Small-cell Carcinoma of the Endometrium Presenting as Cushing's Syndrome

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Abstract. Small-cell carcinoma (SCC) of neuroendocrine type is an uncommon tumor of the endometrium. No previous report has documented Cushing's syndrome due to ectopic ACTH production by SCC of the endometrium. We describe a 56-year-old Japanese woman with SCC of the endometrium and multiple lung metastases presenting as Cushing's syndrome. The patient was referred to our hospital because of general fatigue with facial and leg edema, and multiple nodular lesions in the bilateral lungs on chest X-ray examination. A physical examination revealed that the patient had moon face, buffalo hump, and truncal obesity. Endocrinological examinations confirmed ACTH-dependent Cushing's syndrome. Thoracic computed tomography imaging showed multiple nodular lesions in the bilateral lungs. Abdominal magnetic resonance imaging suggested a malignant tumor of the uterus. The patient received a lung tumor biopsy and surgical hysterectomy. The endometrial carcinoma was histologically a SCC admixed with endometrioid adenocarcinoma. The SCC of the endometrium showed immunoreactivity for pro-opiomelanocortin, ACTH, and vimentin, but not for thyroid transcription factor-1. The lung biopsy specimen had the same features. These findings indicated that the SCC originated from the endometrium, and the ectopic ACTH-producing tumor caused Cushing's syndrome. This study provides the evidence that SCC of endometrial origin was an ectopic ACTH-producing tumor causing Cushing's syndrome.

Key words: Small-cell carcinoma, Endometrium, Ectopic ACTH production, Cushing's syndrome

SMALL-CELL carcinoma (SCC) of the endometrium is a tumor resembling SCC of the lung, but arising in the endometrium [1]. SCCs are aggressive tumors that can occur throughout the female genital tract; the uterine cervix is the region most commonly affected, whereas endometrial involvement is rare [2].

A variable proportion of cervical SCCs exhibits evidence of neuroendocrine differentiation. Cervical SCCs can be associated with Cushing's syndrome due to ectopic ACTH production [3, 4]. However, no previous report has documented Cushing's syndrome due to ectopic ACTH production by a SCC of the endometrium. Here, we describe a case of a SCC of the endometrium causing Cushing's syndrome due to ectopic ACTH production.

Patient and Methods

Case report

A 56-year-old Japanese woman consulted a local clinic because of a two-month history of general fatigue with facial and leg edema. A chest X-ray showed multiple nodular lesions in the bilateral lungs, and therefore the patient was referred to our hospital. A physical examination revealed that the patient had hypertension (167/95 mmHg), facial and leg edema, and features of Cushing's syndrome such as moon face, buffalo hump, and truncal obesity. Her medical history included a right oophorectomy at the age of 20 years. Menarche had occurred at the age of 13 years, and until menopause at the age of 53 years, her menses had been regular with a 28-day cycle and a flow lasting five days. The patient was gravida 2, para 2.

Results of the basal endocrinological examination are summarized in Table 1. Laboratory tests performed at 0800 h showed a plasma ACTH level of 234

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Table 1. Basal endocrinological values

	basal values	normal range
Plasma ACTH at 0800 h (pg/mL)	234	7.4-55.7
Serum cortisol at 0800 h (μg/dL)	54.0	4.0-18.3
Serum DHEAS (ng/mL)	2730	80-1880
PRA (ng/mL/h)	0.7	0.3-5.4
Upright plasma aldosterone (pg/mL)	68	39-307
Blood serotonin (ng/mL)	60	57-230
TSH (μU/mL)	0.028	0.30-4.00
Free T4 (ng/dL)	0.79	0.75-1.75
Urinary free cortisol (μg/day)	1940	11.2-80.3
Urinary 17-OHCS (mg/day)	46.1	2.2-7.3
Urinary 17-KS (mg/day)	35.0	2.4-11.0
Urinary metanephrine (mg/day)	0.05	0.05-0.23
Urinary normetanephrine (mg/day)	0.18	0.07-0.26
Urinary VMA (mg/day)	4.2	1.3-5.1
Urinary 5-HIAA (mg/day)	4.3	1.0-6.0

DHEAS, dehydroepiandrosterone sulfate; PRA, plasma renin activity; T4, thyroxine; 17-OHCS, 17-hydroxycorticosteroids; 17-KS, 17-ketosteroids; VMA, vanillylmandelic acid; 5-HIAA, 5-hydroxyindole acetic acid.

