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Malignant Adrenal Rest Tumor of the Retroperitoneum Producing Adrenocortical Steroids

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Abstract We present a case of a malignant adrenal rest tumor arising from the retroperitoneum with Cushing's syndrome in a 31-year-old female. Her serum cortisol and dehydroepiandrosterone sulfate levels were elevated, while adrenocorticotropic hormone levels were low. Computed tomography scans and magnetic resonance imaging revealed a retroperitoneal tumor with no visible lesions in the adrenal glands and ovaries. From those results and the histopathologic findings following biopsy of an enlarged supraclavicular lymph node, the patient was diagnosed as a malignant adrenal rest tumor of the retroperitoneum. Despite chemotherapy, the patient died of rapid development of multiple metastases. Autopsy revealed a large tumor that extended around the abdominal aorta from the levels of the left kidney to the aortic bifurcation with generalized metastases. Tumor cells were characterized by clear and eosinophilic cytoplasm and atypical nuclei that exhibited frequent and atypical mitoses. Immunohistochemistry regarding steroidogenesis was performed and revealed that the tumor cells were immunopositive for adrenal 4 binding protein/steroidogenic factor-1, cholesterol side-chain cleavage enzyme, 17α-hydroxylase, and 21-hydroxylase. We thus elucidated the adrenocortical steroid production in the tumor cells causing Cushing's syndrome. This case report first demonstrates the steroidogenic capacity in a malignant adrenal rest tumor.

Keywords Adrenal rest tumor · Adrenocortical steroidogenesis · Cushing's syndrome · Immunohistochemistry

Introduction

Although adrenal rest tissue is not infrequent in fetuses and infants (about 10%), adrenal rest tumor is rare [1]. Adrenal rest tumors can occur anywhere along the path of embryonic adrenal migration, such as in the kidney, periadrenal and periaortic fatty tissue, ovary, uterus, broad ligament, spermatic cord, testis, and epididymis [1, 2]. Additional sites include the pancreas, liver, transverse colon, celiac-plexus area, retroperitoneum, placenta, gallbladder, and spinal cord [1–3]. The adrenal rest rarely persists until adulthood as it generally undergoes atrophy and disappears within a few years after birth [2]; however, a neoplasm can arise from the adrenal rest. Their malignant counterpart, however, is extremely rare, and only a few sporadic cases have been reported [2–12].

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We present here the case of a malignant adrenal rest tumor of the retroperitoneum with the symptoms of Cushing's syndrome. In addition to conventional histopathologic procedures, we confirmed adrenocortical steroidogenic activity by immunohistochemistry using adrenal 4 binding protein/steroidogenic factor-1 (Ad4BP/SF-1), cholesterol side-chain cleavage enzyme (P450scc), 17α-hydroxylase ($P450c_{17}$), 3β-hydroxysteroid dehydrogenase (3β-HSD), 21-hydroxylase ($P450c_{21}$), and dehydroepiandrosterone sulfotransferase (DHEA-ST).

Case Report

A 31-year-old woman was admitted because of Cushing's syndrome with signs including acne on the face and trunk, a moon face, leg edema, hirsutism, and mild muscle weakness of the lower extremities. Pigmentation in the face and trunk was absent, and there was no blood pressure elevation. In addition, palpable swelling of a supraclavicular lymph node was found. Computed tomography (CT) scans and magnetic resonance imaging (MRI) demonstrated swelling of supraclavicular lymph nodes 3 cm in diameter and a large retroperitoneal mass along the abdominal aorta, although neither adrenal gland showed abnormalities (Fig. 1a, b). The right ovary exhibited small cystic lesions, but there was no neoplastic lesion. The levels of serum cortisol [30 μg/dl (normal value, 4.0–18.3 μg/dl)] and dehydroepiandrosterone sulfate [DHEA-S, 1,060 μg/dl (normal value, 45–270 μg/dl)] were elevated with low levels of serum adrenocorticotropic hormone (ACTH, <2.0 pg/ml), and then, the absence of a circadian rhythm in serum cortisol concentrations and unresponsiveness of serum cortisol concentrations to the administration of dexamethasone (DEX) were identified.

