Cushing’s Syndrome due to ectopic adrenocorticotropic hormone (ACTH or corticotropin) production (ectopic ACTH syndrome (EAS)) accounts for 10–20% of the cases of Cushing’s syndrome in western countries [1] and for 3.6% of the cases in Japan [2]. The leading cause of EAS is small cell lung carcinoma, accounting for about 50% of all tumors, and other is indolent tumors such as pancreatic, bronchial, and thymic tumors and thyroid medullary carcinoma.

Neuroendocrine tumors (NETs) of the gastro-entero-pancreatic system are rare, with an incidence of 1~2/100,000 individuals per year and are equally distributed between the sexes [3]. Among the pancreatic NETs (p-NETs), insulinoma (31.7%) and gastrinoma (8.6%) are the common islet cell tumors in Japan; less common ones are glucagonoma (4.9%), somatostatinoma (2.3%), and VIPoma (1.2%). There are also nonfunctioning islet neoplasms (47.7%) and other ectopic hormone (such as ACTH)-producing tumors (1.2%) [4]. These p-NETs are generally malignant ex-
cept insulinoma.

European Neuroendocrine Tumor Society (ENETS) and the World Health Organization (WHO) have proposed a strategy for the diagnosis and treatment of NETs. WHO has proposed the classification of NETs on the basis of criteria such as metastasis, Ki-67/MIB-1 index, histology, vascular invasion, and tumor size [5]. The WHO classification of NETs is as follows: (1) well-differentiated NETs (benign or uncertain), (2) well-differentiated neuroendocrine carcinoma, and (3) poorly differentiated neuroendocrine carcinoma, while the ENETS classification of NETs consists of 3 groups, termed G1, G2, and G3, also using Ki-67/MIB-1 index and the number of nuclear mitosis as the criteria, in addition to TNM classification for foregut NETs [6, 7].

EAS occurring from ACTH-producing p-NETs is particularly an aggressive disorder, and metastases is observed in the early phase of the clinical course even before the development of Cushing syndrome’s features [8]. The 2- and 5-year survival rates of patients with p-NET are reportedly 40% and 16%, respectively [9]. The ACTH-producing p-NETs metastasize most frequently to the liver, and the goal of treatment is reduction in the size and prevention of the growth of tumor and control of excessive ACTH and/or cortisol production.

In this study, we report the case of a female patient who presented with p-NETs developing EAS and multiple liver metastases. We treated her liver metastases by transarterial chemoembolization (TACE), administered somatostatin analogue for the stabilization of tumor growth, and metyrapone for controlling her cortisol level. These combined treatments have been successful so far in controlling the hormone levels and tumor size, and the patient has survived more than 20 months without serious complications.

Materials and Methods

Immunohistochemistry

We immunostained paraffin-embedded sections of liver metastatic biopsy samples with rabbit polyclonal anti-ACTH antibody (Dako, Glostrup, Denmark), chromogranin A (Dako), gastrin (Dako), glucagon (Dako), MIB-1 (Dako), somatostatin receptor (SSTR)-2a (Gene Tex, Irvine, CA, USA), using biotin-labeled anti-rabbit IgG antibody and the peroxidase-labeled streptavidin method [10]. Frozen sections of liver metastases were also immunostained with anti-SSTR-3 (Gene Tex) and SSTR-5 (Chemicon International, Inc. Billerica, MA), using Alexa fluor-488 or -546 (Molecular Probes, Inc., Eugene, OR, USA). We counterstained the samples with 4',6-diamidino-2-phenylindole (DAPI) after incubation with the secondary antibody.

Reverse transcription-polymerase chain reaction detection of SSTR subtypes, pro-opiomelanocortin, and gastrin

Total RNA was isolated from S5 and S8 liver metastases with standard protocol using the Rneasy mini extraction kit (QiAgen, Hilden, Germany), according to the manufacturer’s manual. A total of 100 ng of RNA was reverse transcribed using the Rivatra Ace reverse transcriptase (TOYOBO, Tokyo, Japan). The reaction mixture (2.5 µL) was used for polymerase chain reaction (PCR) amplification. cdnA was first denatured at 94°C for 10 min followed by PCR for 35 cycles each consisting of a denaturation step (94°C for 1 min), an annealing step (69°C for 1 min), and a primer extension step (72°C for 1 min). Finally, an elongation step (72°C for 10 min) was performed.

