□ **CASE REPORT** □

Feminizing Adrenocortical Carcinoma with Distinct Histopathological Findings

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Abstract

We herein present a 60-year-old man with adrenocortical carcinoma who had gynecomastia. An endocrinological examination revealed increased levels of serum estradiol and dehydroepiandrosterone-sulfate (DHEA-S) and reduced levels of free testosterone. Magnetic resonance imaging showed an adrenal tumor with heterogeneous intensity. Iodine-131 adosterol scintigraphy showed an increased uptake at the same site. Because feminizing adrenocortical carcinoma was suspected, right adrenalectomy was performed; the pathological diagnosis was adrenocortical carcinoma. The results of immunostaining indicated a virilizing tumor. Aromatase activity was identified on RT-PCR. As disorganized steroidogenesis is pathologically present in adrenocortical carcinoma, this diagnosis should be made with caution.

Key words: adrenocortical carcinoma, aromatase, estrogen-producing tumor, gynecomastia, testosterone

Introduction

Adrenocortical carcinomas are rare tumors, with estrogen-producing feminizing adrenocortical carcinomas being even rarer among hormone-producing tumors, with an incidence of 2% among all adrenocortical carcinomas (1, 2). A normal adrenal gland has little aromatase activity (aromatase converts androgen to estrogen). In contrast, in feminizing adrenocortical carcinoma, oncogenesis leads to aromatase expression and induces estrogen synthesis, resulting in feminization in men.

We herein present a case of feminizing adrenocortical carcinoma that was clinically an estrogen-producing tumor and pathologically a testosterone-producing tumor.

This study design was approved by our hospital ethics committee, and informed consent was obtained from the patient.

Real-time quantitative RT-PCR was performed with the use of fluorescence SYBR green technology (Light Cycler; Roche Molecular Biochemics, Manheim, Germany), and

quantitative measurement of P450 aromatase was performed. Total RNA was extracted from the excised tumor, and cDNA was quantitated. The PCR primers used are shown in Table 1. Real-time quantitative RT-PCR was also performed for a control group including the ovaries, normal adrenal cortex, and adrenal cortex obtained from patients with Cushing syndrome and the adrenal medulla obtained from pheochromocytoma.

Case Report

A 60-year-old man presented with a 3-year history of gynecomastia and right hypochondriac pain. He visited a physician and received famotidine treatment with a diagnosis of gastroenteritis, without relief. The following year, he visited another physician, was found to have a 13 cm right adrenal tumor on abdominal computed tomography (CT), and was referred to our center. At presentation, his body height was 163.7 cm, body weight was 70.7 kg, and body mass index was 26.4 kg/m^2. His blood pressure was 110/60 mmHg and pulse rate was 64 beats per minute, which were normal.

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Table 1. PCR Primer Sets for the Determination of Aromatase Promoter.

	Sense	Antisense
Exon II-III coding region	GACTCTAAATTGCCCCCTCTG	CAGAGATCCAGACTCGCATGA
Exon I.3-specific	CCTTGTTTTGACTTGTAACCA	CAGAGATCCAGACTCGCATGA
Promoter II-specific	AACAGGAGCTATAGATGAAC	CAGAGATCCAGACTCGCATGA

Table 2. Pre- and Postoperative Endocrinological Data.

	pre	post	reference ranges
ACTH (pg/mL)	8.7	25.6	7.2 - 63.3
Cortisole (μg/dL)	10.3	13.1	2.3 - 19.4
DHEA-S (μg/dL)	560	42	38.0 - 313.0
Estradiol (pg/mL)	284	17	15.0 - 35.0
F-testosterone (pg/mL)	<0.6	0.257	5.4 - 16.7
LH (mIU/mL)	0.1	3.6	2.2 - 8.4
FSH (mIU/mL)	0.2	5.4	1.8 - 12.0
PRA (ng/mL·hr)	1.1	0.7	0.3 - 2.9
Aldosterone (pg/mL)	110.0	37.4	35.7 - 240.0

Table 3. Diurnal Rhythm and Dexamethasone Suppression Test (1 mg and 8 mg).

