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Case report

Small cell carcinoma of the ovary: an immunohistochemical and ultrastructural study with a review of the literature

Beatriz Lifschitz-Mercer [1], Raffaele David [2], Muralee Dharan [2], and Bernard Czernobilsky [1]

[1] Department of Pathology, Kaplan Hospital, Rehovot, affiliated with the Medical School of the Hebrew University and Hadassah, Jerusalem, Israel
[2] Central Emek Hospital, Afula, affiliated with the B. Rappaport School of Medicine, the Technion, Institute of Technology, Haifa, Israel

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Summary. This is an immunohistochemical and ultrastructural study of two small cell carcinomas of the ovary with a review of the literature. These cases showed a dimorphic population of small and large cells sharply demarcated from each other. Cytokeratin 18 and vimentin were mainly expressed in the large tumour cells, some of which also stained for alpha-smooth muscle actin. Periodic-acid-Schiff-positive, alpha-1-antitrypsin-positive hyaline globules were present in one case. Ultrastructural findings included filamentous nucleolonema as well as evidence of smooth muscle differentiation. Some of these observations have not been previously reported. Certain of the above features seem to support a germ cell origin of small cell carcinoma, but they cannot be considered specific for germ cell neoplasms. Thus, small cell carcinoma of the ovary cannot be classified into one of the known categories of ovarian tumours at the present time.

Key words: Small cell carcinoma – Ovary – Immunohistochemistry – Ultrastructure – Literature review

Introduction

Small cell carcinoma of the ovary associated with hypercalcaemia is a highly malignant tumour in young females (Dickersin et al. 1982). Although at first the small cell element was considered to constitute the pathognomonic histological feature of this neoplasm, it soon became apparent that 25% or more of the tumours also contained large cells with abundant eosinophilic cytoplasm, which occasionally predominated (Young and Scully 1985; Young et al. 1987). Hypercalcaemia, which was initially reported to be present in all of the patients, was subsequently found to be absent in one-third of the cases (Young et al. 1987).

Correspondence to: B. Lifschitz-Mercer, Department of Pathology, Kaplan Hospital, Rehovot, 76100 Israel

The histogenesis of this neoplasm has not yet been elucidated. Dickersin et al. (1982) seemed to favour an epithelial origin, but also considered the possibility that the tumours are either poorly differentiated granulosa cell or primitive germ cell tumours. Holtz et al. (1979) regarded the lesion as a sex cord-stromal tumour, while Ulbright et al. (1987) proposed a germ cell origin. According to Aguirre et al. (1989) the application of immunohistochemical methods including staining for intermediate filaments has not been helpful in elucidating the nature of the small cell carcinoma of the ovary.

In the present immunohistochemical and electron microscopic study of two small cell carcinomas of the ovary, some novel results were obtained which may contribute to our understanding of the histogenesis of these complex neoplasms.

Case reports

Case 1

A 20-year-old woman was admitted to the hospital with a history of low abdominal pain and a pelvic mass of a few weeks duration. On physical examination a right-sided, somewhat tender abdominal mass was palpated and confirmed by ultrasound examination. The mass was solid with small cystic areas. Laboratory tests were not remarkable. Serum calcium level was not measured.

At laparatomy a right ovarian tumour was found to have ruptured in one area. One litre of bloody abdominal fluid was present. The tumour and right fallopian tube were removed and a wedge biopsy was obtained from the left normal-appearing ovary.

The patient was lost to follow-up 6 months after surgery, during which time no metastases were identified.

Pathological findings. The specimen consisted of a tumour measuring $10 \times 10 \times 4$ cm. The tissue was predominantly solid with small cystic areas. Consistency was soft with friable and haemorrhagic areas.