SSTR primer sets are shown below [11]; amplified sizes are described in parentheses:

- SSTR1-FW 5'-AGACGGCCACCAACATCTAC-3'
- SSTR1-RV 5'-GCACGTAGCACAGGCAGATA-3' (450 bp)
- SSTR2-FW 5'-GACAAGCAATGCAGTCCTCA-3'
- SSTR2-RV 5'-CTGTGTACCAAGCCCCAGAT-3' (500 bp)
- SSTR3-FW 5'-TCTGCTACCTGCTCATCGTG-3'
- SSTR3-RV 5'-TTGAAGCGGTAGGAGAGGAA-3' (290 bp)
- SSTR4-FW 5'-AAGCTCATCAACCTGGGCGTG-3'
- SSTR4-RV 5'-GGGTTCTGGTTGCAGGGCTTC-3' (635 bp)
- SSTR5-FW 5'-CTCTCTGGACCTTGTGCC-3'
- SSTR5-RV 5'-ACGAGCAAACAGGTACGCTT-3' (310 bp)

The following primer sets were used for the detection of pro-opiomelanocortin (POMC) and gastrin mRNA [12, 13].

- POMC gE for 5'-GAGGGCAAGCGCTCCTACTC-3'
- POMC gE rev 5'-GGGGCCCTCGTCCTTCTTCTC-3' (261 bp)
- POMC PX for 5'-CTACGGCGGTTTCATGACCT-3'
- POMC PX rev 5'-CCCTCACTCGCCCTTCTTG-3' (100 bp)
- GasAltF 5'-TGGAGCCACATGGTTCAGT-3'
- HGASL 5'-TCCATCCATCCATAGGCTTC-3' (360 bp); common primer for Alt and Ens
- GasEnsF 5'-CCACACACCTCCAGAGCTT-3' (300 bp)
- We used 2.0 µL reaction mixture for POMC and...
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Gastrin PCR amplification. cDNA was first denatured at 95°C for 5 min followed by PCR for 30 cycles each consisting of a denaturation step (95°C for 45 s), an annealing step (58°C for 1 min), and a primer extension step (72°C for 2 min). Finally, an elongation step (72°C for 7 min) was performed.

Case Report

A 64-year-old woman was referred to our hospital in July 2007. In 2001, she was incidentally diagnosed with a pancreatic tumor when computed tomography (CT) was performed. The tumor size was estimated to be about 20 × 20 mm.

Since then, the patient was healthy and did not show any symptoms, until she finally noticed facial plethora, generalized edema, and fatigue in 2007. She visited a general hospital, and a CT scan revealed a 30 × 25 mm pancreatic tumor and multiple liver metastases. Marked elevations of ACTH (735.0 pg/mL) and cortisol (34.7 µg/dL) with a hypokalemia (2.7 mEq/L) and hyperglycemia (postprandial blood glucose, 387 mg/dL) were observed. Insulin treatment was required for to control blood glucose levels (HbA1c 7.7% on regular insulin 32 units/day). The attending physician suspected pituitary Cushing disease with a possibility of EAS and referred the patient to our hospital.

The patient showed following features on physical examination upon admission: Cushingoid appearance with centropetal obesity (body mass index (BMI) = 29.4), moon face, facial plethora, abdominal skin striae, proximal muscle weakness, and psychotic symptoms, such as impaired memory and emotional lability. Blood pressure level was 162/90 mmHg.

Initial endocrine studies revealed high ACTH (340.0 pg/mL) and cortisol (26.9 µg/dL) levels, lack of circadian rhythm, and non-suppressibility by low-dose (1 mg) or high-dose (8 mg) dexamethasone administrations. Urinary excretion of free cortisol was 893 µg/day. ACTH and cortisol levels were high and did respond to a provocative test by 100 µg of corticotropin-releasing hormone (CRH). These data strongly suggested EAS rather than pituitary Cushing disease.

To assess the effectiveness of medications to suppress cortisol levels, metyrapone and octreotide loading tests were performed. Metyrapone suppressed cortisol levels sufficiently after an oral 250-mg administration up to 8 h (from 41.2 to 5.2 µg/dL at the nadir point), while an injection of 50 µg octreotide did not suppress them at all (from 48.9 to 46.8 µg/dL, nadir).