		8 :00	14 :00	23 :00	1 mg	8 mg
ACTH	(pg/mL)	8.7	10.1	2.5	<2.0	<2.0
Cortisol	(μg/dL)	10.3	6.9	6.2	7.9	7.3

Table 4. Pre- and Postoperative Urinary Steroid Profile.

urinary metabolites(Ms)	pre	post	reference ranges
Σpregnenolone Ms[1]	18.858	0.026	0.097 - 0.422
progesterone M[2]	3.223	0.151	0.084 - 0.371
DOC M[3]	0.016	0.004	0.002 - 0.007
Σcorticosterone Ms[4]	0.074	0.257	0.206 - 0.646
aldosterone M[5]	0.012	0.010	0.003 - 0.026
Σ17OHpregnenolone Ms[6]	65.045	0.163	0.049 - 0.246
Σ17OHprogesteone Ms[7]	9.745	0.585	0.333 - 1.272
11-deoxycortisol M[8]	1.990	0.075	0.035 - 0.198
Σcortisol Ms[9]	9.120	6.183	4.739 - 11.343
ΣDHEA Ms[10]	65.092	0.380	0.065 - 1.313
Σandrostenedione Ms[11]	5.492	1.209	0.751 - 2.583
Σestrogen Ms[12]	0.955	0.001	0.001 - 0.015

1) pregnenolone,3β,20α-pregnenediol, 2) 3α,20α-pregnanediol, 3) tetrahydro-11-deoxycorticosterone, 4) 5β-tetrahydrocorticosterone, 5α-tetrahydrocorticosterone, 5β-tetrahydro-11-dehydrocorticosterone, 5α-tetrahydro-11-dehydrocorticosterone, 5) 5β-tetrahydroaldosterone, 6) 17α-hydroxypregnenolone, 3β,17α,20α-pregnenetriol, 7) 5β-17α-hydroxypregnanolone, 5α-17α-hydroxypregnanolone, 5β-3α,17α,20β-pregnanetriol,5β-3α,17α,20α-pregnanetriol, 5α-3α,17α,20α-pregnanetriol, 8) 5β-tetrahydro-11-deoxycortisol, 9) cortisol, 6β-hydroxycortisol, 5β-tetrahydrocortisol, 5α-tetrahydrocortisol, 20α-cortol, 20β-cortol, 20α-dihydrocortisol, 20β-dihydrocortisol, cortisone, 5β-tetrahydrocortisone, 5α-tetrahydrocortisone, 20α-cortolone, 20β-cortolone, 5α-20β-cortolone, 20α-dihydrocortisone, 20β-dihydrocortisone, 10) DHEA, 3β,17β-androstenediol, 16α-hydroxy-DHEA, 16β-hydroxy-DHEA, 16-oxo-androstenediol, 3β,16α-17β-androstenetriol, 11) androsterone, etiocholanolone, 12) estrone, estradiol, estriol.

There were no abnormalities in his breath or heart sounds. Palpation revealed a tumor in the right hypochondriac region, but no superficial lymph nodes. The patient did not have any Cushingoid features such as moon face, central obesity, or buffalo hump. A gynecomastia was detected in the breast, corresponding to Tanner developmental stage 2 with concomitant libido decrement. There was no notable medical history, however, the patient's mother had valvular heart disease. He drank socially and never smoked.

The complete blood cell count was within the normal range. There were no abnormalities in the levels of electrolytes or blood glucose. An endocrinological examination revealed elevated levels of DHEA-S and estradiol, and reduced levels of testosterone, LH, and FSH (Table 2). Considering the hypothalamo-pituitary-adrenal axis, the circadian rhythm of cortisol was lost, and no cortisol suppression was identified on the low-dose (1 mg) and high-dose (8 mg) dexamethasone suppression tests (Table 3).

