedded in paraffin. Sections 4 μm thick were prepared, and stained with hematoxylin and eosin. Immunohistochemical staining was performed for ACTH (monoclonal 02A3, 1:200, DAKO, Kyoto, Japan), chromogranin A (monoclonal DAK-A3, 1:50, DAKO), synaptophysin (rabbit polyclonal, 1:50, DAKO), and neuron-specific enolase (NSE) (monoclonal BBS/NC/VI-H14, 1:50, DAKO) as neuroendocrine markers. Antigen retrieval was performed as described elsewhere [5].

For electron microscopy, the specimens were fixed with 2.5% glutaraldehyde and post-fixed with 1% osmium tetroxide. After processing and embedding in epoxy resin, ultrathin sections stained with uranyl acetate and lead citrate were examined using an electron microscope.

RT-PCR and PCR of SSTR2

Total RNA was extracted from the formalin-fixed, paraffin-embedded specimen and incubated for 3 hours with proteinase K. TRIzol reagent (Invitrogen, CA, USA) was used for RNA isolation. RNA was incubated with RNase-free deoxyribonuclease-I (Promega, WI, USA) to eliminate any contaminating genomic DNA. For synthesis of the first-strand cDNA, the RNA was reverse-transcribed by incubation with Oligo (dT) 12–18 Primer (Invitrogen) and StrataScript transcriptase (Stratagene, CA, USA).

PCR reactions were performed on a Mastercycler ep (Eppendorf, Hamburg, Germany) using AmpliTaq Gold PCR Master mix (Applied Biosystems, CA, USA). PCR amplification was run for 40 cycles at 95°C for 15s, 56°C for 15s, and at 72°C for 30s, followed by a final extension step for 7 min at 72°C. The following PCR primers were used: SSTR2 forward 5´-GGT CAG GAG TTC GAG ACC AG-3´ and reverse 5´-CTC CCG AGC AGC TAG GAT TA-3´. The PCR product was anticipated to be 112 base pairs (bp) and was visualized after 2% agarose gel electrophoresis in TBE buffer and ethidium bromide staining. For PCR, the positive control was human pituitary and the negative control was distilled water.

The investigation was performed in accordance with the principles of the Declaration of Helsinki and approved by the institutional review board committee at Tokai University School of Medicine.

RESULTS

Microscopically, thymic carcinoid tumor exhibited a solid growth pattern, three mitoses per 2 mm^2, but no necrosis (Fig. 3). These features were compatible with atypical thymic carcinoid. Immunohistochemically, the tumor cells were positive for ACTH (Fig. 4–A), chromogranin A (Fig. 4–B) synaptophysin (Fig. 4–C) and NSE (Fig. 4–D). Ultrastructurally, the tumor cells contained small dense-cored neurosecretory granules (Fig. 5). Electrophoresis of the PCR product showed a band of 112 bp, suggesting expression of SSTR2 mRNA in the carcinoid cells (Fig. 6).

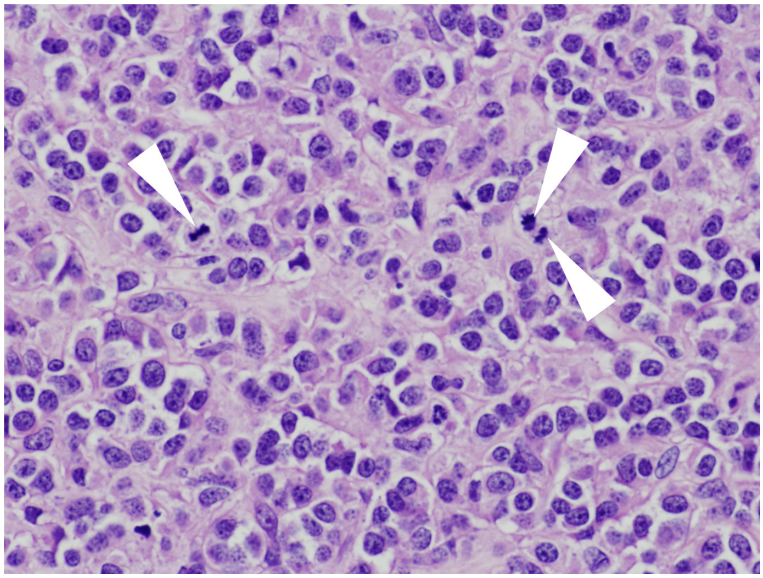

Fig. 3 Histological appearance of the atypical thymic carcinoid. The tumor exhibited a solid growth pattern with three mitoses per 2 mm^2, but no necrosis (hematoxylin-eosin stain). White arrowheads indicate mitoses of the carcinoid cells.

