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Immunohistochemical and ultrastructural localization of tissue polypeptide antigen in human ovarian tumours

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Summary. Forty specimens of benign and malignant ovarian tumours were studied for localization of tissue polypeptide antigen (TPA) at light and electron microscopic levels by an indirect immunoperoxidase technique. Of the 30 ovarian carcinomas, 23 (77%) were positive and 7 (23%) were negative for TPA, while of the 10 benign ovarian tumours 3 (30%) were positive and 7 (70%) were negative. Positive reaction did not correlate with the tumour grade. Of the 10 patients with metastasis, 8 (80%) had positive tumours. Staining for TPA was observed at the intraluminal cell surfaces and peripheral cell membranes. The ultrastructural localization of TPA revealed electron-dense reaction products at the cell surface and microvillous surfaces. These results provide confirmatory and supplementary evidence to support the previous findings of TPA in the serum and suggest that testing for TPA in ovarian tumors has a limited prognostic importance and a poor diagnostic value. The surface property of TPA suggests that the cell membrane is involved in secretion and probably synthesis of TPA.

Key words: Ovarian tumours – Tissue polypeptide antigen – Light and electron microscopy – Immunohistochemistry

Introduction

Many tumour markers have been assessed for their ability to detect cancer or predict the clinical course of the disease, but few have been found to be significantly helpful in practice. Ovarian cancer comprises 3 to 15 cases per 100,000 women per year according to the geographical distribution, and despite various modes of therapy, its prognosis remains uniformly poor (Anteby et al. 1983; Silverburg and Lubera 1988). Prognostic clues can be sought by accurate staging and histological grading of the tumour, but there is considerable variation in the biological behaviour of the tumours. Functional markers may provide clinically useful information. For practical purposes, a marker substance should be expressed in detectable amounts by malignant cells only and preferably by all malignant cells in a tumour, including its metastases, and should be detectable in the serum.

Previous investigators have demonstrated the existence of common tumour antigenicity and purified the tissue polypeptide antigen (TPA) from extracts of pooled human cancer tissues from various sites (Bjorklund and Bjorklund 1957; Bjorklund et al. 1973). Elevated levels of TPA have been found in the serum and body fluid of cancer patients, especially those with active disease (Bjorklund et al. 1973; Bjorklund 1980; Menendez-Botet 1978). More than 80% of patients with cancers of lung, colon, pancreas, as well as lymphoma have been shown to have elevated levels of serum TPA (Menendez-Botet 1978).

Very few preliminary studies on serum TPA levels in ovarian cancer have been reported. In one study, 33% of low grade malignant and 73% of malignant ovarian tumours had elevated TPA levels (Inoue et al. 1985). Immunohistochemical evidence of TPA in ovarian tumours is lacking and no ultrastructural study of TPA has been reported. In this study, an indirect immunoperoxidase technique was used at light and electron microscopical levels to assess the expression of TPA in tissue sections of ovarian tumours and correlate the find-

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ings with the tumour grade and metastases. Reference is made to the incidence, distribution and cellular location of this antigen.

Materials and methods

Formalin-fixed, paraffin-embedded and frozen sections were used. There were 10 benign and 30 malignant ovarian tumours including 10 cases with lymph node or more advanced metastases (Table 1). Paraffin-embedded tissues had been fixed in 10% formalin. Haematoxylin and eosin stained sections from the lesions were reviewed to verify the histological types and grades. Histological classification was assessed using the World Health Organization (W.H.O.) criteria and grading was done according to the international Federation of Gynecology and Obstetrics (FIGO) grading system. Frozen tissue sections from 4 cases of ovarian carcinom were used for ultrastructural study.

An indirect immunoperoxidase technique was used for the localization of TPA at light and electron microscopical level. Rabbit anti-TPA, subunit B_1 antibody was kindly supplied by the Nippon Radioisotope (distributor of AB Sangtec Medical, Sweden). Normal goat serum and peroxidase-labeled goat anti-rabbit IgG, F $(ab')_2$ fraction were obtained from commercial sources.

For light microscopy, 4 μ paraffin sections were deparaffinized in xylene and rehydrated in graded alcohol to phosphate buffered saline (PBS), pH 7.4. The sections were incubated in 0.3% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase activity, washed in PBS and pretreated with 0.1% solution of protease type IIV (Sigma Chemical Co., St. Louis, MO) for 15 min to make the antigenic determinants available. Nonspecific immunoglobulin binding sites were blocked by incubating the tissue sections with 1:20 dilution of normal goat serum for 30 min. The sections were then treated with 1:5 dilution of rabbit anti-TPA:B_1 at 4° C overnight, washed in 2 changes of PBS and incubated with 1:50 dilution of peroxidase-labeled goat anti-rabbit IgG, $F(ab')_2$ for 1 h at room temperature. After washes in PBS, the sections were treated with 0.05% (wt/v) diaminobenzidine (DAB) and 0.03% (v/v) hydrogen peroxide in PBS, pH 7.4, for 10 min, washed in distilled water, counterstained with haematoxylin, dehydrated, cleared and mounted.