pg/mL (normal range, 7.4-55.7), a serum cortisol level of 54.0 μg/dL (normal range, 4.0-18.3), a serum dehydroepiandrosterone sulfate (DHEAS) level of 2730 ng/mL (normal range, 80-1880), plasma renin activity level of 0.7 ng/mL/h (normal range, 0.3-5.4), upright plasma aldosterone level of 68 pg/mL (normal range, 39-307), blood serotonin level of 60 ng/mL (normal range, 57-230), TSH level of 0.028 μU/mL (normal range, 0.30-4.00), and free thyroxine (T4) level of 0.79 ng/dL (0.75-1.75). The urinary free cortisol level was 1940 μg/day (normal range, 11.2-80.3). The urinary 17-hydroxycorticosteroids level was 46.1 mg/day (normal range, 2.2-7.3). The urinary 17-ketosteroids level was 35.0 mg/day (normal range, 2.4-11.0). Levels of urinary fractionated catecholamines were revealed as follows: urinary metanephrine 0.05 mg/day (normal range, 0.05-0.23), urinary normetanephrine 0.18 mg/day (normal range, 0.07-0.26), and urinary vanillylmandelic acid 4.2 mg/day (normal range, 1.3-5.1). The urinary 5-hydroxyindole acetic acid level was 4.3 mg/day (normal range, 1.0-6.0). These results indicated ACTH-dependent hypercortisolism. A circadian rhythm analysis showed that her serum cortisol level at 0000 h was 61.0 μg/dL (Table 2). These results suggested that the normal plasma ACTH level

Table 2. Circadian rhythms of ACTH and cortisol

Clock time	0000 h	0600 h	1600 h
Plasma ACTH (pg/mL)	155	140	199
Serum cortisol (μg/dL)	61.0	56.0	57.0

Table 3. Overnight low-dose (1 mg) dexamethasone suppression test

	value	normal response
Serum cortisol (μg/dL)	69.2	<5

Table 4. Overnight high-dose (8 mg) dexamethasone suppression test

	value	normal response
Serum cortisol (μg/dL)	83.0	<50% of baseline in Cushing's disease

Table 5. CRH stimulation test

	basal value	30 min	60 min	120 min
Plasma ACTH (pg/mL)	151	154	147	142

Normal response, ACTH increase >50% over baseline value

and cortisol circadian rhythm had been lost because serum cortisol level is highest early in the morning and reaches a nadir at around midnight in normal subjects. After an overnight low-dose (1 mg) dexamethasone suppression test, her serum cortisol level was 69.2 μg/dL (normal response, <5) at 0800 h (Table 3). After an overnight high-dose (8 mg) dexamethasone suppression test, the serum cortisol level was 83.0 μg/dL (less than 50% of baseline in Cushing's disease) at 0800 h (Table 4). A human CRH stimulation test (100 μg, i.v.) showed that her peak ACTH level was 154 pg/mL, suggesting no plasma ACTH response (normal response, ACTH increase >50% over baseline value) (Table 5).

Magnetic resonance imaging (MRI) of the pituitary gland revealed no pituitary adenoma. These findings supported a diagnosis of Cushing's syndrome caused by an ectopic ACTH-producing tumor. Thoracic computed tomography (CT) imaging showed multiple nodular lesions in the bilateral lungs (Fig. 1A). Abdominal MRI suggested a malignant tumor of the uterus, and the patient was diagnosed as FIGO stage IVb. Subsequently, she was referred to the departments of thoracic surgery and gynecology for evaluation of the lung lesions and malignant tumor of the

uterus, respectively. Video-assisted thoracoscopic surgery (VATS) to evaluate the lung lesions and hysterectomy with right salpingectomy and left salpingo-oophorectomy were performed. The uterine body contained a mass lesion, which was a solid whitish nodular mass measuring 45×32 mm, and showed endophytic growth (Fig. 1B).

The postoperative course was uneventful and the patient received chemotherapy including cisplatin and etoposide, but the levels of plasma ACTH and serum cortisol remained high. The patient was orally administered metyrapone, which inhibits 11β -hydroxylase and, consequently, her serum cortisol level decreased to the normal range. Thereafter, the physical features of Cushing's syndrome gradually ameliorated. However, the patient died of respiratory failure 3 years after hysterectomy.