The corticosteroid profile from 24-hour urine collection (Table 4) revealed increased levels of pregnenolone, progesterone, deoxycorticosterone, 17-hydroxyprogesterone, androstenedione, and estrogen, suggesting an adrenal tumor with reduced activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 11-β-hydroxylase.

Abdominal CT and magnetic resonance imaging showed a massive tumor, measuring 16×11×14 cm in size, suggesting an intratumoral hemorrhage and necrosis. There was no radiological evidence indicating metastasis to the liver, right kidney, or surrounding tissue. Iodine-131 adosterol scintigraphy revealed an uptake at the site corresponding to the right adrenal gland and suppression on the left side (Fig. 1).

Adrenocortical carcinoma was suspected according to the endocrinological and imaging studies, and right adrenalectomy was performed. After surgery, the blood levels of estradiol and DHEA-S were reduced, and levels of free testosterone were elevated (Table 2). The urinary steroid profile was improved (Table 4).

Postsurgical adjuvant therapy was not performed, because of the patient's refusal, until 11 months after surgery, when metastases to the locoregional lymph nodes and peritoneum and increased levels of estradiol were observed. In addition,
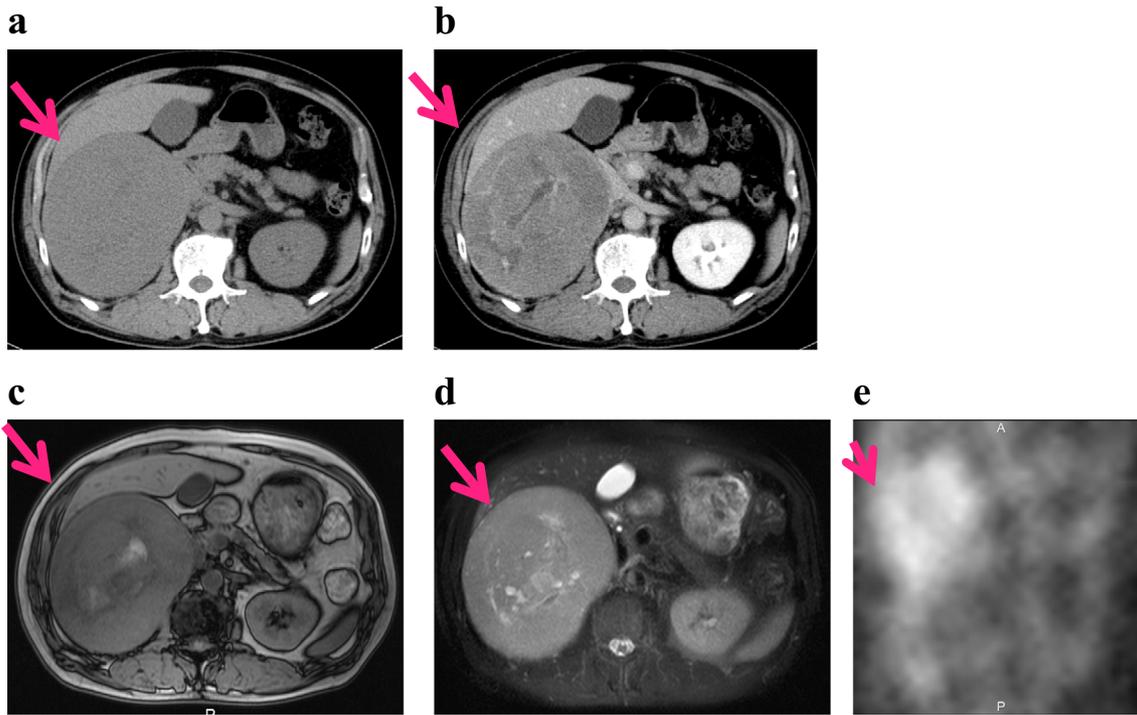
Figure 1. **(a) Computed tomography (CT) showed a heterogeneous mass measuring approximately 16×14×11 cm between the right kidney and liver. (b) Enhanced CT showed that the adrenal gland was unevenly stained. (c) T1-weighted magnetic resonance imaging (MRI) showed high-signal intensity within the tumor, indicating necrosis and bleeding. (d) T2-weighted MRI showed high-signal intensity within the entire tumor. (e) I-131 adosterol scintigraphy showed an increased uptake corresponding to the right adrenal gland, with suppression on the other side.**