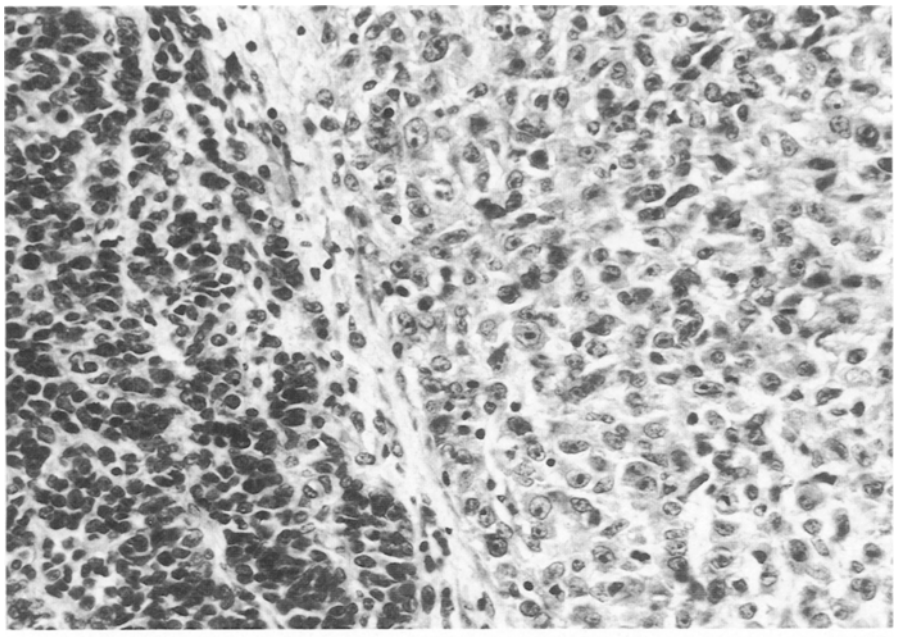

Fig. 1. Small cell carcinoma showing dimorphic population of small and large cell sharply demarcated from each other (case 1). H & E, ×150

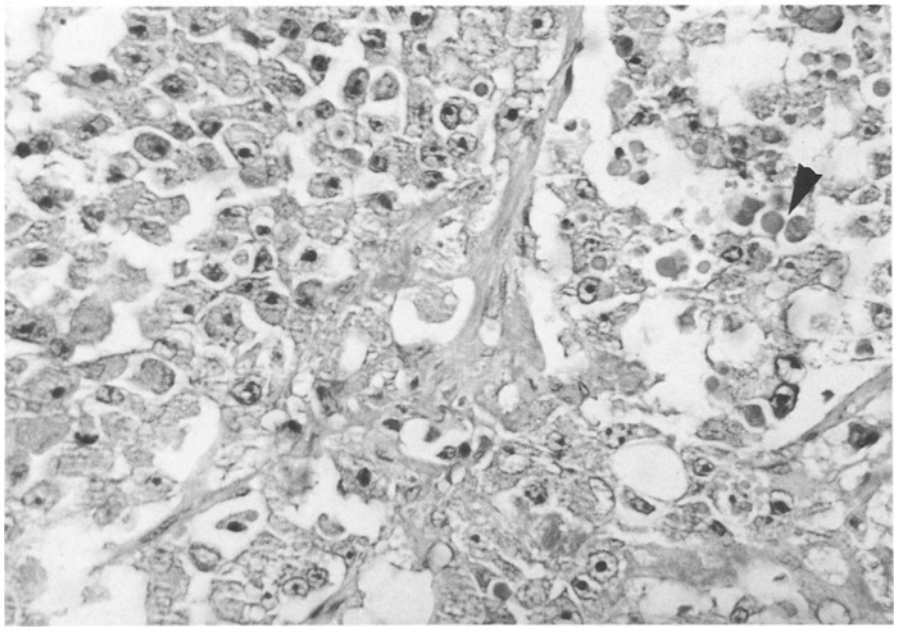

Fig. 2. Large, "luteinized" type tumour cells with numerous intra- and extracellular hyaline globules (*arrowhead*) (case 2). H & E, ×200

Microscopic examination revealed the tumour to be composed of a dimorphic population of small and large cells showing numerous mitotic figures. The small cells were rather uniform and had round, oval or spindle-shaped hyperchromatic nuclei usually containing a single nucleolus. The cytoplasm was inconspicuous. The large cells showed round, vesicular nuclei with prominent nucleoli. The cytoplasm of the latter cells was abundant and occasionally foamy. Some of these cells resembled luteinized cells.

In most areas the small and large cells were sharply demarcated from each other (Fig. 1). Occasionally mixtures of small and large cells were seen. In both populations of cells there were scattered glandular or small cystic structures containing eosinophilic fluid. Foci of necrosis were also present. Invasion of medium-size blood vessels by large tumour cells was observed.

Some small and large cells showed periodic acid-Schiff (PAS)-positive, diastase-resistant intracytoplasmic material. Groups of small cells were surrounded by reticulin fibres.

Case 2

A 19-year-old woman was admitted to the hospital because of abdominal pain and dysuria of a few weeks' duration. Physical and ultrasound examinations re-

vealed a pelvic mass. Laboratory tests were normal except for a blood calcium level of 14.1 mg, which dropped to 9.2 mg postoperatively.

At laparoscopy a right ovarian mass was identified and a right salpingo-oophorectomy was performed.

