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CASE REPORT

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Small cell neuroendocrine carcinoma of the ovary

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Abstract A 64-year-old woman (gravida 0, para 0) had a unilateral ovarian mass measuring 14 cm in its greatest diameter, which was mostly solid. Microscopically, the tumour was characterized by two predominant proliferating patterns: a carcinoid-like pattern with trabecular, tubular, glandular, or insular arrangements and a closely packed nesting pattern with central coagulation necrosis and occasional glandular arrangements. These two patterns were intermingled, and numerous mitotic figures were present. Electron microscopy showed neurosecretory granules in the cells, which were argyrophilic and positive for neuroendocrine markers (chromogranin, leu 7, neuron-specific enolase, and synaptophysin). The tumour was aneuploid by flow cytometry. The patient received chemotherapy postoperatively, developed brain and multiple bone metastases and died of disease 10 months after surgery. This tumour must be distinguished from other small cell neoplasms, especially ovarian small cell carcinoma of the hypercalcaemic type.

Key words Neuroendocrine carcinoma · Small cell carcinoma · Ovarian tumour

Introduction

The first 11 cases of primary ovarian small cell carcinoma of the pulmonary type were described by Eichhorn et al. in 1992 [2]. This tumour is different clinicopathologically from another type of ovarian small cell carcinoma, which occurs in young women and is associated with hypercalcaemia in approximately two thirds of cases [2]. Small cell carcinoma of the pulmonary type possesses microscopic features and the neuroendocrine profile of pulmonary small cell carcinoma [2]. Primary ovarian neoplasms of this type have rarely been reported [1, 9]. To our knowledge, in the English language literature, there has been only one case report, by Khurana et al. [6], since the 1992 study by Eichhorn et al. In the present report, we describe a case of small cell neuroendocrine carcinoma of the ovary, and we discuss the main differential diagnosis and its possible histogenesis.

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Case history

A 64-year-old Japanese women (gravida 0, para 0) had a 2-month history of vaginal bleeding and lower abdominal pain. Pelvic examination showed a tumour the size of a grapefruit in the left ovary. Laboratory data showed elevated serum levels of tumour markers (CEA, 48 ng/ml; CA19-9, 760 U/ml; CA125, 450 U/ml). A total hyperectomy, bilateral salpingo-oophorectomy, partial omentectomy, and pelvic and para-aortic lymphadenectomy were performed. Intraoperatively, there was no conspicuous macroscopic abnormality in the abdominal cavity. Chest X-ray showed no significant abnormalities. The patient had no endocrine symptoms related to her tumour. Postoperatively, combined chemotherapy with cyclophosphamide and *cis*-diamine-dichloroplatinum II was administered. The patient developed brain and multiple bone metastases and received radiation therapy, but died of disease 10 months after surgery. An autopsy was not performed.

Materials and methods

The resected tissues were fixed in 10% buffered formalin, routinely processed and paraffin embedded. Sections were examined with haematoxylin and eosin (H&E), periodic acid–Schiff (PAS) with and without diastase, Fontana-Masson stains, and the argyrophilic staining technique of Grimelius.

Formalin-fixed, paraffin-embedded tissue sections were evaluated immunohistochemically with the antibodies listed in Table 1, using the peroxidase – antiperoxidase (PAP) technique or avidin – biotin peroxidase complex (ABC) technique with an ABC kit (Vector Laboratories, Burlingame, Calif.). The PAP technique was used for S-100 protein (S-100), chromogranin, synaptophysin, somatostatin, calcitonin, adrenocorticotropic hormone (ACTH), glucagon, and gastrin immunostaining. The ABC technique was used for CAM 5.2 (keratin of 40 kDa, 45 kDa, and 52 kDa mol. wt.), epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), vimentin, leu 7, neuro-specific enolase (NSE), and neurofilament (NF) immunostaining. Appropriate positive and negative control experiments were also performed.