Fig. 1. Diagnostic imaging of ectopic ACTH syndrome
A: Primary pancreatic tumor, which irregularly stained 25 × 25 mm size, located at the pancreatic head (arrow) in an enhanced computed tomography (CT) scan.
B: Post-treatment CT scan indicated no changes in the primary pancreatic tumor.
C: Multiple liver metastatic lesions were visualized by enhanced CT.
D: Shrinkage of or diminishing metastatic liver tumors were detected in enhanced CT scan after the treatment
Fig. 2. Positron emission tomography scan of ectopic ACTH syndrome
Positron emission tomography (PET) scan displayed abnormal accumulations of fluorodeoxyglucose (FDG) in the pancreatic head (circled), distribution at multiple sites in the liver, and enlargement of both adrenal glands (arrows).

Fig. 3. ACTH levels in venous sampling
Schematic representation of the ACTH levels in the venous sampling test. The ratio represents plasma ACTH level, obtained from selective venous sampling, divided by the ACTH level of the distal IVC.

Fig. 4. Histopathological analyses of liver metastatic lesions.
Hematoxylin and eosin (HE) staining and immunohistochemical analysis of ACTH, chromogranin A (CGA), gastrin, glucagon, Ki-67/MIB-1, somatostatin receptor (SSTR)-2a, SSTR-3, and SSTR-5 of liver samples were performed.
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expression of ge isoform was greater in the S8 tumor, but that of PX was almost comparable between the S5 and S8 tumors (Fig. 5b). As for gastrin mRNA expression, ensemble transcript (Ens: 310 bp) and an alternative one (Alt: 360 bp) could be detected. The expression of both Alt and Ens transcripts were greater in the S5 than that in the S8 tumor (Fig. 5b). These data identified the heterogenic production of POMC and gastrin in metastatic liver tumors.

The treatment goal was initially aimed at 1) radical resection of the primary pancreatic endocrine carcinoma, 2) regression of the metastatic hepatic tumors, and 3) control of ectopic ACTH production and/or adrenal cortisol oversecretion. The surgical team, however, decided not to operate on the pancreatic tumor because of highly predicted post-operative complications, including hepatic abscess formation.

Since the tissues of the hepatic tumors expressed SSTR-5, which is one of the targets of the somatostatin analogue, we started treatment of octreotide long-acting release (LAR) (20 mg/month) in order to stabilize tumor cell growth and/or to decrease ectopic hormone production. Along with the somatostatin analogue injections, oral administration of metyrapone (750 mg/day) was started, which resulted in a remarkable decrease of plasma cortisol levels from 30~40 to 10~15 µg/dL. Then, TACE (cysplatin 100 mg + epirubicin hydrochloride 20-35 mg + lipiodol 2~3.5 mL) was initiated for the multiple hepatic tumors, with intervals of 4 to 6 weeks.

These combined treatments dramatically decreased
p-nET rarely produces ACTH with symptoms of Cushing’s syndrome. This EAS p-nET accounts for less than 1.2% of all p-nETs in the Japanese population [4], and it represents about 3.6% of the causes of EAS [2]. EAS-p-nETs are usually malignant, and liver metastases are often identified at the initial diagnosis [8]. Because of the faster progression of the disease, the prognosis of EAS-p-nETs is poor.

Our patient was diagnosed with a pancreatic tumor 6 years prior to obvious Cushingoid symptoms, suggesting that the tumor had little endocrine activity, at least at the time of initial diagnosis. When Cushing’s syndrome developed, the patient was found to have multiple hepatic tumors from the primary pancreatic tumor with the diagnosis of EAS-p-NET. Histopathology and immunostaining for ACTH and gastrin proved hepatic tumors to be compatible as ACTH- and gastrin-producing p-NET. The result of venous sampling suggested ACTH production by the liver tumors. We speculated that the metastatic tumors were the main sources of ACTH secretion causing EAS.