Open biopsy of the left supraclavicular lymph node was performed. On the basis of the endocrinological, radiological, and histopathologic findings, the patient was diagnosed as having a malignant adrenal rest tumor of the retroperitoneum with Cushing's syndrome. Despite extensive chemotherapy for 1 month, rapid development of multiple metastases occurred. The patient consequently suffered multiorgan insufficiency and died of this condition at 16 months after admission.

Materials and Methods

Tissues were fixed in 10% formalin and cut into 4-mm slices. They were embedded in paraffin, cut into 3-μm-thick sections, and stained with hematoxylin–eosin (H&E). Ultrastructural studies of biopsied supraclavicular lymph node tissue were performed on glutaraldehyde-fixed samples routinely processed, epon embedded, and stained with uranyl acetate and lead citrate using a JEM-1400C transmission electron microscope (JEOL, Tokyo, Japan). For immunohistochemistry of autopsied tissues, after deparaffinization, all the sections were treated with 3% hydrogen peroxide in water for 5 min to block endogeneous peroxide. They were then pretreated in citrate buffer (10 mmol/L, pH 6.0) at 95°C for 10 min in a microwave for antigen retrieval. Rabbit anti-α-inhibin monoclonal antibody (mAb; R1, 1:50 dilution, DakoCytomation; Carpinteria, CA, USA), anti-Ki67 mAb (MIB1, 1:100 dilution, DakoCytomation), anti-NSE mAb (BBS/NC/VI-H14, 1:100 dilution, DakoCytomation), and CD31 mAb (JC/70A, 1:100 dilution, DakoCytomation) antibodies were then used for immunostaining. In addition, to examine steroidogenesis, the sections were further immunostained with anti-P450scc polyclonal antibody (pAb) [13] (1:1,000 dilution), 3β-HSD pAb [14] (1:1,000 dilution), $P450c_{21}$ pAb [15] (1:15,000 dilution), $P450c_{17}$ pAb [16] (1:1,000 dilution), DHEA-ST pAb [17] (1:1,000 dilution), and Ad4BP/SF-1 pAb [18–20] (1:500 dilution). Next, sections were incubated with respective antibodies at 4°C overnight and processed with an EnVision+ kit (DakoCytomation) or Histfine kit (Nichirei Co. Ltd., Tokyo, Japan) in accordance with the manufacturer's instructions. Immunoproducts were visualized using diaminobenzidine tetrahydrochloride, and the sections were counterstained with hematoxylin.

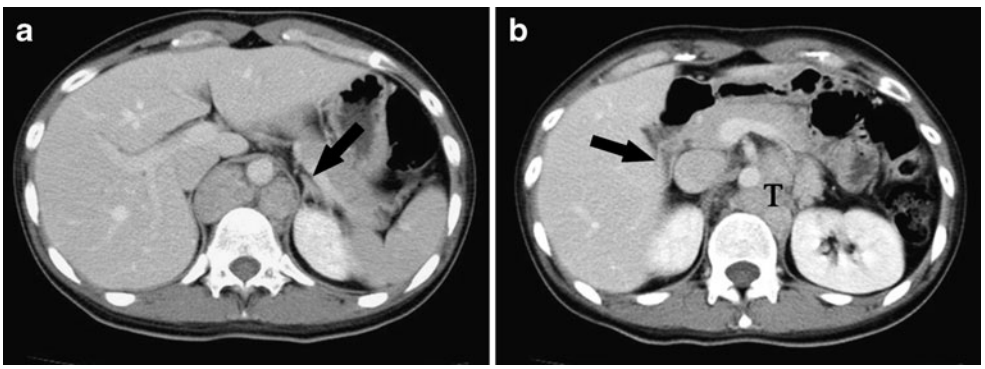

Fig. 1 Abdominal computed tomography scan showing a retroperitoneal tumor around the abdominal aorta (*T*) at the time of admission. The left (**a**, *arrow*) and right (**b**, *arrow*) adrenal glands have no primary tumor and are not involved by the retroperitoneal tumor

Results

Biopsy of Supraclavicular Lymph Node

Macroscopically, the tumor was yellowish and friable with hemorrhage. Histopathologically, it exhibited a uniform distribution of atypical cells that had clear and eosinophilic cytoplasm and large round nuclei (Fig. 2a). Mitotic figures were frequently observed. Ultrastructurally, the tumor cells were polygonal with cytoplasm that included well-developed smooth endoplasmic reticulum, abundant mitochondria and lipid droplets, and large irregular nuclei (Fig. 2b). Moreover, developed rough endoplasmic reticulum and microvilli on the tumor cell surface were also evident. Secretory granules were not found. These findings by light and electron microscopy were considered characteristic of steroid-producing cells.

