CASE REPORT

Multiple Endocrine Neoplasia Type I and Cushing’s Syndrome Due to an Aggressive ACTH Producing Thymic Carcinoid

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Abstract

Thymic carcinoid in multiple endocrine neoplasia type 1 (MEN 1) is previously reported as a non-ACTH producing tumor. The present case is a 39-year-old man with mortal outcome from thymic carcinoid and Cushing’s syndrome with high plasma ACTH. The symptom was first observed at age 29 and was relieved after extended thymectomy, with reduction of ACTH level. The tumor was positive for ACTH, Grimelius silver staining and Chromogranin A. The finding of primary hyperparathyroidism, pituitary adenoma, and a novel germline nonsense mutation (W423X) established the diagnosis of MEN 1. Cushing’s syndrome due to ACTH producing thymic carcinoid should be also considered as one phenotype of the MEN 1 spectrum.

Key words: Thymic carcinoid, MEN 1, Ectopic ACTH, Malignant, Cushing’s syndrome

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Introduction

Multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominantly inherited syndrome predisposing to tumor development in three principal glands, i.e. the parathyroids, the anterior pituitary, and the endocrine pancreas and duodenum. In addition, a range of other tumors have been reported to occur in MEN 1 patients, such as adrenal cortical tumors, angiofibroma, collagenoma, lipoma, carcinoids, leiomyoma and thyroid tumors (1). The disease gene, MEN1, is a tumor suppressor gene located on chromosome 11q13 (2), that encodes a 610 amino acid protein termed menin (3). Following its identification in 1997, mutation detection in classical MEN 1 have helped to clarify the mutation spectra and associated phenotypes. Perhaps the best example is that of thymic carcinoid, that in the last years has become clearly linked to the MEN 1 phenotype and is now regarded as a strong indicator of a genetic MEN 1 predisposition. Thymic carcinoid is a rare endocrine tumor originating from the foregut, and that is recognized in both sporadic and familial forms. In sporadic cases, carcinoid syndrome due to ectopic production of diverse neuroendocrine transmitters has been reported. However, MEN 1-related carcinoid rarely involves the carcinoid syndrome, and in particular, thymic carcinoid in MEN 1 with ectopic production of ACTH has not been previously reported. In this study we report a unique case of MEN 1 presenting with malignant thymic carcinoid producing ACTH, and resulting in Cushing’s syndrome.
Case Report

The reported patient is a Japanese man with a smoking history of 10 years. At 23 years of age he developed persistent urinary lithiasis and was five years later diagnosed with primary hyperparathyroidism (PHPT). Neck surgery was performed with removal of 3 1/2 parathyroid glands, the largest of which was the size of a thumb. His family history revealed that his mother had also been treated for PHPT. Soon after the operation at age 29, the patient gradually developed hypertension and moon face. Central obesity or other pathological finding was not seen. In the laboratory data, serum potassium was as low as 2.9 mEq/l. And serum
calcium was 5.6 mg/dl, which remained low after subtotal parathyroidectomy, and normalized two years later after the operation. There was no other remarkable abnormality, concerning the general laboratory data, including glucose. On admission for further investigation, a high level of plasma ACTH and serum cortisol were detected in agreement with Cushing’s syndrome (Table 1), and diagnostic imaging also showed complete absence of tumor foci. The patient did not undergo hormone replacement therapy, as the serum cortisol level was within the normal range after thymectomy. Postoperative treatments consisted of irradiation and chemotherapy with the combination of doxorubicin, cisplatin (CDDP) and etoposide. In addition, etoposide was administered for the following four years and ten months. At age 35, three months after the arbitrary withdrawal, enlargement of the left supraclavicular lymph node was noticed, and chest CT revealed a tumor recurrence in the pleural cavity. Serum neuron-specific enolase (NSE) was 13.0 ng/ml, which slightly increased. Although radiation therapy and chemotherapy with CDDP, VP, and methylprednisolone were introduced, the lesion was found to expand. Subsequent chemotherapy with CDDP, Adriamycin, vincristine, and capreomycin was also without effect. Plasma ACTH and serum cortisol levels gradually increased (Table 1), which exacerbated the Cushing’s syndrome. In spite of multiple chemotherapies, the disease progressed with expansion of the primary lesion and development of multiple metastases. As a conservative treatment subcutaneous injections of Octreotide (300 μg/day) were introduced. Plasma ACTH was decreased to 85 pg/ml at day 15 after initiation of Octreotide (Table 2). At day 27 of Octreotide injection, concentrations of serum cortisol and urine 17-OHCS were decreased, and the gradual restoration of muscle strength appeared (Table 2). Serum potassium was relatively low in the initial stage of Octreotide treatment, inclined to increase in the intermedial stage, and again decreased in the terminal stage. During this