Table 1 Results of immunohistochemical study (++ many tumour cells staining, + some tumour cells staining, – no tumour cells staining)

Antibody	Monoclonal polyclonal	Dilution	Source[a]	Results
Cytokeratin, CAM 5.2	M	1:1	Becton Dickinson	++
Epithelial membrane antigen (EMA)	M	1:400	Dakopatts	++
Carcinoembryonic antigen (CEA)	M	1:30	Dakopatts	+
Vimentin	M	1:100	Dakopatts	–
S 100 protein (S-100)	P	1:200	Dakopatts	+
Chromogranin	P	1:500	Incstar	++
Leu 7	M	1:30	Becton Dickinson	++
Neuron-specific enolase (NSE)	M	1:150	Dakopatts	+
Neurofilament (NF)	M	1:100	Immunobiology Laboratory	–
Synaptophysin	P	1:1,500	Euro-Diagnostica BV	+
Somatostatin	P	1:400	Dakopatts	–
Calcitonin	P	1:400	Dakopatts	–
ACTH	P	1:200	Dakopatts	–
Glucagon	P	1:400	Dakopatts	–
Gastrin	P	1:300	Dakopatts	–

[a] Becton Dickinson, San Jose, Calif.; Dakopatts, Glostrup, Denmark; Immunobiology Laboratory, Takasaki, Japan; Incstar, Stillwater, Minn.; Euro-Diagnostica BV, Apeldoom, The Netherlands

Samples for electron microscopy were taken from a formalin-fixed specimen. They were re-fixed with 1.2% glutaraldehyde, and post-fixed with 1% osmium tetroxide. After dehydration in a graded ethanol series, they were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and were observed at 50 kV with an electron microscope (Hitach HS-9, Tokyo, Japan).

Flow cytometry was performed on the formalin-fixed, paraffin-embedded tissue blocks (two blocks). The technique of Hedley et al. [5] was used for DNA analysis, with some minor modifications. The methods used have already been described elsewhere [3].

Pathological findings

Macroscopically, the left ovarian tumour, measuring 14.0×12.0×9.0 cm and weighing 620 g, was mostly solid. The cut surface was yellowish white and soft with a minor cystic component, necrosis, and haemorrhage. There was no capsular invasion. Microscopically, the tumour was characterized by two predominant proliferating patterns of small to medium-sized cells: a carcinoid-like pattern with trabecular, tubular, glandular, or insular arrangements (Fig. 1) and a closely packed nesting pattern with central coagulation necrosis and occasional glandular arrangements (Fig. 2). These two patterns were intermingled. The tumour cells had round to oval hyperchromatic nuclei containing dispersed chromatin and inconspicuous nucleoli. Some cells had pleomorphic nucleic with single small nucleoli. The cytoplasm was scanty and pale, and eosinophilic or amphophilic (Fig. 3). The cytological atypia was moderate. The mitotic activity of these cells was 38 per 10 high-power fields. The stromal matrix were fibrous and cellular with occasional oedema and hyalinization. In small foci, a mucinous tumour of borderline malignancy (Fig. 4) and moderately differentiated endometrioid carcinoma (Fig. 5) were observed adjacent to the small to medium cell tumour. No teratomatous element was identified. In small to medium cell areas, the tumour cells were negative for PAS and Fontana-Masson stains. Grimelius stain showed many tumour cells with argyrophilic granules (Fig. 6). Some cells in the mucinous tumour of borderline malignancy were argyrophilic. There was a microscopic focus of metastasis in the omentum with histology identical to that of the ovarian tumour. The right ovary contained endometriotic cysts. The endometrium showed simple hyperplasia without atypia. There were no metastatic lesions in the lymph nodes.

The immunohistochemical findings are summarized in Table 1. The tumour cells were diffusely and strongly positive for CAM 5.2 and EMA. CEA, S-100, chromogranin (Fig. 7), leu 7, synaptophysin, and NSE were each detected in a modrate number of cells. The tumour cells were uniformly negative for vimentin, neurofilament, calcitonin, ACTH, somatostatin, glucagon, and gastrin.