the fluorodeoxyglucose uptake was shown on fluorodeoxyglucose-positron emission tomography at the locoregional lymph nodes and peritoneum. Mitotane (o,p'-DDD) was orally administered according to the diagnosis of metastases. After the initiation of oral mitotane, the metastatic lesions shrunk and the estradiol level decreased. Treatment with etoposide, doxorubicin, and cisplatin (i.e., the EDP regimen) is planned in the case of tumor regrowth.

Pathology

The resected tumor measured 13×12 cm in diameter and was yellow-tan in color. On pathological examination, a solid tumor was found adjacent to the normal adrenal gland, with cellular atypia. A thick capsule was present at the rim of the tumor. The tumor included cellular components with a clear cytoplasm forming a"Zellballen"structure, and cells with eosinophilic abundant cytoplasm were associated with the thin fibrotic vascular interstitium (p0, T2, N0, M0) (Fig. 2).

As shown in Fig. 2, the tumor satisfied seven of nine items in the Weiss criteria: (1) nuclear atypia, (2) mitosis, (3) capsular invasion, (4) confluent necrosis, (5) cytoplasm, (6) architecture, and (7) sinusoidal invasion, indicating that the adrenal tumor might be malignant. The adrenal tissue was positive for SF-1, suggesting that the tumor originated from the adrenal cortex. The Ki67-labeling index was 18% in the hot spot, indicating an extremely high-grade malig-

nant tumor. Regarding steroidogenic enzymes, a disorganized steroidogenesis pattern was observed, without the expression of aromatase, 17-β hydroxysteroid dehydrogenase 1 (17-β-HSD1), STS, or 5-alpha-reductase 1 in most tumor cells, and with the expression of STS 5-alpha-reductase 2 in some parts of the tumor cells. The diffuse expression of 17-β hydroxysteroid dehydrogenase 5(17-β-HSD5) was present in the tumor, pathologically indicating a testosterone-producing tumor (Fig. 3).

RT-PCR

Real-time quantitative RT-PCR for exons was performed to identify the expression of exon II, promoter II, and exon I.3 (Fig. 4). In the tested ovary, the expression of exon II, promoter II, and exon I.3 was positive. Laboratory samples from the control group, including the normal adrenal cortex, and those obtained from patients with Cushing syndrome were weakly positive. The tested pheochromocytoma was negative. In contrast, samples from the patient showed mRNA levels higher than that of the control group.

In addition, upon measuring the actual steroid production of the adrenocortical carcinoma tissue, the estrogen level was relatively higher than the testosterone level (Table 5).