Our speculation was endorsed with a reported patient resembling our case [14]. The patient with as-

plasma ACTH and cortisol levels in 3 months (Fig. 6) and also normalized serum potassium concentrations; therefore, metyrapone and insulin doses were readily tapered. HbA1c levels went down from 7.8% using insulin injections (with acarbose 100 mg/day) to 5.5% without insulin therapy. Biochemical parameters reached steady state, and physical Cushingoid features and psychological complaints completely disappeared within 3 months. During the treatment, serum gastrin level decreased from 550 to 280 pg/mL, and glucagon was also reduced from 180 to 110 pg/mL. After discharge, the patient has continued to regularly visit our hospital for TACE and octreotide LAR injections and been well for more than 20 months. Serum ACTH and cortisol levels had been maintained in the normal range, and liver metastatic lesions have been reduced in number and size (Fig. 1D). The primary pancreatic tumor has remained because TACE against the pancreatic tumor has not been successful during this time (Fig. 1B). As for the remaining primary tumor in the pancreatic head, radical surgical treatment is currently being planned. Careful observation with TACE and octreotide LAR treatment has been continued.

Discussion

Clinical course of this patient during the treatments

Clinical management and the normalization of biochemical markers, such as ACTH and cortisol, were indicated up to 3 months of the clinical course.

Fig. 6. Clinical course of this patient during the treatments

Clinical management and the normalization of biochemical markers, such as ACTH and cortisol, were indicated up to 3 months of the clinical course.
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symptomatic p-NET, who underwent successful radical resection of the pancreatic tumor, developed overt Cushing’s syndrome and hepatic tumors after the operation [14]. The pancreatic tumor was negative for ACTH or cortisol staining, whereas hepatic metastases were strongly positive for ACTH [14]. There might have been some transformation in the histopathological and/or endocrinological nature of the pancreatic islet tumor cells when hepatic metastases occurred. In addition to the case report, our analysis of SSTR, POMC, and gastrin expression on metastatic tumors also indicated the possibility that such transformation might occur during the metastatic process of NETs, which may have the divergent characteristics. If so, careful examinations, including biopsies with immunohistochemical analyses, may be crucial for precise diagnosis and determination of treatment strategies.

Various therapeutic modalities have been tried for treating metastatic tumors, such as TACE [15], systemic chemotherapy [16], somatostatin analogues [17,18], interferon [19], and most recently total hepatectomy with living donor transplantation [20]. These efforts, however, seem to have limited effects. ACTH and/or cortisol normalization by tumor ablation, surgically or chemotherapeutically, is the most preferred treatment goal.

Octreotide, a somatostatin analogue, has been shown to decrease hormone secretion from a variety of NETs, such as pituitary adenomas and islet cell tumors [21,22]. Such inhibitory effects are mediated through SSTR subtypes 2, 3, and 5 [22]. In general, the inhibiting effect for hormone secretion is mainly mediated through SSTR-2, while apoptosis induction and tumor growth suppression are through SSTR-3 and -5 [22]. We began to treat the patient with octreotide LAR, expecting the effects to stabilize the tumor growth since SSTR-5 expression had been confirmed as far as examined. There was a report that the somatostatin analogue itself was effective in reducing ACTH levels in a similar EAS patient [18]. In the case, SSTR-2 and POMC mRNA were abundantly expressed in both primary pancreatic tumor and liver metastases [18].

Our treatment, combined with TACE and metyrapone administration, was almost completely successful, as shown in the clinical course (Fig. 6). Metyrapone suppressed cortisol synthesis so quickly that the patient’s general condition improved dramatically to endure stress by TACE therapy. It is essential to correct hypercortisolemia in EAS patients as quickly as possible because persisting hypercortisolemia often leads to aggravating complications, such as diabetes, hypertension, gastric ulcer, psychiatric disorders, and opportunistic infections. Fortunately for the patient, both ACTH and cortisol levels gradually decreased and metyrapone was successfully withdrawn after administration for 3 months (Fig. 6). During these 20 months of the treatment course, TACE (10 times) and octreotide LAR injections (15 times) have been given, both of which were considered to be the clue to successful normalization of ectopic ACTH production and stabilization of tumor growth. Although it is difficult to precisely evaluate the proportion of contributions by each treatment, the clinical course for more than 20 months has shown significant effectiveness of the treatment strategy with octreotide LAR, TACE, and metyrapone.

In conclusion, we have reported a patient with EAS-p-NET whose clinical course has been remarkably fine, indicating the usefulness of our diagnosis and treatment strategy. We would also like to emphasize the possibility of its changeable nature in the tumor growth or metastatic process, yielding diverse and heterogeneous primary and/or metastatic tumors. Even though NETs exhibit an indolent clinical course, many of them have malignant potential. Therefore, we should manage patients with NETs with highly individualized care.

References