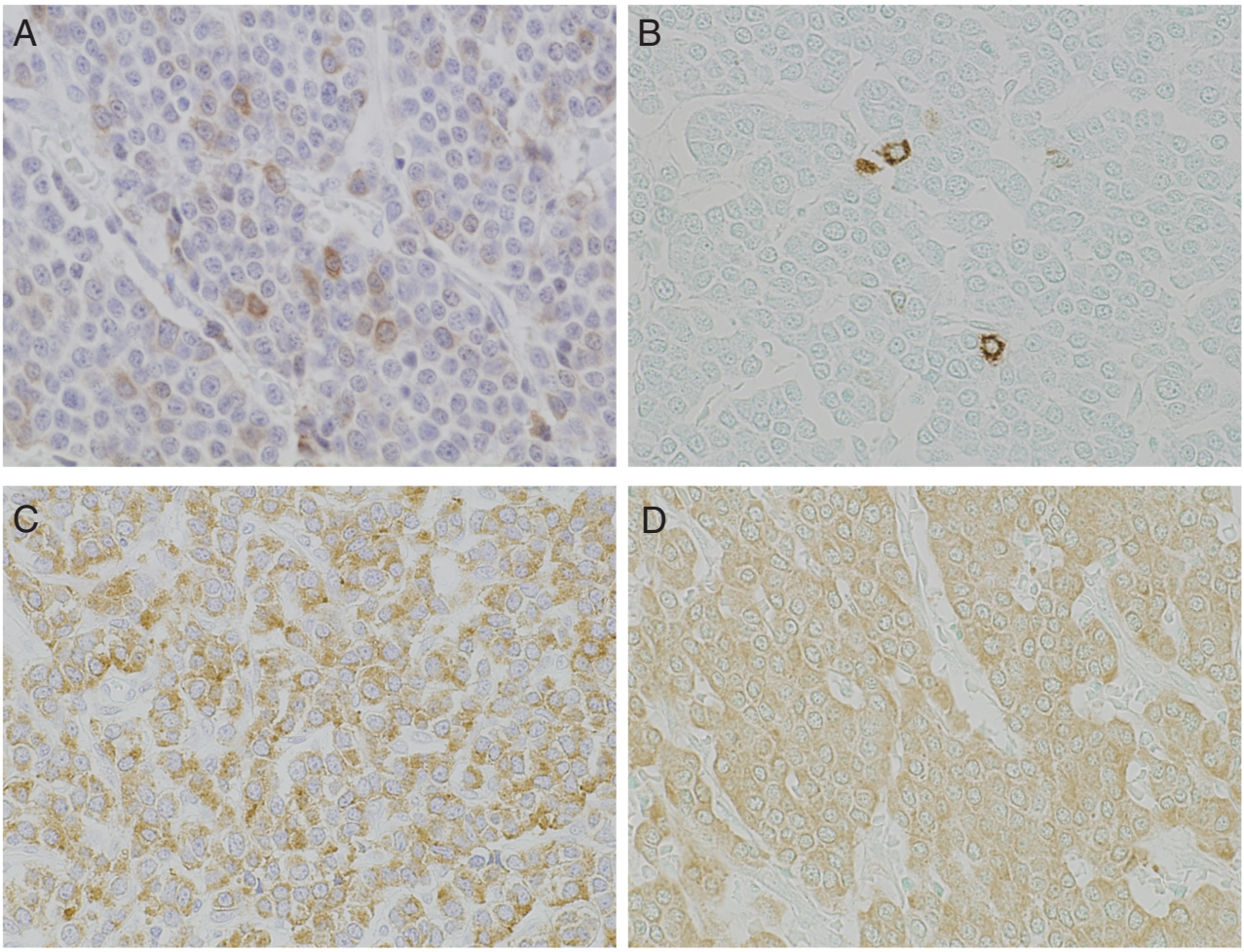

Fig. 4 The carcinoid cells show immunoreactivity for ACTH (A), chromogranin A (B), synaptophysin (C), and NSE (D). ACTH, adrenocorticotropic hormone; NSE, neuron-specific enolase.