The patient developed widespread metastases and expired 30 months after diagnosis.

Pathological findings. The right ovarian mass measured $13 \times 8 \times 7$ cm. The external surface was bosselated but smooth. Cut section revealed a fleshy, soft, mostly solid, partially haemorrhagic mass with scattered cystic areas.

On microscopic examination the tumour was composed of small and large cells. In some areas cells intermediate in size and nuclear characteristics could be identified, suggestive of a transitional phase between the two main groups of cells. The luteinized character of the large cells was particularly striking in this case. Many of the latter cells were also vacuolated.

The large tumour cells were predominantly arranged in an alveolar fashion with occasional central cavity formation. Areas of necrosis were also present. The lumens of some medium-sized blood vessels were infiltrated by large tumour cells. Sharply demarcated from the large cells were small tumour cells which grew in a solid or trabecular fashion. Mitoses were numerous in both small and large cells.

Some of the small and large tumour cells showed intra- and extracytoplasmic hyaline globules, which appeared as round or oval eosinophilic bodies (Fig. 2). These hyaline globules stained strongly with PAS, which was diastase resistant. The globules also stained positively with phosphotungstic acid haematoxylin and Ziehl-Neelsen stains. Masson trichrome stained the globules brick-red and with luxol fast blue stain a bright blue colour was obtained. The globules also displayed a yellow autofluorescence.

There was PAS-positive, diastase-resistant staining of some of the large tumour cells. Reticulin fibres surrounded groups of small and large cells, but this was less conspicuous in the latter.

Materials and methods

Immunohistochemical studies on formalin-fixed paraffin-embedded tissue were carried out using standard immunoperoxidase techniques (Lifschitz-Mercer et al. 1991).

The primary monoclonal antibodies used are listed in Table 1. The numbering of the various cytokeratin polypeptides (CK) is according to Moll et al. (1982).

The bound antibodies were visualized by applying the avidin-biotin peroxidase complex (ABC) protocol (Hsu et al. 1981) using the Vectastain, Elite, ABC Kit (Vector Lab., Burlingame, Calif., USA).

For ultrastructural studies samples of one tumour (case 2) taken from different areas were cut into blocks approximately 1 mm in size and fixed in 2.5% phosphate-buffered gluteraldehyde (pH 7.2), washed in phosphate buffer and post-fixed in 1% osmium tetroxide. Following dehydration in graded alcohols, the tissue was put through propylene oxide and embedded in Epoxy resin (Polybed 812). Sections 1 μm thick were stained with toluidine blue and blocks were selected for thin sectioning using an LKB ultramicrotome. These thin sections were double stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

Table 1. Primary monoclonal antibodies used

Antibody	Antigen recognized	Source
AE1, AE3	Broad range of cytokeratin	Biogenix Labs, San Ramon, Calif., USA
CY-90	Cytokeratin 18	BioMakor, Rehovot, Israel
Ks 19.2.105	Cytokeratin 19	Progen, Heidelberg, FRG
Vimentin V9	Vimentin	BioMakor
Desmin	Desmin	BioMakor
Alpha-SM actin	Alpha-smooth muscle actin	BioMakor
S-100 protein		Dakopatts, Glostrup, Denmark
Neurone-specific enolase		Dakopatts
Chromogranin A		Biogenix
Alpha-fetoprotein		Dakopatts
Alpha-1-antitrypsin		Dakopatts
Carcinoembryonic antigen		Dakopatts

Results

In the two cases, groups of and scattered large tumour cells stained positively with the broad spectrum CK antibody as well as with the antibody to CK 18 (Table 2). The small tumour cells showed occasional staining in isolated cells only. There was no staining for CK 19 in any of the tumour cells (Fig. 3A, B). Staining of large tumour cells with the vimentin antibody was patchy in one case and diffuse in the other small tumour cells showed staining in both cases (Fig. 3C). Alpha-smooth muscle (SM) actin stained scattered large tumour cells in a wide area in one case (Fig. 3D). Focally, large cells stain was seen in the other case. Isolated small tumour cells stained for alpha-SM actin in one of the cases.