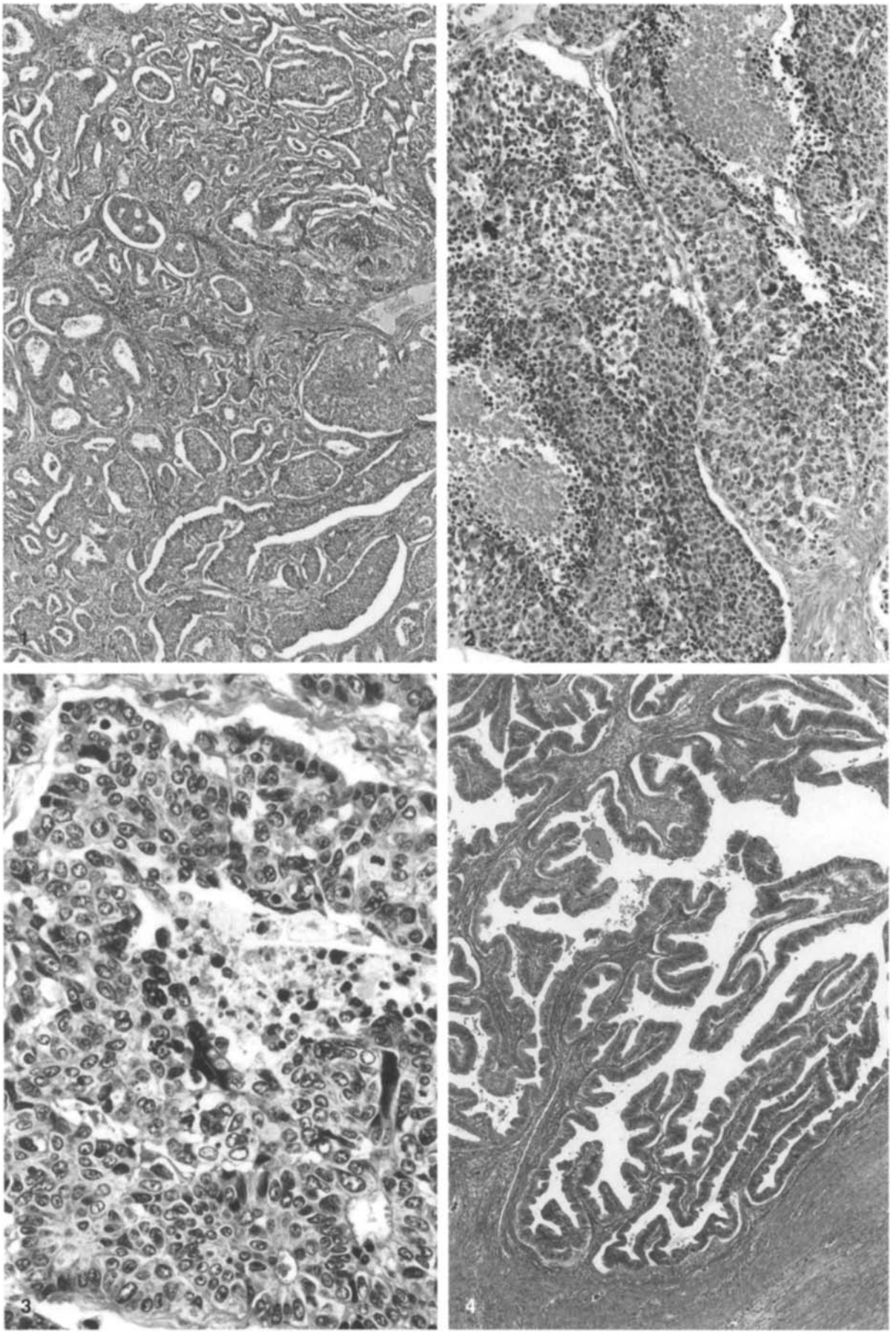
The preservation of the ultrastructure was less than optimal, owing to the primary fixation of the tissue in formaldehyde. The tumour cells had ovoid nuclei with peripheral chromatin condensation and small nucleoli. In the cytoplasm of these cells, rough endoplasmic reticulum, mitochondria, and lipid droplets were observed. Electron-dense granules measuring from 150 to 450 nm in diameter and surrounded by membrane-like