Table 2  Laboratory and Clinical Findings After the Initiation of Subcutaneous Injection of Octreotide

<table>
<thead>
<tr>
<th>Laboratory parameter</th>
<th>Plasma</th>
<th>Serum</th>
<th>Urine</th>
<th>Muscle strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment period with octreotide (days)</td>
<td>ACTH (pg/ml)</td>
<td>Cortisol (μg/dl)</td>
<td>Sodium (mEq/l)</td>
<td>Potassium (mEq/l)</td>
</tr>
<tr>
<td>0</td>
<td>139</td>
<td>37.7</td>
<td>143</td>
<td>3.7</td>
</tr>
<tr>
<td>15</td>
<td>85</td>
<td>45.1</td>
<td>140</td>
<td>3.6</td>
</tr>
<tr>
<td>27</td>
<td>77</td>
<td>24.9</td>
<td>137</td>
<td>3.7</td>
</tr>
<tr>
<td>127</td>
<td>65</td>
<td>31.1</td>
<td>147</td>
<td>3.8</td>
</tr>
<tr>
<td>196</td>
<td>67</td>
<td>20</td>
<td>138</td>
<td>4.1</td>
</tr>
<tr>
<td>294</td>
<td>48</td>
<td>27.2</td>
<td>144</td>
<td>3.9</td>
</tr>
<tr>
<td>378</td>
<td>46</td>
<td>25.5</td>
<td>145</td>
<td>3.3</td>
</tr>
<tr>
<td>442</td>
<td>70</td>
<td>38.1</td>
<td>145</td>
<td>2.8</td>
</tr>
</tbody>
</table>

- : not determined; E: inability; A: ability.
treatment, at age 39, a pituitary tumor was detected by head MRI (Fig. 2). Two years later the patient died from multiple metastases of the thymic carcinoid and autopsy was not performed.

### Methods

Before the study informed consent was obtained from the patient in accordance with the guidelines of the institutional ethical committee.

**Histopathology and immunostaining**

Specimens of the primary mediastinal tumor were obtained at surgery at Fujita Medical University, embedded in paraffin and sectioned. Histopathological evaluation of the tumor morphology was performed on slides stained with HE. Immunostaining for argyrophilic cells was carried out with the Grimelius technique. Expression of chromogranin A and ACTH was analyzed by immunohistochemical detection with antibodies against human chromogranin A and adrenocorticotropic hormone (ACTH) 1-17 (Biogenesis England, UK) according to the protocol recommended by the manufacturer.

**MEN1 mutation detection**

Genomic DNA was extracted from peripheral leukocytes using a standard method. In brief, leukocytes were separated from whole peripheral blood using the Ficoll/Conray gradient method. Genomic DNA was extracted from obtained leukocytes by proteinase K digestion, Phenol-Chloroform extraction and ethanol precipitation. The MEN1 coding region, exons 2 to 10, were amplified as 15 different fragments by PCR and directly sequenced. Fourteen pairs of primers were according to Lemmens et al (4), and one pair of primers was designed for exon 2 and 5′-flanking intronic sequences: Forward, 5′-TGTGCGTGTGTCGGGGCGG-3′; Reverse, 5′-CCAGCTCGGCAGCAAACAGG-3′. In each reaction 100 ng of genomic DNA was amplified according to the manufacturer’s protocol (AmpliTaq gold™).

The thermocycling conditions consisted of an initial denaturation for 10 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 62°C (67°C for the new primer pair), and 30 sec at 72°C, and a final extension for 10 min at 72°C. The PCR products were then electrophoresed in 1.5% agarose gel, dissected as bands from the gel, and purified. Sequencing of both strands was performed using the Big Dye Terminator Cycle Sequencing kit (both Perkin-Elmer, Applied Biosystems) and an ABI PRISM 310 analyzer.

### Results

The patient reported here was clinically and genetically diagnosed with MEN 1. In addition to the typical findings of PHPT and prolactinoma he was diagnosed with Cush-
Cushing’s syndrome and a thymic carcinoid at age 29. Histopathological examination after HE staining of the thymic tumor demonstrated features typical of a thymic carcinoid including the formation of rosettes (Fig. 3a). Further analysis showed a positive reaction of the argyrophilic tumor cells with the Grimelius technique, (Fig. 3b) as well as examination of Chromogranin A by immunohistochemistry (Fig. 3c). These characteristic findings constituted the diagnosis of thymic carcinoid. In addition, the majority of tumor cells showed a positive expression of ACTH (Fig. 3d) by immunohistochemistry. This demonstrated a clinical diagnosis of Cushing’s syndrome due to ACTH production from the thymic carcinoid. Consequently, Cushing’s syndrome disappeared after initial surgical removal of the tumor, and re-occurred at tumor relapse (Table 1). Direct sequencing of the coding region of MEN1 revealed a heterozygous nonsense mutation in the patient’s constitutional DNA. As illustrated in Fig. 4, the mutation constitutes a single base substitution TGG -> TGA in exon 9. On the protein level this would correspond to an alteration of amino acid 423 from tryptophan to the introduction of a premature stop codon.