Table 2. Summary of positive immunohistochemical results in tumour cells

Antibody	Case 1		Case 2	
	SC	LC	SC	LC
$AE_1 + AE_3$ (broad spectrum CK)	±	+	±	+
CY90 (CK 18)	±	±	−	+
Vimentin	±	+	±	+
Alpha-SM actin	−	+	±	+
S-100 protein	−	−	−	+
NSE	±	+	−	−

SC, Small cells; LC, large cells, +, positive in groups and/or scattered cells; ±, positive in isolated cells; CK, cytokeratin; NSE, neurone-specific enolase

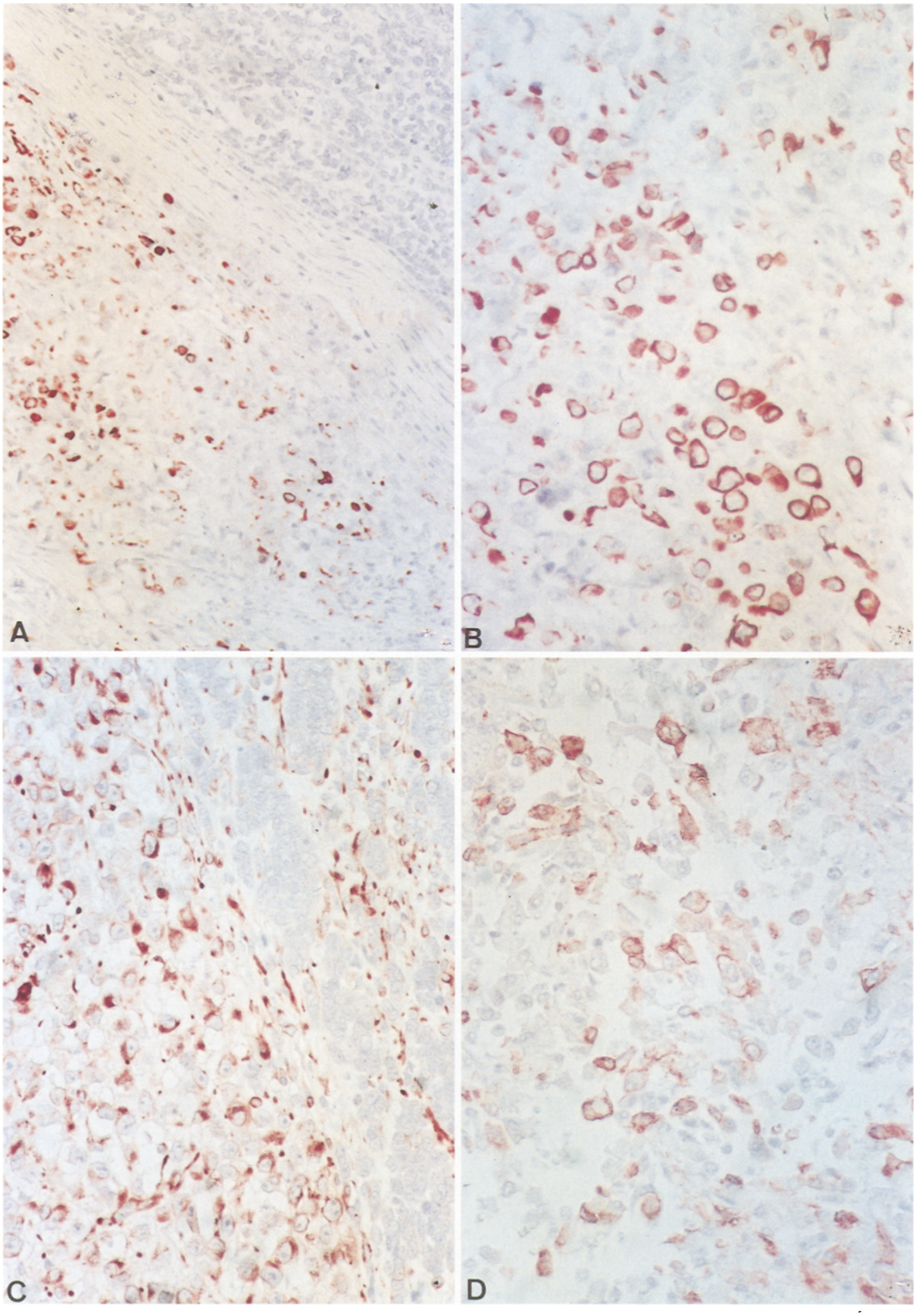

Fig. 3A–D. Small cell carcinoma stained for cytoskeletal proteins using immunoperoxidase method on formalin-fixed material. **A** Large tumour cells only staining for CK 18. Small cells remain unstained (*upper right corner*) (case 2). ×120. **B** Higher magnification of large tumour cells staining for CK 18 (case 2). ×150. **C** Diffuse staining of large tumour cells for vimentin. In area of small tumour cells (*right side*) positive staining is limited to stromal cells and blood vessels (case 1). ×150. **D** Large tumour cells staining for alpha-smooth muscle actin (case 2). ×200