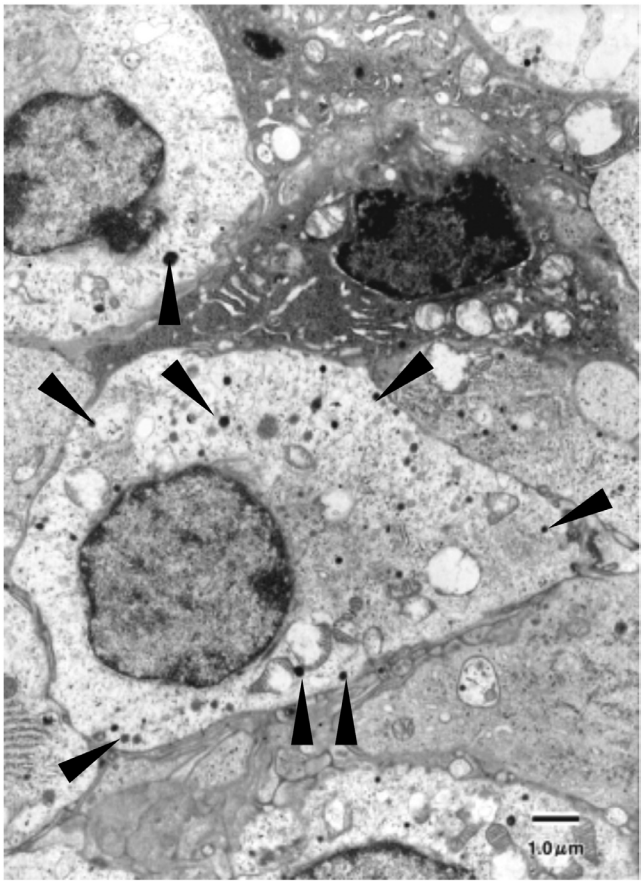

Fig. 5 Many small electron-dense neurosecretory granules are evident in the tumor cells (black arrowheads).

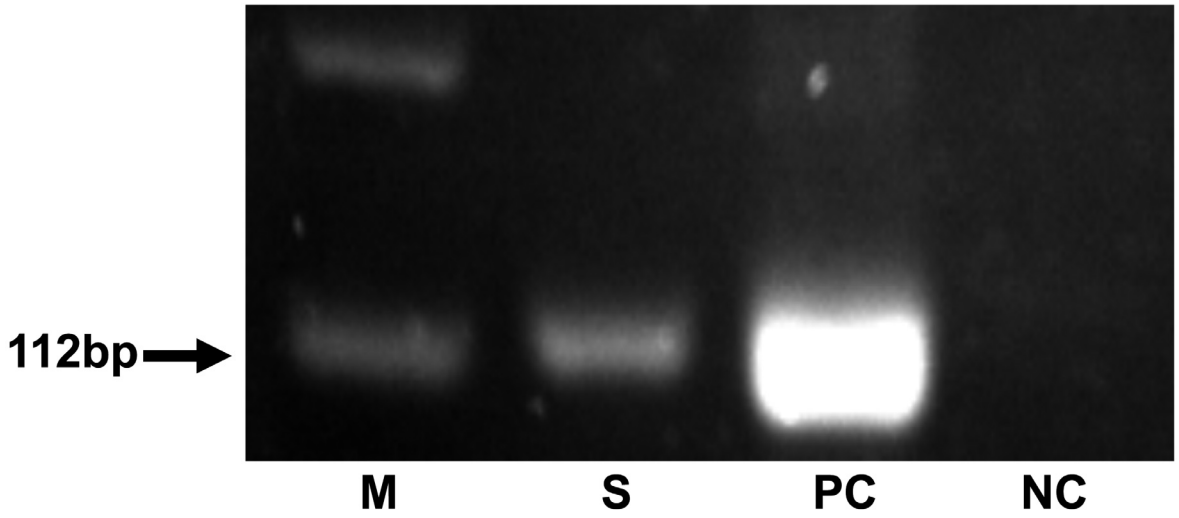

Fig. 6 RT-PCR shows expression of SSTR2 mRNA. Lane S is a sample from the thymic carcinoid of the patient. The presence of a 112-base-pair DNA fragment indicates the expression of SSTR2 mRNA.
RT-PCR, reverse transcription-polymerase chain reaction; SSTR2, somatostatin receptor 2; bp, base pair; M, marker (100 base pairs); S, sample from the patient (112 base pairs); PC, positive control (normal human pituitary); NC, negative control (distilled water).