Therefore, adrenocortical carcinoma in this patient was considered to have an aromatase activity.
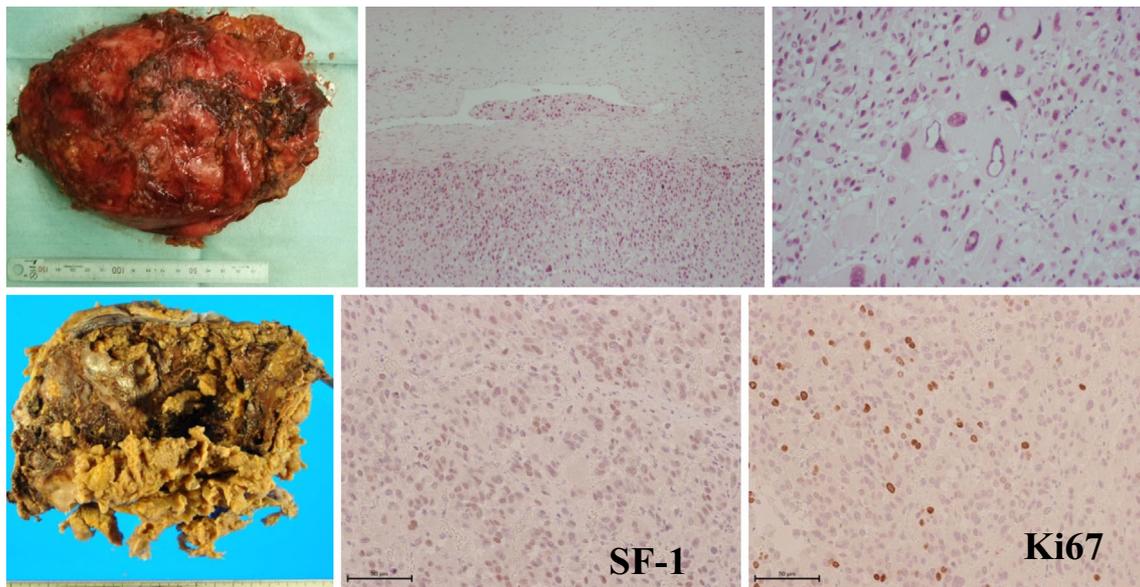
Figure 2. **The left upper panel shows the excised adrenal gland. The left lower panel shows a section of the adrenal gland. The middle upper panel shows Hematoxylin and Eosin (H&E) staining of the adrenal gland (400×), indicating vascular invasion. The middle lower panel shows immunostaining with an antibody against SF-1. The right upper panel shows H&E staining of the adrenal gland (400×). The right lower panel shows immunostaining with an antibody against Ki67; the Ki67 labeling index of the hot spot was 18%.**
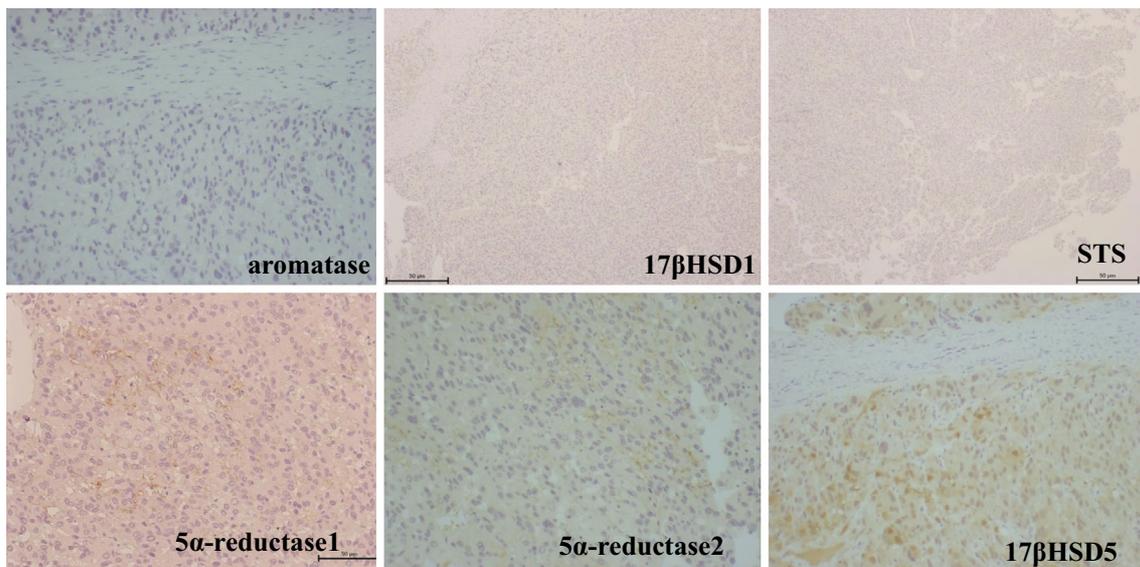
Figure 3. **A steroidogenic enzyme pattern indicating disorganized steroidogenesis. Aromatase, 17βHSD1, STS, and 5α-reductase 1 were not expressed in most tumor cells. However, STS and 5α-reductase 2 were expressed in some tumor cells. 17βHSD5 was diffusely expressed in all tumor cells. The immunohistological studies strongly suggested the production of testosterone by the adrenal tumor.**

Discussion

Adrenocortical carcinoma is a rare tumor with an incidence of 0.5-2 cases per million persons, while accounting for only approximately 0.2% of total cancer deaths (2-5).