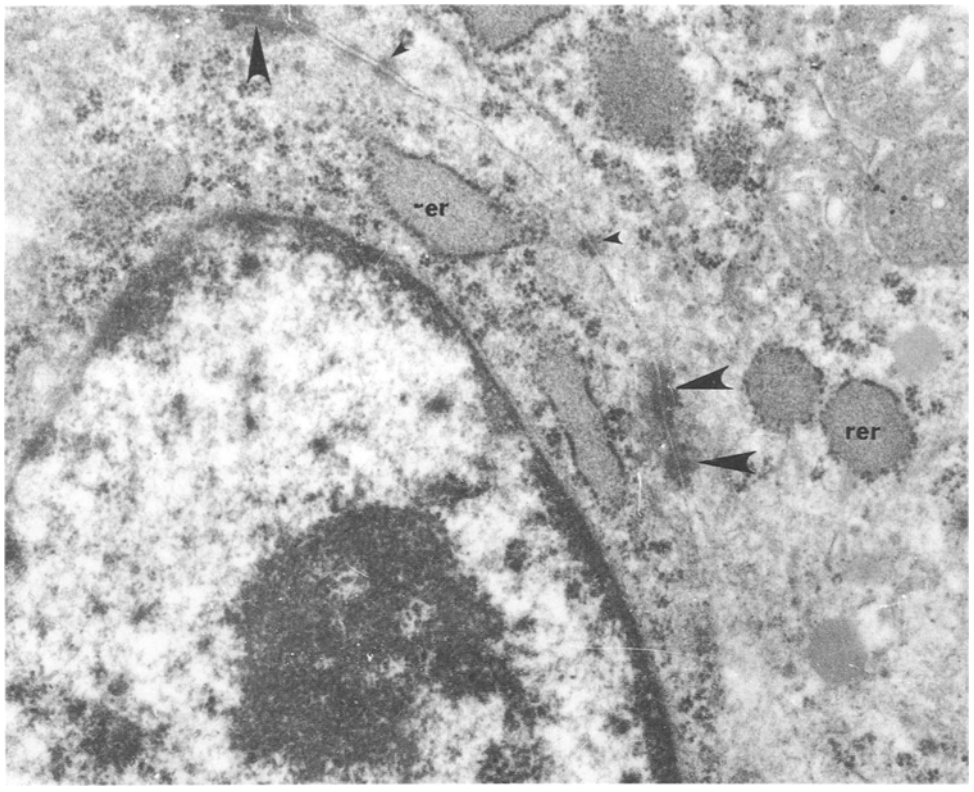

Fig. 4. Electron micrograph of two adjacent small tumour cells showing well-developed desmosomes (*large arrowheads*) and less well developed cellular junctions (*small arrowheads*). Note cisternae or rough endoplasmic reticulum. ×39600