Autopsy

A large irregular mass was readily found below the left kidney and extended to the level of the aortic bifurcation. The tumor measuring 25×16×12 cm was multinodular and fused together with the aorta, inferior vena cava, jejunum, pancreatic tail, and bilateral ureters. It was difficult to identify precisely the primary site within the retroperitoneal mass. The tumor mass varied from light yellowish to grayish and had a lobular architecture. Some areas of the tumor were friable and necrotic. The right adrenal gland located in the normal anatomical site was atrophic (4.5 g) and was not invaded by the tumor. In addition, although the left adrenal gland was invaded by the tumor (Fig. 3a), the atrophic cortex was noticeably unaffected, with the tumor invasion presenting as many small nodules located mainly in the medulla and around the cortex. The lesions were considered to be metastases to the adrenal cortex by venous invasion from the retroperitoneal tumor. Metastases were also found in the liver, lung, bilateral ovaries, and bone marrow and were disseminated in the pericardium with pleural and peritoneal effusion. Metastases were present in both ovaries, but not externally including the broad ligament of the uterus. The large mass of the retroperitoneum exerted pressure directly on the right ureter, and an abscess was present in the right renal pelvis.

Tumor cells were predominantly large and uniform in size with large round nuclei and were mixed with various bizarre cells (Fig. 3b). The cytoplasm was not only clear and vacuolated but also eosinophilic. Mitotic figures were frequent, and some atypical mitoses were also found. MIB1-positive nuclei were numerous in the tumor cells (about 10%). Tumor architecture was primarily diffuse, but varied sporadically with cord and nest formations. There were many necrotic and fibrotic areas that were partially regressive possibly induced by chemotherapy. In and around the tumor, venous and lymphatic invasion was frequently noted. The lesions of the left adrenal gland comprised mainly venous invasion of the medulla, and the atrophic cortex remained. Lesions of the left adrenal gland were therefore considered metastatic lesions. In the liver and lung, tumor cells not only formed nodular lesions but also filled the sinusoids and blood vessels. By immunohistochemistry, tumor cells were diffusely positive for α-inhibin, NSE, Ad4BP/SF-1 (Fig. 3c), P450scc, and $P450c_{21}$ (Fig. 3d). Immunopositivity of Ad4BP/SF-1 was revealed in the cytoplasm and nucleus of the tumor cells, and immunopositivity for the others was found only in the cytoplasm. In addition, $P450c_{17}$-positive cells were focally scattered in the tumor, but DHEA-ST and 3β-HSD were not expressed. The immunopositivity of all metastatic lesions in the ovaries was the same as that of the retroperitoneal tumor.

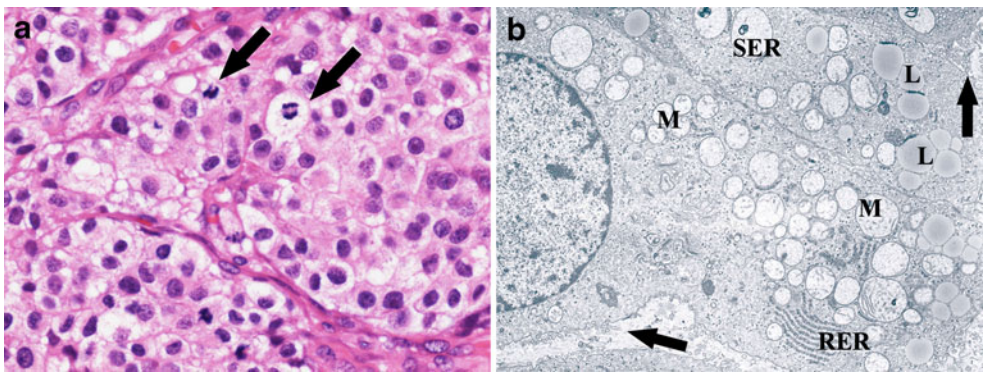

Fig. 2 **a** Histopathologic findings of biopsied supraclavicular lymph node (H&E stain). The tumor cells that have clear and eosinophilic cytoplasm with atypical large nuclei exhibiting mainly a diffuse growth pattern. Mitotic figures are frequently found (*arrows*). **b** Electron microscopic features of tumor cells. The tumor cell has cytoplasm that contains abundant smooth endoplasmic reticulum (*SER*), mitochondria (*M*), lipid droplets (*L*), and well-developed rough endoplasmic reticulum (*RER*). At the surface of tumor cells, microvilli are well developed (*arrows*)