DISCUSSION

The present patient showed the features of CS, including moon face, central obesity, and buffalo hump. Results of endocrinological examinations were compatible with ACTH-dependent CS caused by an ectopic ACTH-producing tumor. MRI of the pituitary, and the cavernous sampling test, ruled out ACTH-producing pituitary adenoma. Thus, it was indicated that an ectopic ACTH-producing tumor had caused the CS in this case.

Thoracic CT revealed a mediastinal tumor. After

resection, the tumor was confirmed to be an atypical thymic carcinoid. Immunohistochemical studies confirmed that the carcinoid cells were positive for ACTH, and electron microscopy showed that the carcinoid cells contained dense-cored neurosecretory granules. These findings confirmed that an ACTH-producing atypical thymic carcinoid had caused the CS.

The clinical course of atypical thymic carcinoid is very aggressive. Since the disseminated lesions are diffuse and multiple, it is difficult to control the hypercortisolism, which causes hypertension, hyperglycemia, and an immunocompromised state, thus worsening the condition of the patient. Systemic chemotherapy is the only treatment that can control tumor progression and paraneoplastic syndrome. Several chemotherapeutic regimens for metastatic carcinoids, including streptozocin, cyclophosphamide, fluorouracil and/or doxorubicin, have been reported, but the response rates seldom exceed 30% and complete responses are uncommon [13, 14].

SSTRs are widely expressed in many tissues, freaquently as multiple subtypes that coexist in the same cell. The five receptors share common signaling pathways such as the inhibition of adenylyl cyclase, activation of phosphotyrosine phosphatase, and modulation of mitogen-activated protein kinase (MAPK) through G-protein-dependent mechanisms. SSTRs block cell secretion by inhibiting intracellular cAMP and Ca^{2+} and by a receptor-linked distal effect on exocytosis. SSTR1, 2, 4, and 5 induce cell cycle arrest via phosphotyrosine phosphatase-dependent modulation on MAPK, associated with induction of the retinoblastoma tumor suppressor protein and p21. SSTR3 triggers phosphotyrosine phosphatase-dependent apoptosis accompanied by activation of p53 and the proapoptotic protein Bax [9]. SS analogues mainly target SSTR2 [10].

As with other NECs, the number of SS receptors on carcinoid tumors determines the suppressive effect of therapy with octreotide, an SS analogue, on hormonal hypersecretion by the tumors [15]. It was demonstrated that expression rate of SSTR1, 2, 3, 4, and 5 at mRNA level in the carcinoid were 76%, 80%, 43%, 68%, and 77%, respectively. Studies using antibodies against SSTR1 and SSTR2 demonstrated that expression rate of SSTR1 and SSTR2 were 88% and 78%, respectively [16].

It was reported that the symptoms, such as diarrhea and flushing attacks, caused by an overproduction of serotonin or tachykinin were controlled in 70 to 90% of patients with metastatic carcinoid [17]. And, octoreotide temporarily inhibits tumor growth, and in about 20% of patients, enlarged lymph nodes and liver metastases shrink [17]. Furthermore, most important is the improvement in the quality of life during octreotide therapy, and preliminary evidence suggests that such therapy may prolong survival [18]. In addition, it has been suggested that octreotide has some side effects, but that these are usually well tolerated [19]. In the present case, we confirmed the expression of SSTR2 in the carcinoid cells. Thus, our data suggest that octreotide therapy should be considered in patients with metastatic carcinoid if the tumor expresses SSTR2.

In conclusion, we have described a case of atypical thymic carcinoid associated with CS. Our data suggest that evaluation of SSTR2 expression is worthwhile in patients with aggressive and metastatic atypical carcinoid, as treatment with an SS analogue may be useful for control of tumors that express SSTR2.

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