Immunohistochemistry

The specimens were fixed in 10% buffered formalin, processed, and embedded in paraffin. Four- μ m-thick sections were stained with hematoxylin and eosin. Antigen retrieval was performed using an autoclave (10 min at 121°C in 10 mM citrate buffer). Immunohistochemical staining was performed for pro-opiomelanocortin (POMC) (mouse monoclonal 3A6, 1:50, Biogenesis, Poole, UK), prohormone convertases 1/3 (PC1/3) (rabbit polyclonal, 1:500, established in our laboratory) [5], ACTH (rabbit polyclonal, ready-to-use, DAKO, Kyoto, Japan), glucagon (rabbit polyclonal, 1:200, DAKO), insulin (rabbit polyclonal, 1:100, DAKO), calcitonin (rabbit polyclonal, 1:200, DAKO), serotonin (mouse monoclonal 5HT-H209, 1:10, DAKO), somatostatin (rabbit polyclonal, 1:300, DAKO), pancreatic polypeptide (PP) (rabbit polyclonal, 1:1000, DAKO), gastrin (rabbit polyclonal, 1:500, DAKO), gastrin-releasing peptide (rabbit polyclonal, 1:200, DAKO), erythropoietin (EPO) (goat polyclonal, N-19, 1:50, Santa Cruz Biotechnology, Inc. California, USA), parathyroid hormone (PTH) (goat polyclonal, N-18, 1:50, Santa Cruz Biotechnology Inc.), vimentin (mouse monoclonal V9, 1:100, DAKO), and thyroid transcription factor-1 (TTF-1) (mouse monoclonal SPT24, 1:100, Novocastra, Newcastle, UK).

The investigation was performed in accordance with the principles of the Declaration of Helsinki and approved by the institutional review board committee

Fig. 1

A, Thoracic computed tomography image showing multiple nodular lesions in the bilateral lungs.

B, Macroscopic appearance of the surgical specimen. Arrows pointing to a solitary tumor of the endometrium measuring 45×32 mm.

at Tokai University School of Medicine.

Results

Microscopic features

The endometrial tumor cells were comprised of two morphological components. Most were poorly differentiated and intermediate in size, forming sheets and nests with small, round, and hyperchromatic nuclei, indistinct nucleoli, and scanty cytoplasm with a high nucleus/cytoplasm ratio (Fig. 2A). The other component was characterized by some glandular or villoglandular structures (Fig. 2B). These findings were compatible with SCC of the endometrium admixed with endometrioid adenocarcinoma. Tumor cells did not show Crooke's hyaline change.

The tumor cells in the lung possessed the same microscopic features as those in the endometrium. The crashed spindle shape of the nuclei appeared to be a

Fig. 2

A, Hematoxylin and eosin (H&E)-stained section of the small-cell carcinoma component showing that the tumor cells are poorly differentiated, small to intermediate in size, forming sheets and nests, and containing small, round hyperchromatic nuclei with indistinct nucleoli and scanty cytoplasm; B, H&E-stained section of the adenocarcinoma component showing that the other tumor component is an endometrioid adenocarcinoma characterized by the presence of some glandular or villoglandular structures. Original magnification $\times 40$.

VATS artifact (Fig. 5A).

Immunohistochemistry

Both the SCC and endometrioid adenocarcinoma of the endometrium were immunoreactive for POMC, PC1/3, ACTH, and glucagon (Fig. 3A–H). Immunoreactivities for insulin, calcitonin, serotonin, somatostatin, PP, gastrin, GRP, EPO, and PTH were negative in both the SCC and endometrioid adenocarcinoma of the endometrium (not shown).

Immunoreactivity for vimentin was positive in both the SCC (Fig. 4A) and endometrioid adenocarcinoma of the endometrium (Fig. 4B). Immunoreactivity for TTF-1 was negative in both the SCC (Fig. 4C) and endometrioid adenocarcinoma (Fig. 4D) of the endometrium. Both the SCC and endometrioid adenocarcinoma of the lung were immunoreactive for POMC, PC1/3, ACTH, glucagon, and vimentin (not shown).

The SCC of the lung was not immunoreactive for TTF-1; however, nuclei of the alveolar epithelial cells (internal positive control) showed immunoreactivity for TTF-1 (Fig. 5B).

Discussion

The following diagnostic criteria for SCC of the endometrium have been proposed: 1) unequivocal evidence of endometrial origin, 2) dense sheet-like growth of morphologically similar small to intermediate-sized tumor cells in standard hematoxylin and eosin-stained sections, and 3) immunohistochemical

staining for one or more neuroendocrine markers [6]. Furthermore, it is possible for SCC of the endometrium to be combined with endometrioid adenocarcinoma [1, 2]. Therefore, the present case satisfied the criteria for SCC of the endometrium admixed with endometrioid adenocarcinoma.