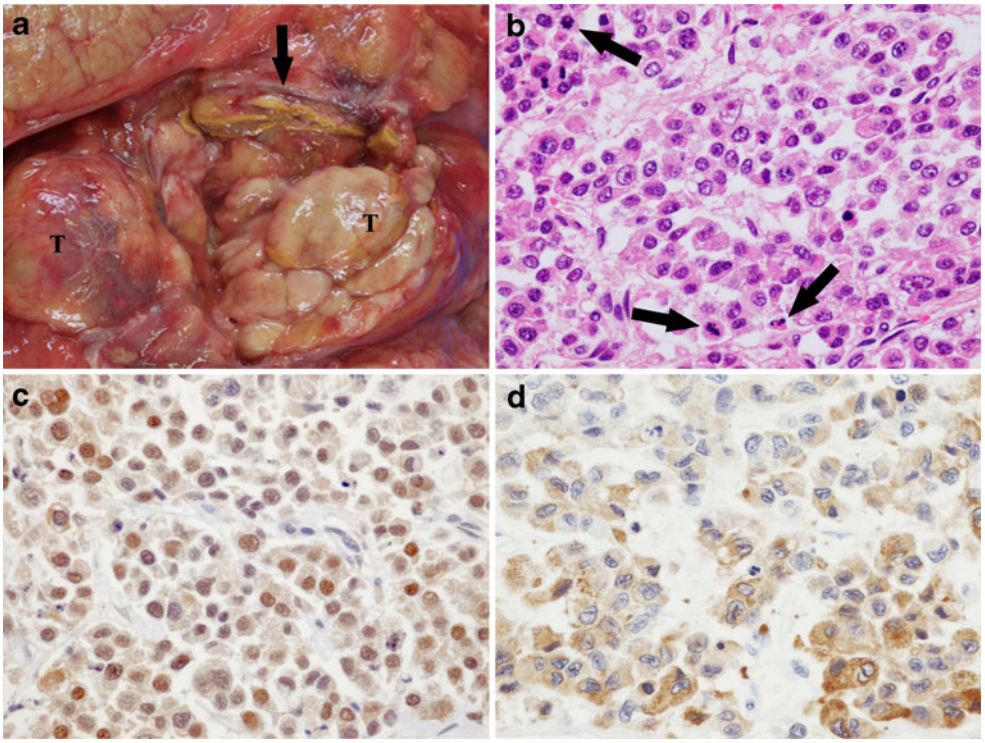

Fig. 3 **a** Macroscopic findings at autopsy. The large tumor (*T*) is present around the left adrenal gland (*arrow*), but the adrenal cortex is free from the tumor. **b** Histopathologic findings of tumor tissue at autopsy (H&E stain). The tumor cells have conspicuous atypical nuclei with marked nucleoli and diffusely extended. Mitotic figures are present (*arrows*). **c**, **d** Immunohistochemistry for Ad4BP (**c**) and P450c$_{21}$ (**d**). The immunopositivity of Ad4BP is observed in the nuclei and cytoplasm. The expressions of P450c$_{21}$ are found diffusely in the cytoplasm of the tumor cells

Discussion

In the present study, the patient exhibited typical symptoms of Cushing's syndrome with high serum cortisol and DHEA-S levels, loss of cortisol circadian rhythm, and unsuppressed serum cortisol levels following DEX administration. By CT and MRI, a large retroperitoneal tumor metastasizing to a supraclavicular lymph node was found without evidence of primary adrenocortical and ovarian lesions. From these results and the immunohistochemical analysis designed to elucidate adrenocortical steroid hormone production, the tumor was considered as a malignant bioactive adrenal rest tumor of the retroperitoneum. However, at autopsy, the left adrenal cortex was partially involved by the tumor, and therefore, it is possible that the tumor may have arisen from an extra-adrenal nodule. To our knowledge, there have been so far only four case reports in which steroidogenic capability was found in an adrenal rest tumor [7, 21–23]. The present case, however, is the first to confirm steroidogenic capability in a malignant tumor by immunohistochemistry.