For electron microscopy, fresh tissue samples of ovarian carcinoma were fixed in periodate-lysine-paraformaldehyde (PLP) solution for 4 h at 4° C, washed in 2 changes of PBS and treated with increasing concentration of sucrose (10, 15, 20 and 25%) in PBS at 4° C each for 4 h. The tissue samples were placed in OCT compound (Miles Lab., Ill.) and snap-frozen in *n*-hexane precooled in dry ice-acetone. Frozen sections of 4 μ thick were cut in cryostat, taken onto albumin-coated glass slides and air-dried. The sections were washed in PBS and immunostained for TPA as for paraffin sections. After DAB reaction, the tissues were post-fixed in 1% osmium tetroxide in phosphate buffer (PB) for 1 h and dehydrated in increasing percentages of ethanol up to absolute ethanol. Epon-filled EM-embedding capsules (TAAB) were inverted over the sections and the assembly was polymerized. The blocks were detached from the slides by heating on a hot plate. Ultrathin sections from areas of carcinoma were cut, placed on the grids and observed both unstained or double-stained with uranyl acetate and lead citrate.

Controls included omission of the anti-TPA antisera or its substitution with nonimmune normal rabbit serum. Tissue sections were recorded as positive if more than 10% of the glandular epithelium showed specific brown staining. Immunostaining also was classified according to the approximate percentage of the tissue area stained positively.

Table 1. TPA staining in ovarian tumours

Histology	TPA-Neg.	TPA-Pos. (% area stained)			Totals
		10–50	50–80	80–100	
Benign tumours	7	2	1	0	10
Serous cystadenoma	2	0	1	0	3
Mucinous cystadenoma	3	1	0	0	4
Dermoid cyst	1	1	0	0	2
Fibroma	1	0	0	0	1
Malignant tumours	7 (2)[a]	6 (3)	11 (4)	6 (1)	30 (10)
Serous cystadenocarcinoma	4 (1)	3 (2)	5 (3)	2	14 (6)
Mucinous cystadenocarcinoma	3 (1)	3 (1)	6 (1)	4 (1)	16 (4)
Grades of malignant tumours					
Grade 1, well-differentiated	1 (1)	1 (1)	3	3	8 (2)
Grade 2, moderately differentiated	3	2	5 (2)	2 (1)	12 (3)
Grade 3, poorly differentiated	3 (1)	3 (2)	3 (2)	1	10 (5)

[a] Numbers in brackets indicate cases with metastasis

Results

The normal ovarian surface mesothelium, occasionally present in the sections of benign lesions, was uniformly negative for TPA on light microscopy. Of the 10 benign ovarian tumours, 3 (30%) were positive and 7 (70%) were negative for TPA (Table 1). Positive tumours included one dermoid cyst and one each of the serous and mucinous cystadenomas. In the dermoid cyst, positive reactions were observed at the epithelium of some cystic glands (Fig. 1). In the cystadenomas, weak TPA staining was found at the intraluminal and peripheral cell membranes.

Of the 30 ovarian carcinomas, 23 (77%) were positive and 7 (23%) were negative for TPA (Table 1). Most positive cases (17/23) had staining of more than 50 percent of the tumour areas (Fig. 2). Both intraluminal and peripheral cell membranes were stained. The staining intensity varied among tumours as well as within the individual tumours. No correlation was found between the TPA positivity and tumour grade. Of the 10 patients with metastasis, 8 (80%) had tumours positive for TPA.