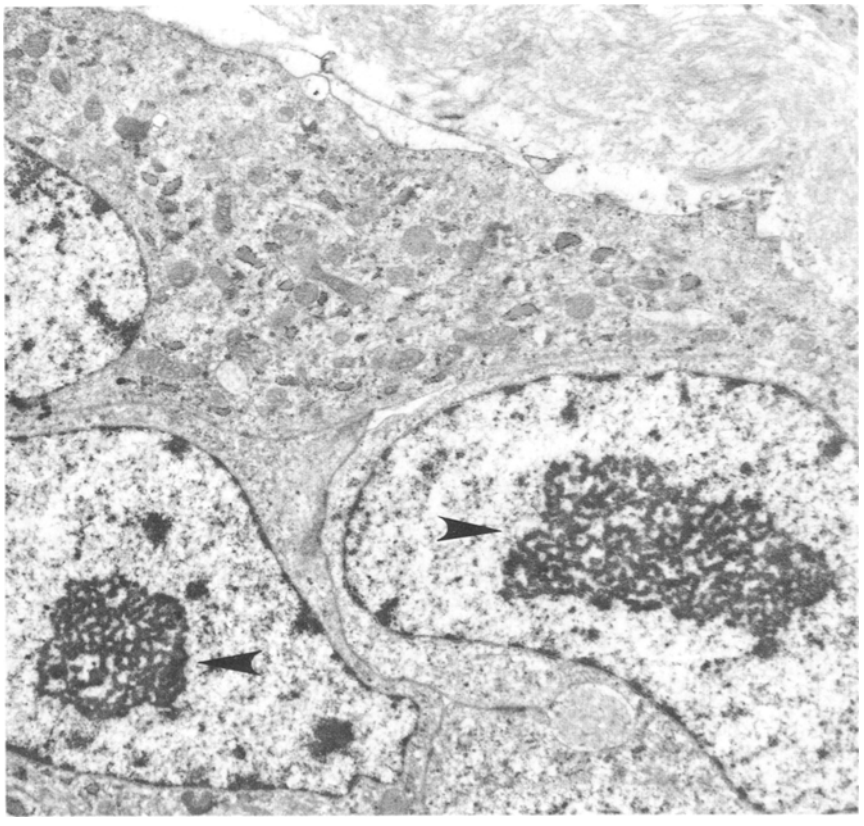

Fig. 5. Electron micrograph of large tumour cells showing nuclei with distinct nucleolonema (*large arrowhead*). ×8900

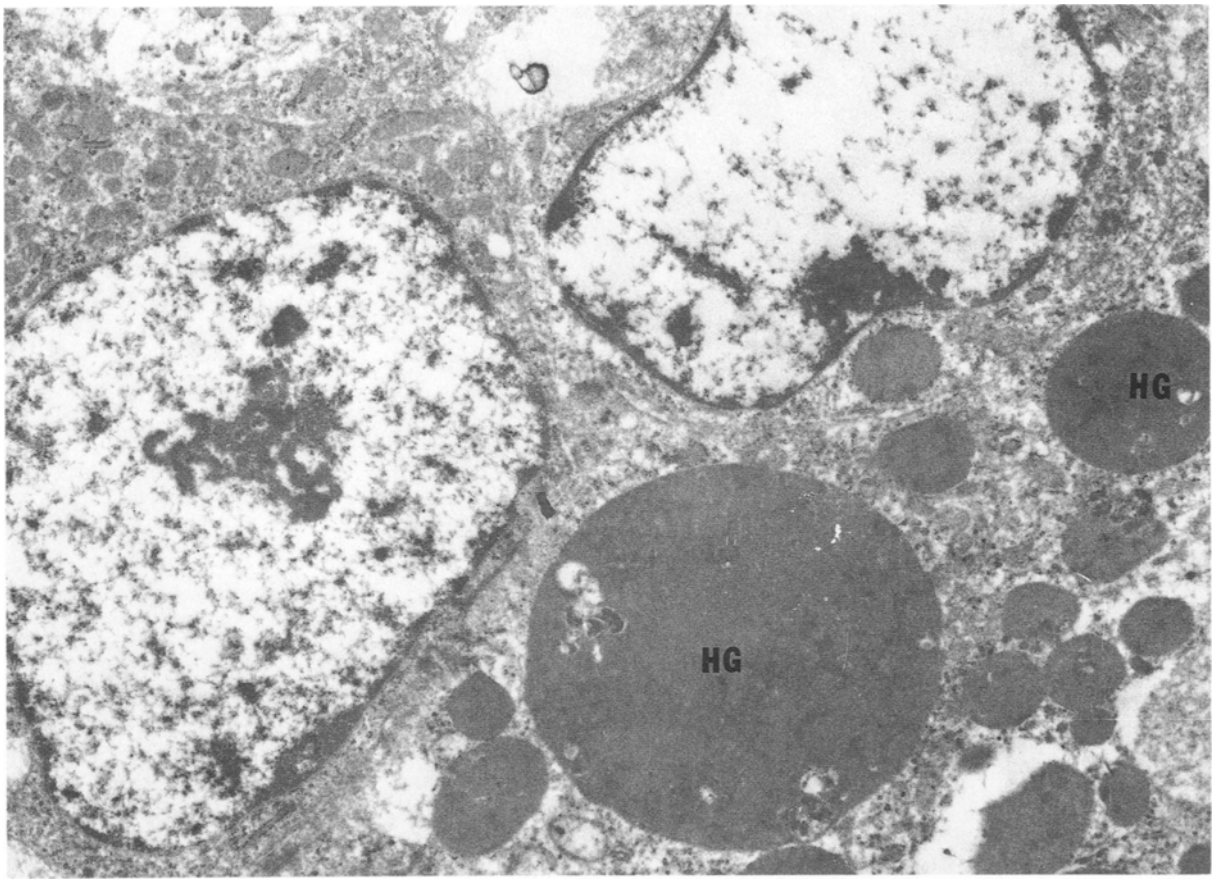

Fig. 6. Electron micrograph of large tumour cells with intracellular hyaline globules (*HG*). ×11100

S-100 protein was detected in scattered large cells in one case. Groups of large cells and isolated small tumour cells stained for neurone-specific enolase (NSE) in one case.