Fig. 1 Carcinoid-like lesion with trabecular, tubular, glandular, and insular arrangements. H&E, ×100 ▶

Fig. 2 Packed nesting pattern with central coagulation necosis. H&E, ×200

Fig. 3 Small solid nests with occasional glandular arrangements, intermingled pleomorphic cells, and necrotic areas. H&E, ×400

Fig. 4 Areas of mucinous tumour of borderline malignancy. H&E, ×100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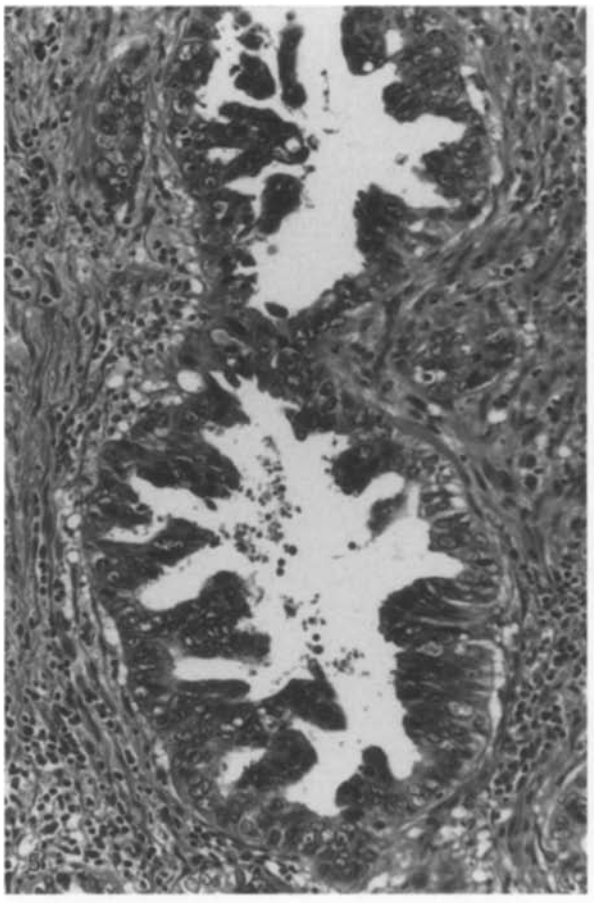