Ultrastructural localization of TPA revealed electron-dense reaction products at the cell surfaces (Fig. 3). The tumour cells possessed abundant

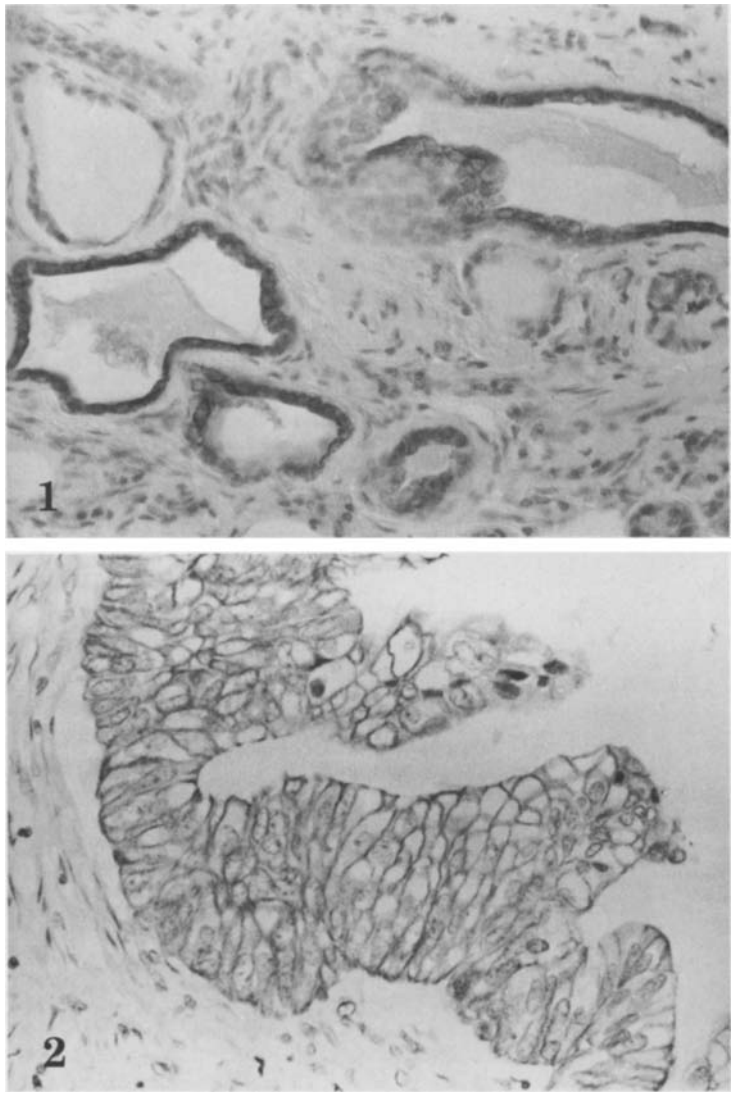

Fig. 1. Dermoid cyst of the ovary. Positive TPA staining is seen in the epithelium of some cystic glands. (Immunoperoxidase stain ×100)

Fig. 2. Serous cystadenocarcinoma of the ovary. Intraluminal and peripheral cell membrane staining of TPA are seen. (Immunoperoxidase stain ×100)

microvilli. Heavy staining was noted on these microvillous surfaces (Fig. 4). The staining appeared to be uniform in some areas whereas in others it had a beaded appearance. No other site of distinct immune reaction for TPA was seen in the cells.

Discussion

Tissue polypeptide antigen (TPA) is a complex protein which was originally isolated from a pool of homogenized human cancer tissues of different sites and types (Bjorklund and Bjorklund 1957; Bjorklund et al. 1973; Bjorklund 1980). Biochemically, 4 subunits of A, B_1, B_2, and C have been isolated after dissociation of TPA, of which subunit B_1 has been found potentially to be the major antigenically active material (Luning et al. 1980). Subunit B_1 of TPA can also be purified from liver metastases of bronchial carcinoma. It has an apparent molecular weight of 43000 daltons (Wiklund et al. 1981). Using a haemagglutination inhibition assay (Bjorklund et al. 1973) or, more recently, a radioimmunoassay (Bjorklund et al. 1980), elevated levels of TPA have been found in the sera and other body fluid of patients with malignant tumours and much less frequently in those with benign tumours, infectious diseases and normal subjects (Bjorklund et al. 1973; Menendez-Botet et al. 1978; Inoue et al. 1985). More than 80% of patients with carcinomas of the lung, colon, pancreas, and lymphomas have been found to have elevated levels of serum TPA (Bjorklund 1980).