Scattered large tumour cells and hyaline globules stained for alpha-1-antitrypsin in case 2. No alpha-fetoprotein or carcinoembryonic antigen could be detected in this tumour.

Numerous blocks from case 2 were examined by electron microscopy, including the areas of large cells and small cells. The surfaces of the large tumour cells were generally smooth with close apposition of their plasma membrane that was frequently joined by desmosomes in varying stages of development (Fig. 4). The most regular ultrastructural feature of the large cells was the presence of an oval or round and sometimes polyhedral nucleus with one or two prominent nucleoli. The latter were distinctive in that a remarkably well-developed filamentous nucleolonema was almost invariably present (Fig. 5). Foci of heterochromatin were evenly scattered. In striking contrast, the nuclei of the small cells disclosed great irregularity of the nuclear shape and much more abundant heterochromatin. Nucleoli with the complex twisted filamentous strands as seen in the large cells were never present in these cells. The cytoplasm of both types of cells had prominent rough endoplasmic reticulum (RER) with frequent cystically dilated cisternae containing moderately electron-dense granular material.

The intracellular eosinophilic bodies were represented by moderately electron-dense granular material without any limiting membrane (Fig. 6). At the periphery of these globules a row of ribosomes could be seen and often short segments of RER were noted adjacent to the globules. The globules were sharply demarcated and relatively homogeneous. Frequently, within the small cells multiple globules of different sizes were present and the larger ones appeared to arise from confluence of smaller ones. There was some variability in electron density from one globule to another.

In addition to the RER and hyaline bodies, both the large and the small cells had numerous polyribosomes and mitochondria sometimes showing a ring-shaped profile with partial transformation of cristae.

Occasional large and small cells showed bundles of 10 nm filaments, randomly distributed but with a radial and/or arcuate pattern. The bundles appeared relatively thick, gently curved and branched. They were densely packed laterally and therefore displayed a relatively high electron density.

Occasional heterophagolysosomes were seen. These phagolysosomes showed a very condensed nuclear fragment as well as cytoplasmic organelles in varying stages of degradation. Membrane-bounded globules were also present extracellularly.

Some of the tumour cells displayed subplasmalemmal bundles or aggregates of microfilaments, some with

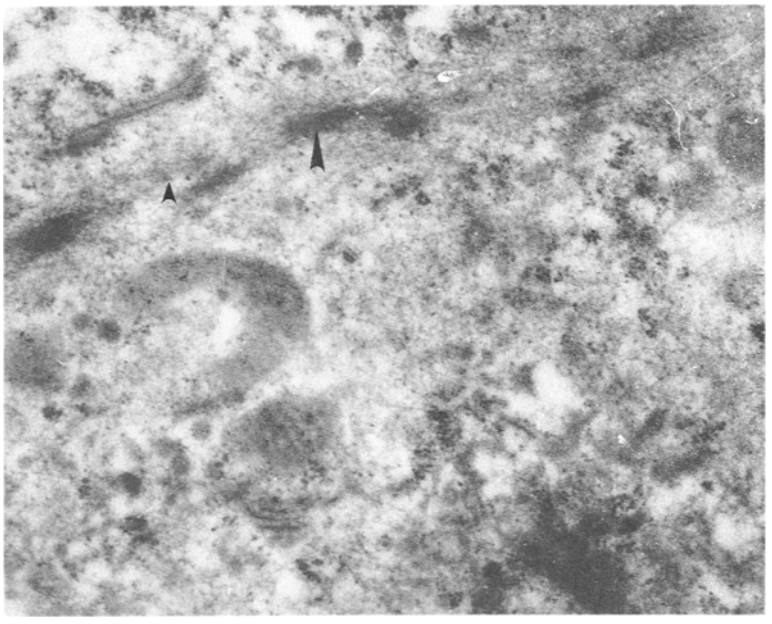

Fig. 7. Electron micrograph showing subplasmalemmal bundles of microfilaments (*small arrowhead*) with small fusiform dense bodies (*large arrowhead*). ×31680

small fusiform dense bodies (Fig. 7). Isolated tumour cells were partially enveloped by discontinuous basal lamina, but no pinocytic vesicles were found. Glycogen, microvilli or mucus granules could not be detected in any of the cells.

Discussion

In spite of the great interest engendered by the so-called small cell carcinoma of the ovary since it was first recognized as an entity (Dickersin et al. 1982), the histogenesis of this neoplasm is still the subject of speculation. It has been considered to belong to each of the categories of ovarian tumours in turn, namely epithelial, sex cord-stromal and germ cell neoplasms (Dickersin et al. 1982).