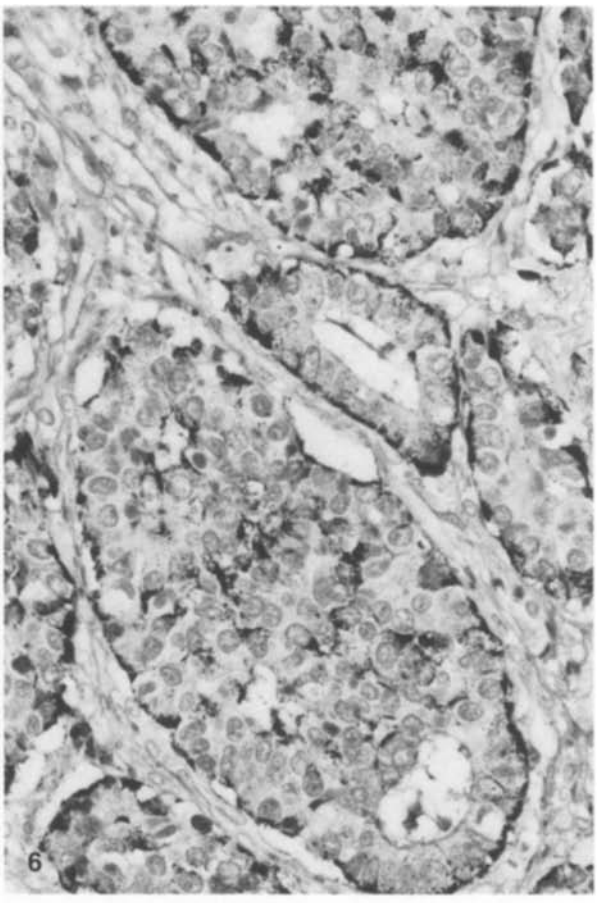

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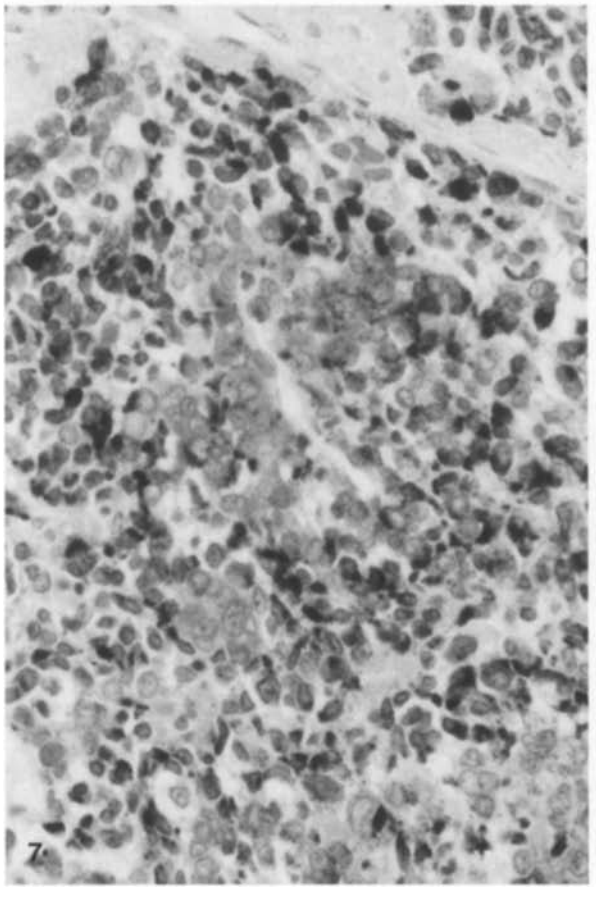

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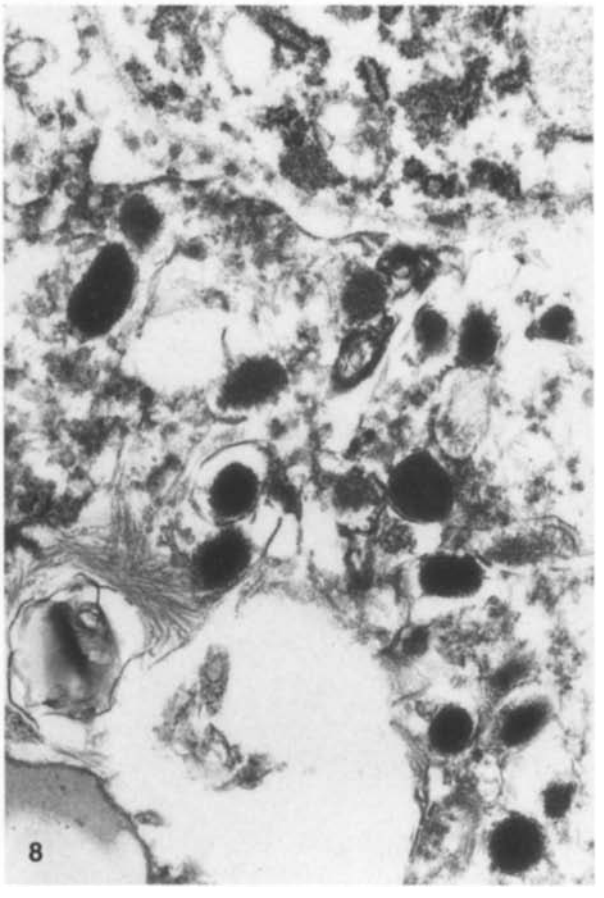

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material and intermediate filaments were also identified (Fig. 8).