Adrenocortical carcinoma is classified into hormonally functional and nonfunctional tumors, and approximately 60% of patients have clinical symptoms caused by excessive steroidenesis (6). Major symptoms with excessive hormone secretion include Cushing syndrome due to excess cortisol (50%), virilization due to excessive androgen (34%), and the combination thereof (11%); however, feminization is rare (2-6%) (7, 8).
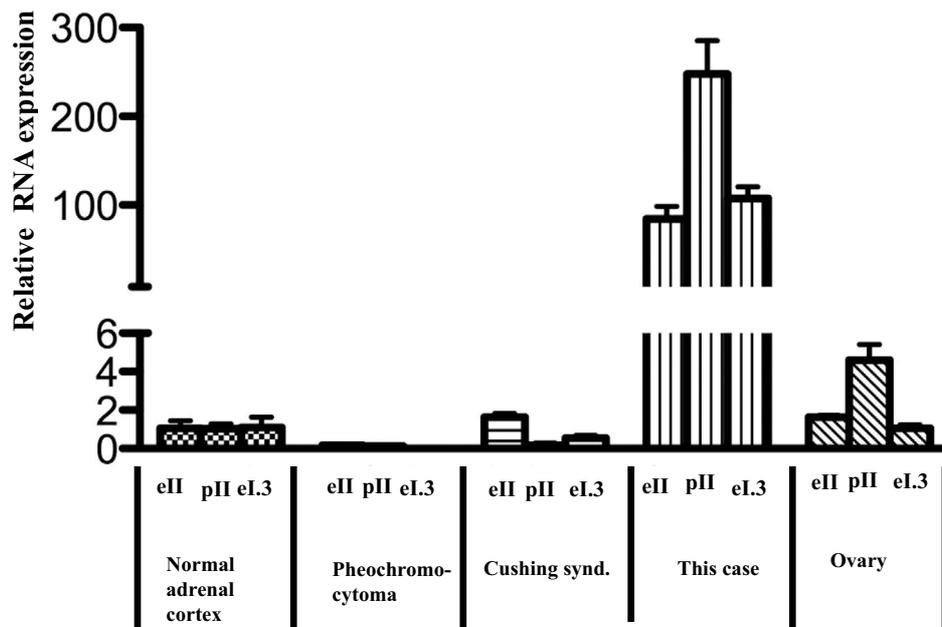
Figure 4. RT-PCR demonstrated the expression of exon II, promoter II, and exon I.3 for each sample. eII: exon II, pII: promoter II, eI.3: exon I.3