The features cited in the literature favouring a possible epithelial derivation of this tumour include light microscopic appearance of the neoplastic cells (Dickersin et al. 1982) as well as electron-microscopic findings described by McMahon and Hart (1988). These authors demonstrated desmosome-like junctions, partial investment with basal lamina, and abundant dilated RER forming large vesicles, all of which supported in their opinion the epithelial nature of small cell carcinoma of the ovary.

While the above-cited studies failed to provide any evidence of specific lines of differentiation of the epithelial cells forming the neoplasms, Abeler et al. (1988) concluded that small cell carcinomas of the ovary are neuroendocrine neoplasms on the basis of positive staining for NSE and the presence of neuroendocrine granules by electron microscopy. This was consequently refuted by Scully and Dickersin (1989), who stressed the non-specificity of positive enolase staining and pointed out the fact that neither they nor others have been able to identify dense-core granules in these neoplasms (Abeler et al. 1988; Holtz et al. 1979).

The theory of a germ cell origin of small cell carcinoma has been presented by Ulbright et al. (1987), who studied six cases and applied immunohistochemical methods in addition to light and electron microscopy. Their cases showed a dimorphic population of small and large cells, and some contained PAS-positive, diastase-resistant hyaline globules. Immunohistochemical stains showed expression of CK and vimentin. In three of their cases some tumour cells also stained for alpha-1-antitrypsin. Electron microscopic features included granular material in dilated RER intracytoplasmic dense globules, maculae adherens and extracellular basement membrane-like material.

Immunohistochemical studies of ovarian small cell carcinoma were carried out by Young et al. (1987) and Aguirre et al. (1989), who showed focal CK and vimentin staining of the tumour cells. One third of the cases exhibited cellular reactivity with anti-epithelial membrane antigen (Aguirre et al. 1989). The majority of the small cell tumours in the latter study stained for NSE and chromogranin A. S-100 protein was negative. A comparison of these results with granulosa cell tumours, both adult and juvenile, as well as with Sertoli cell tumours did not yield significant differences. Aguirre et al. (1989) concluded that their immunohistochemical study failed to elucidate the nature of the small cell carcinoma of the ovary.

In the present study of two tumours, small and large cells were present in each of the cases. While in case 1 some intermingling of small and large tumour cells occurred, in most areas the small and large cell populations were sharply separated from each other. An intermediate sized cell population, possibly representing a transition between the small and large cells was present in one case. There were hyaline globules; intracellular ones were represented by non-membrane bound moderately electron-dense granular material, while the extracellular globules consisted mainly of cellular organelles in varying stages of degradation.

Of particular interest was our observation that CKs as well as vimentin were expressed, mainly in the large tumour cell population. The small cells remained unstained or showed focal staining only. Alpha-SM actin decorated scattered large tumour cells in both cases and isolated small cells in one case. This difference in cytoskeletal profile of the small and large cells suggests that the dimorphic population may denote the presence of lesser and more highly differentiated cells in what is basically an undifferentiated neoplasm. Thus, the small cells which are almost devoid of cytoskeletal elements may represent the most anaplastic cell population, whereas the expression of the cytoskeletal components in large cells may indicate a somewhat higher degree of differentiation potential in the latter. In epithelial tumours of the ovary, CKs are expressed in both well and poorly differentiated tumour cells (Moll et al. 1983).

Some of our data seem to support a germ cell origin of small cell carcinoma. These features include alpha-1-antitrypsin-positive hyaline globules (a yolk sac tumour marker; Beilby et al. 1979), the presence of filamentous nucleolonema in the tumour cells which have been described in dysgerminoma (Listrom et al. 1986) and in spermatocytic seminoma (Rosai et al. 1969), as well as evidence of a heterologous differentiation potential by the tumour cells, similar to that observed in testicular germ cell tumours (Fogel et al. 1990; Lifschitz-Mercer et al. 1991). None of the features can be considered specific for germ cell neoplasms, however, since some of them have also been observed in other ovarian tumours.

In conclusion, small cell carcinomas of the ovary present a myriad of light microscopic, immunohistochemical and ultrastructural features, some of which can be found in epithelial, sex cord-stromal and ovarian germ cell tumours. A combination of these morphological elements, as seen in some small cell carcinomas, is not characteristic for any of the above-listed categories of ovarian neoplasms. It is for this reason that, in spite of the suggestion of a germ cell origin, the histogenesis of small cell carcinoma can still not be determined. Our results may be helpful in the frequently difficult differential diagnosis between small cell carcinoma and other ovarian tumours.

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