The tumour was DNA aneuploid with S-phase fractions (SPF) of 14.0% and 11.6%. The DNA indexes and coefficients of variation were 1.52 and 1.53, and 2.5% and 2.6%, respectively.

Discussion

The current neoplasm was microscopically and immunohistochemically identical to the previously reported cases [2, 7]. This tumour showed a range of histological patterns similar to the atypical carcinoid – small cell undifferentiated carcinoma spectrum of pulmonary neoplasms, with two predominant characteristic patterns, a carcinoid-like pattern and a closely packed nesting pattern with central necrosis. A similar tumour associated with carcinoid syndrome and Cusihng's syndrome was documented by Brown and Lane [1]. In our case, the neuroendocrine differentiation was manifested by a histological growth pattern, argyrophilia, ultrastructural demonstration of neurosecretory granules, and expression of several neuroendocrine markers.

Of the 13 reported cases, including ours, a component of endometrioid carcinoma was present in 5 tumours, 1 other tumours showed squamous differentiation, 3 contained atypical mucinous glands, and 2 others were associated with a Brenner tumour [2, 7]. Small cell neuroendocrine carcinomas of the type described here have been documented in the uterine cervix and endometrium [4, 6, 12]. These were often associated with other types of carcinoma, such as squamous cell carcinoma and adenocarinoma. This situation is similar to that found in other locations, such as the lung and gastrointestinal tract.

The differential diagnosis of this neoplasm includes metastatic small cell carcinoma from the lung or another site, ovarian small cell carcinoma of the hypercalcaemic type, carcinoid, and primitive neuroectodermal tumour. Metastatic small cell carcinoma can be ruled out because the patient had no pulmonary tumour and there was associated mucinous borderline tumour and endometrioid carcinoma. Ovarian small cell carcinomas of the hypercalcaemic type are characterized by their occurrence in premenopausal patients, the presence of follicle-like spaces, large pleomorphic cells with prominent nucleoli and clumped chromatin distribution, and abundant rough endoplasmic reticulum [8, 13]. The tumour of our patient, which was not associated with a teratomatous element, is different from insular and trabecular carcinoids: these carcinoids are composed of monotonous small cells and lack necrosis, haemorrhage, high mitotic activity, and nuclear pleomorphism. Strumal and mucinous (goblet cell) carcinoids are also ruled out by the absence of thyroid component and small nests of glands composed of goblet cells. Primitive neuroectodermal tumours typically exhibit a wide range of differentiation, including neural, glial, ependymal, and medulloepithelial cells, alone or in combination.

The histogenesis of small cell neuroendocrine carcinoma of the ovary remains uncertain. We consider that this type of tumour may arise from stem or multipotential cells within the surface epithelium that have a capacity for neuroendocrine as well as glandular differentiation. It may also arise from neuroendocrine-type (argyrophilic) cells within non-neoplastic surface epithelium or neoplastic glands, including mucinous or endometrioid tumours [10, 11]. The possibility of teratomatous origin cannot be ruled out.

The current case had an aneuploid DNA content. In the study by Eichhorn et al. [2], five of their eight cases were aneuploid and the remaining three were diploid. In contrast, none of 23 ovarian small cell carcinomas of the hypercalcaemic type examined was aneuploid [13]. Whether a flow cytometric analysis is a good tool to predict the biological behaviour of ovarian small cell neuroendocrine carcinomas remains unclear because of the paucity of cases. Since these tumours usually behave very aggressively despite postoperative chemotherapy [2, 7], diagnosis of the type of ovarian small cell carcinoma is essential for optimal patient management.

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◀ **Fig. 5** Endometrioid carcinoma. H&E, ×250

Fig. 6 Grimelius stain showing many cells with argyrophilic granules. Grimelius stain, ×400

Fig. 7 Immunostaining of chromogranin. Note the paranuclear dot-loke staining. Immunostaining, ×400

Fig. 8 Electron micrograph showing electron-dense granules surrounded by membrane-like material and intermediate filaments. ×2400

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