The lung is a common site of metastatic disease as well as a primary site for SCC. Because SCC of the endometrium can be combined with endometrioid adenocarcinoma and SCC of the lung can be combined with adenocarcinoma [7], it was clinically important in the present case to determine whether the endometrium or the lung was the primary site of the SCC.

The tumor in the endometrium was macroscopically solitary, whereas CT imaging revealed multiple tumors in the lung, suggesting that the latter were metastases. It has been reported that both SCC and endometrioid adenocarcinoma of the endometrium express vimentin [1, 8, 9]. In contrast, vimentin is expressed in adenocarcinoma of the lung, but not in SCC of the lung [10]. In the present case, the SCC components in both the endometrium and lung expressed vimentin. TTF-1 is widely used in the diagnosis of thyroid and lung carcinomas [11, 12]. In the present case, both the SCC and endometrioid adenocarcinoma components showed no TTF-1 immunoreactivity. These findings suggested that the endometrium, and not the lung, was the primary site of the SCC.

On initial physical examination, the patient showed features typical of Cushing's syndrome such as moon face, buffalo hump, and truncal obesity. Because the MRI revealed no pituitary adenoma, the overnight high-dose dexamethasone suppression test failed to

Fig. 3

Immunohistochemistry stains of the small-cell carcinoma are positive for pro-opiomelanocortin (POMC) (A), prohormone convertase 1/3 (PC 1/3) (C), ACTH (E), and glucagon (G). Immunohistochemistry stains of the adenocarcinoma component are positive for POMC (B), PC 1/3 (D), ACTH (F), and glucagon (H). Original magnification $\times 40$.

Fig. 4

Immunohistochemistry stain of vimentin was positive in both the small-cell carcinoma (A) and the adenocarcinoma component (B). But immunohistochemistry stain of thyroid transcript factor-1 was negative in both the small-cell carcinoma (C) and the adenocarcinoma component (D). Original magnification $\times 40$.

Fig. 5

- A, H&E-stained section showing metastatic small-cell carcinoma of the lung. The crashed shape of the nuclei appears to be an artifact due to biopsy. Original magnification $\times 40$.
- B, The small-cell carcinoma showing no immunoreactivity for thyroid transcript factor-1 (TTF-1). Nuclei of alveolar epithelial cells (internal positive control) showing immunoreactivity for TTF-1 (arrows). Original magnification $\times 40$.

suppress serum cortisol and plasma ACTH did not respond to CRH, the results were compatible with ACTH-dependent Cushing's syndrome due to an ectopic ACTH-producing tumor. The SCC of the endometrium showed immunohistochemical staining for ACTH, suggesting that it was responsible for ectopic ACTH production and causing Cushing's syndrome.

ACTH is synthesized as a precursor molecule, POMC. In the human pituitary, POMC is enzymatically processed to pro-ACTH, N-terminal POMC (N-POC), β -lipotropic hormone, ACTH (39 amino-acid residues), and β -endorphin. Processing of POMC in the pituitary takes place by PC 1/3 located within mature dense core granules via the regulated secretory pathway [13, 14]. Furthermore, it was reported that expression of PC1/3 was investigated in various endocrine tumors [15]. In contrast, it was indicated that aberrant processing of POMC might be observed in ectopic ACTH producing tumors [16]. Immunohistochemical analysis in this study revealed that immunostaining for POMC, PC1/3, and ACTH were positive in the tumor cells. These data suggested that the tumor cells produced POMC with cleavage to ACTH by PC1/3. In addition, it was suggested that ACTH produced by the tumor had biological activity

because ACTH caused ACTH-dependent Cushing's syndrome in the patient.

The endometrioid adenocarcinoma component expressed POMC and ACTH. POMC is primarily synthesized in the pituitary gland, but its transcripts and ACTH-related peptides are detected in some tumors not associated with ectopic ACTH syndrome, and even in normal tissues as well [17–20]. Endometrioid adenocarcinoma may express ACTH [21], which would provide a possible rationale for the tumor cells of the

endometrioid adenocarcinoma being responsible for ectopic ACTH production in the event of dysregulation of POMC transcription.

In conclusion, this is the first report describing a SCC of the endometrium that was responsible for ectopic production and secretion of ACTH, resulting in Cushing's syndrome. SCC of the endometrium can be one site for ectopic ACTH production when POMC transcription is dysregulated.

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