Table 5. Steroid Contents of Tumor Tissue.
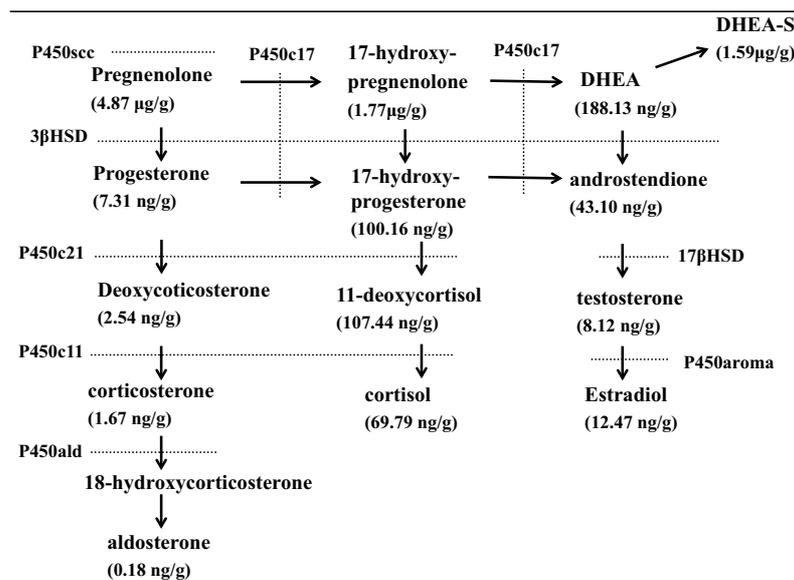
Two possible mechanisms have been reported by which the blood level of estrogen is elevated in feminizing adrenocortical carcinoma. Androgen is converted to estrogen by aromatase. Aromatase is highly expressed in granular layer cells of the developing follicle and syncytium cells of the placenta, and there is very little aromatase activity in the normal adrenal gland (9). Therefore, androgen produced in adrenal carcinoma was previously considered to be converted to estrogen in the peripheral tissues including adipose tissues (10). This is similar to the fact that when high doses of anabolic steroids are administered to men, the conversion of excessive androgen to estrogen is promoted in the peripheral tissues, leading to gynecomastia (11, 12), as reported in doping cases. However, in recent years, aromatase activity has been reported in feminizing adrenocortical carcinoma, and a study showed that the adrenal tumor itself converts androgen to estrogen (13). Normally, the aromatase expression in human tissues is partially regulated by tissue-specific promoters. They are known to be located in exon I.1 in the placenta, exon I.3 and promoter II in the ovaries, and promoter I.4 in adipose tissues (9). However, some studies have shown that the tumor cells of feminizing adrenocortical carcinoma have exon II, promoter II, and exon I.3 and aromatase activity and show intratumoral estrogen production (14, 15). Advani et al. described the presence of promoter II in feminizing adrenocortical caricoma (16).

Preoperative urinary steroid profiling revealed reduced levels of decreased 3β-HSD in addition to decreased 11β-hydroxylase.

In general, if the activity of 3β-HSD decreases, then transformation from DHEA to androstenedione becomes difficult, and consequently the estrogen production decreases.

However, since progesterone, 17-OH progesterone and androstenedione levels were high, and pregnenolone, 17-OH pregnenolone and DHEA levels were also abnormally high, we speculated that this patient did not have sufficient 3β-HSD amounts to metabolize to progesterone, 17-OH progesterone or androstenedione in a proportion similar to normal adrenal gland. That is, it was thought to indicate a relatively low 3β-HSD activity.

In addition, according to the fact that the levels of metabolites of androstenedione were twice the reference range upper limit and a high concentration of estrogen was also found, we speculated that 3β-HSD produced substantial androstenedione metabolites, and was further metabolized to estrogen.

Furthermore, in addition to a decrease in activity of 21-hydroxylase in feminizing adrenocortical carcinoma, a decrease in the activity of 11β-hydroxylase has been noted (17-19).

According to the above findings, we suspected estrogen-producing feminizing adrenocortical carcinoma in this patient. In contrast, no aromatase expression was observed in the adrenal tissue according to the pathological findings, while the expression of 17βHSD5 was observed.

Hence, this patient was diagnosed with a testosterone-secreting tumor. Furthermore, these findings raised the possibility that excessive androgen produced in adrenocortical carcinoma was metabolized to estrogen in the peripheral tissues.

Due to the discrepancy between the clinical and histopathological data, we examined the presence of mRNA for aromatase in the adrenal tumor by RT-PCR in order to confirm the conversion to estrogen in adrenocortical carcinoma. RT-PCR revealed the expression of promoter II and exon I.3 in the patient's adrenocortical carcinoma. Therefore, estrogen production was suspected in adrenocortical carcinoma of this patient.

In addition, as shown in Table 5, intratumoral steroids revealed a relatively higher level of estrogen than testosterone. Moreover, when examining the aromatase activity in tissues, the stable isotope 13C3-T was used as the substrate and purified 13C3-E2 was quantified in order to distinguish the originally present endogenous E2.