The mutation has not been previously reported in Japanese or in other MEN 1 patients. The detection of a constitutional MEN1 mutation confirmed the diagnosis of MEN 1 that was clinically considered based on the finding of PHPT, pituitary tumor and thymic carcinoid in the patient and the history of PHPT in his mother.

**Discussion**

In typical MEN 1, PHPT is the most common endocrinopathy observed, followed by endocrine pancreatic tumors and pituitary adenoma. The case reported here was diagnosed with PHPT at age 28 and pituitary adenoma at 39, following initial symptoms of these lesions for several years. In addition, the clinically most significant MEN 1 feature was a thymic carcinoid, which is found in only about 4% of MEN 1 patients (5).

On the other hand, MEN 1-related thymic carcinoid constitutes 25% of all thymic carcinoids (6), making it a strong indicator of MEN1 genetic screening, especially in the presence of any classical MEN 1 feature in the patient or his family.

Thymic carcinoid is a major cause of death in MEN 1 (7), and often shows local invasion, recurrence, and metastasis. In previous studies, thymic carcinoid was preferentially found in 40- to 50-year-old patients, with the youngest reported patient being diagnosed at 31 years of age (6). Recently, even younger cases with MEN 1-related thymic carcinoid have been described (8). Similarly the patient reported here was diagnosed at a relatively young age, 29 years, in spite of being the first identified MEN 1 case in this family. Furthermore, the aggressive features of the thymic carcinoid in this patient emphasize the need for early chest screening in MEN 1 patients.

In agreement with other reports of thymic carcinoid in smoking MEN 1 patients, the present case had a 10-year history of smoking.

Another remarkable feature of the present case is the production of ACTH by the MEN 1-related thymic carcinoid. The positive immunohistochemistry for ACTH, the prompt decrease of circulating levels of ACTH, cortisol, and the disappearance of Cushing’s syndrome after the initial extended thymectomy strongly supported the idea of a tumor producing ectopic ACTH. Among thymic carcinoids, there are various reports of ectopic production of ACTH (9), concomitantly with CRH (10), or with GHRH (11).

These cases are reported as sporadic, however, with some uncertainty due to the lack of genetic analyses.

Cushing’s syndrome due to ectopic ACTH secretion has been described as one of the endocrine symptoms in sporadic cases of thymic carcinoid (12). Furthermore, thymic carcinoid comprises 10% of the extrapituitary sources of ACTH resulting in Cushing’s syndrome (13). In contrast to the sporadic cases, thymic carcinoid encountered in MEN 1 has not been reported to present as Cushing’s or carcinoid syndromes, and MEN 1-related thymic carcinoid in particu-
lar has been described to be non-ACTH producing (14). Gibril et al also reported that thymic carcinoid with MEN1 is hormonally inactive, and rarely accompanied by excessive hormone (15). It is supposed that the lack of hormonal excess contributes to the fact that thymic carcinoid is insidious. And the consensus is that thymic carcinoid is not the initial manifestation of MEN 1, due to the late onset (15).

We described that MEN 1-related thymic carcinoid with ectopic ACTH production appeared as the first manifestation of MEN 1. The reported case here underlines the need to consider all variants of thymic carcinoid as putative components of the MEN 1 phenotype. Since the identification of the MEN1 gene in 1997 constitutional mutations, mostly different, have been described in a few hundred probands. In the present case, a novel nonsense mutation W423X was detected. Diverse MEN1 germline mutations (8, 16), have previously been described in MEN 1-related thymic carcinoid patients.

Almost all of these mutations are of the truncating type, however located in different parts of the gene. As in thymic carcinoid with ectopic hormone production, a GHRH producing tumor with germline mutation Q209X has been described (17)). However, the reported number of MEN 1-related thymic carcinoids with ectopic hormone production is rare. Moreover, ACTH producing MEN 1-related thymic carcinoid has not been previously described. Thus, ACTH-producing thymic carcinoid should be considered as another phenotype of MEN 1 for further research.

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References


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