Here, tumor cells showed remarkable atypia, but they retained the characteristics of the adrenal cortex in light and electron microscopic findings. Furthermore, clinical data and histopathologic findings circumstantially suggested that the tumor produced steroid hormones. However, the tumor's steroidogenic capability became directly evident following immunohistochemical staining. Tumor cells were immunopositive for Ad4BP/SF-1, P450scc, P450c$_{21}$, and partial expression of P450c$_{17}$. Ad4BP is a pivotal transcription factor for all steroidogenesis and is demonstrated in most tumor cells of adrenocortical carcinoma [18]. However, it is also present in other organs including the gonads [20]. P450scc catalyzes the conversion of cholesterol to prognenolone, the initial and rate-limiting step in steroidogenesis in the adrenal gland [13]. P450c$_{17}$, 3β-HSD, and P450c$_{21}$ are also key enzymes in the steroidogenic pathway. P450c$_{17}$ is responsible for the synthesis of glucocorticoids and sex hormones, and is produced only in the adrenal cortex and gonads [16]. DHEA-ST catalyzes the conversion of dehydroepiandrosterone to DHEA-S in the adrenals [17]. DHEA-ST and P450c$_{21}$ are expressed in the adrenal cortex, but not in the gonads [15, 17]. In the present case, adrenocortical steroidogenesis of the tumor was conclusively confirmed by the immunoreactivity of P450c$_{21}$, but DHEA-ST was not expressed in the tumor tissue at autopsy. Although serum DHEA-S level was elevated from the first admission, the discrepancy between serum DHEA-S elevation and immunohistochemical results for DHEA-ST suggested that tumor cell damage may have resulted from chemotherapy or postmortem autolytic change. In addition, the immunoreactivity of tumor cells in the ovary was consistent with that in the retroperitoneum. Taken together, our immunohistochemical results demonstrated that the tumor produced the adrenocortical steroids. Furthermore, we excluded the possibility of metastasis arising from the gonadal tumor.

The criteria proposed by Weiss [24, 25] are currently the most widely employed in the distinction between benign and malignant adrenal cortical tumors, using multiple histopathologic parameters [26]. It has also been reported that a MIB1 labeling index of >2.5% is suggestive of adrenocortical carcinoma [26]. Whether these criteria can be applied to adrenal rest tumors has never been assessed. In the present case, supraclavicular lymph node metastasis was found from the time of first admission confirming malignancy, and the histopathologic findings including the MIB1 index (about 10%) were consistent with a malignant diagnosis according to Weiss.

To our knowledge, only 11 cases of malignant adrenal rest tumors (with the exception of the present case) have been so far reported [2–12]. In these cases, the primary sites of malignant adrenal rest tumor were the testis, liver, retroperitoneum, kidney, and spinal cord, and seven cases showed hormonal symptoms. The primary tumor in the three deceased cases was considered highly malignant arising in the testis. Three out of 11 tumors were consistent with the diagnosis of malignancy by only tumor size and weight [2, 8, 12], and malignancy in the other case appears to be determined only on the basis of extensive mitoses [11]. Moreover, in one case that arose from the testis, the authors themselves stated that the possibility of a malignant Leydig cell tumor with ectopic cortisol production could not be fully ruled out. Therefore, the immunohistochemical examinations for $P450c_{21}$ and DHEA-ST are necessary to distinguish adrenocortical tumor from gonadal tumor.

To conclude, we present a case of a malignant adrenal rest tumor of the retroperitoneum and provided a brief literature review. The clinical and various histopathologic findings elucidated the autonomous secretion of cortisol and associated hormones and enzymes from the tumor cells. As adrenal rest tumor ubiquitously arises along a developmental and migration pathway extending from the gonads to the upper retroperitoneal space, immunohistochemical examination for adrenocortical steroidogenesis is useful to discriminate the adrenal rest tumor from other candidate steroid-producing tumors.

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