Incubation of 500 ng of 13C3-T with 25.4 mg equivalent weight of tissue homogenate at 37℃ for 2 hours (2 mg of NADPH added) was found to form 430.16 pg of 13C3-E2.

This, on converting to per gram of tissue weight, becomes 16.935 ng/g.

When the said measurement conditions are used, the amount of 13C-E2 formed corresponds to about 100 ng/g in rat ovary and about several dozen pg/g in human fat, which

is known to have an aromatase activity.

Additionally, when tissue devoid of any aromatase activity is used, it is below the quantification limit.

Therefore, this value of 16.935 ng/g is considered to confirm a fairly strong aromatase activity, although it is somewhat less than that of the at ovary.

For the pathological analysis, we used immunohistochemistry to analyze the expression pattern of steroidogenic enzymes. This method is used to show elevated synthesis/secretion of steroid hormones in the adrenal cortex (20).

In adrenocortical adenomas, due to the systematic expression of steroidogenic enzymes, a homogeneous expression of steroidogenic enzymes is observed on immunohistochemistry. In contrast, in adrenocortical carcinomas, enzymes are heterogeneously expressed and various intermediate metabolites are secreted. Thus, on immunohistochemistry, all the steroidogenic enzymes are not homogeneously expressed in cells from adrenocortical carcinoma, but the differential expression of 3β-HSD, P450-HSD, and DHEAS-T is observed (13, 20). This heterogeneous expression of steroidogenic enzymes is referred to as "steroid disorganization" (21). Sasano et al. reported a case of adrenocortical carcinoma with Cushing syndrome, but were unable to detect steroidogenic enzymes on immunohistochemistry (22). Another study showed that, even among the cases of adrenocortical carcinoma expressing the same steroidogenic enzymes, the blood/urinary steroid profiles varied in each case. Some cases of adrenocortical carcinoma do not result in clinical symptom even if steroids are synthesized, in which each tumor cell synthesizes steroids at levels below the measurement sensitivity. This type of steroid-producing tumor sometimes exhibits symptoms as the tumor becomes larger (1, 22), which is known to result from the heterogeneous expression of steroidogenic enzymes in the cells of adrenocortical carcinoma. Because immunohistochemistry is incapable of detecting all tumor cells, the expression of steroidogenic enzymes differs at the protein and mRNA levels (22), i.e., in some cases, even if aromatase is negative on the pathological analysis, immunohistochemistry may not detect it when each cell expresses a very small amount. Thus, immunohistochemistry does not detect all the cells of adrenocortical carcinoma, which may lead to a discrepancy between the results of immunohistochemistry and the clinical features. Hence, the expression of steroidogenic enzymes differs from the clinical features depending on the portion of the tumor analyzed by immunohistochemistry.

In this patient, the aromatase expression in the tumor was presumably heterogeneous; thus, an extremely low expression of aromatase in the portion subjected to the pathological analysis resulted in a discrepancy from the clinical features. As the promoter II and exon I.3 expression was detectable in the adrenal tumor by RT-PCR, measuring the levels of aromatase expression in the whole tumor by RT-PCR is useful for the diagnosis in order to correct the discrepancy between the pathological results and clinical features.

Immunohistochemistry showed a testosterone-producing

tumor, which differed from the clinical features. This was presumably caused by steroid disorganization at the site analyzed pathologically. Due to heterogeneity of steroid synthesis in the adrenocortical carcinoma, the expression of steroidogenic enzymes may not be consistent with the clinical data, depending on the portions screened during the pathological analysis.

In this case, we observed the expression of promoter II and exon I.3 in the adrenal tumor, which presumably induced estrogen to cause feminization. As there are many discrepancies between the clinical symptoms and pathological features on a functional analysis of adrenocortical carcinoma, thorough endocrine testing is thus necessary before surgery.

The authors state that they have no Conflict of Interest (COI).

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