Previous investigators reported that 11 of 89 patients with benign ovarian tumours (12%), 6 of 18 with low grade malignant tumours (33%) and

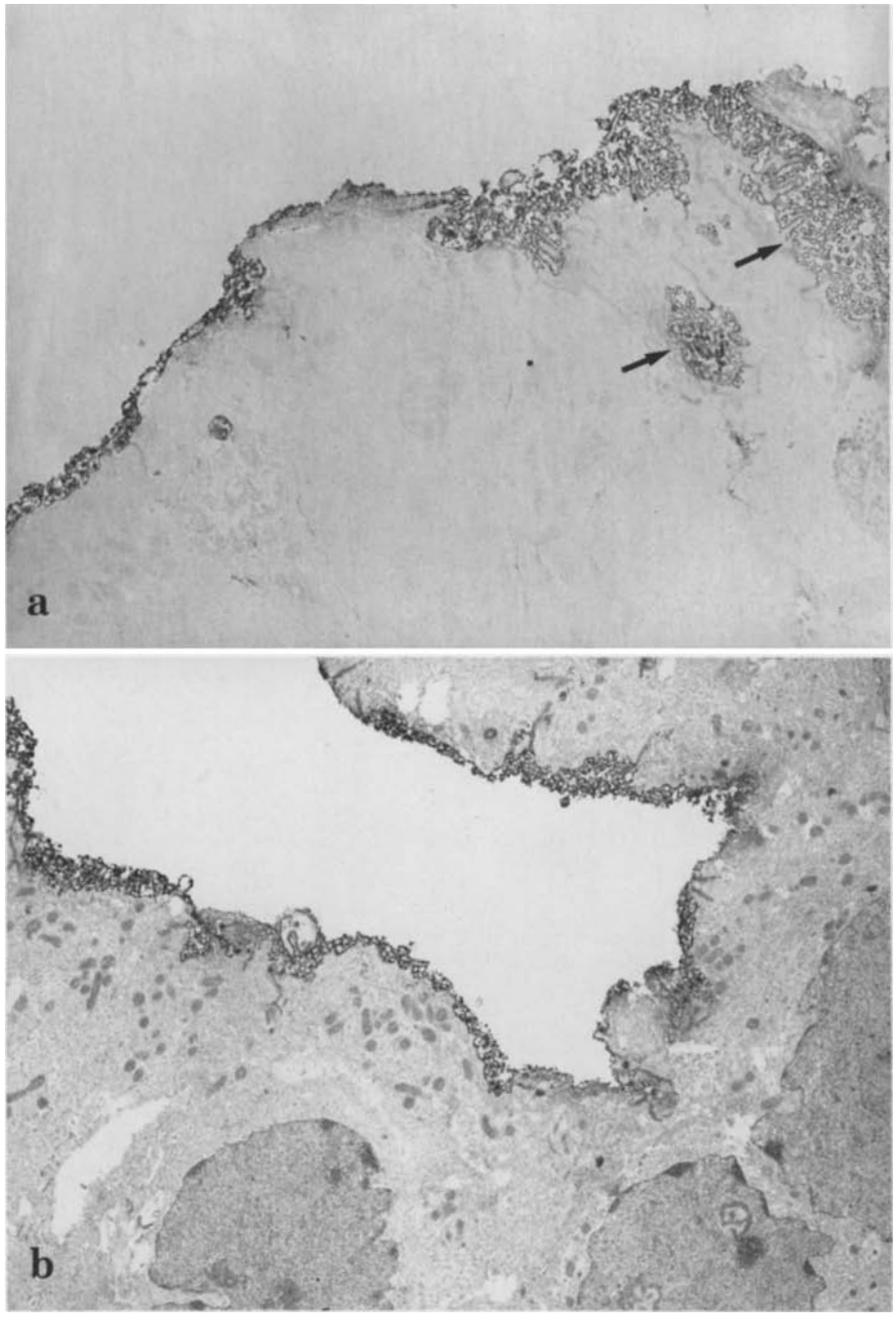

Fig. 3a, b. Electron micrograph of an ovarian cystadenocarcinoma. **a** Electron opaque reaction products of TPA are present at the cell surface and microvillous surfaces. Immunostaining is also seen in the intercellular spaces (*arrows*) where the membranes of two adjacent cells have separated. **b** Another area of tumour cells shows staining of TPA at the cell surfaces. The section is also stained with uranyl acetate and lead citrate. (Immunoperoxidase stain ×8000)

48 of 66 with malignant tumours (73%) had elevated serum TPA levels (Inoue et al. 1985). Among patients with malignant tumours the percentage of those having elevated TPA values increased in parallel with the stage of the disease and were 44% in stage I, 63% in stage II, and 88% in stage III–IV diseases.

The present immunohistochemical study revealed that 77% of the malignant and 30% of the benign ovarian tumours were TPA positive, which was in accord with the previous serum findings (Bjorklund 1980; Inoue et al. 1985). Of the patients with metastases, 80% had TPA positive tumours. These results were confirmatory and complementary to the findings of TPA in the serum and suggest that testing for TPA in ovarian tumours has a limited prognostic importance and poor diagnostic value. In this series, the serum TPA levels were not available to allow a reliable correlative study in the majority of cases.

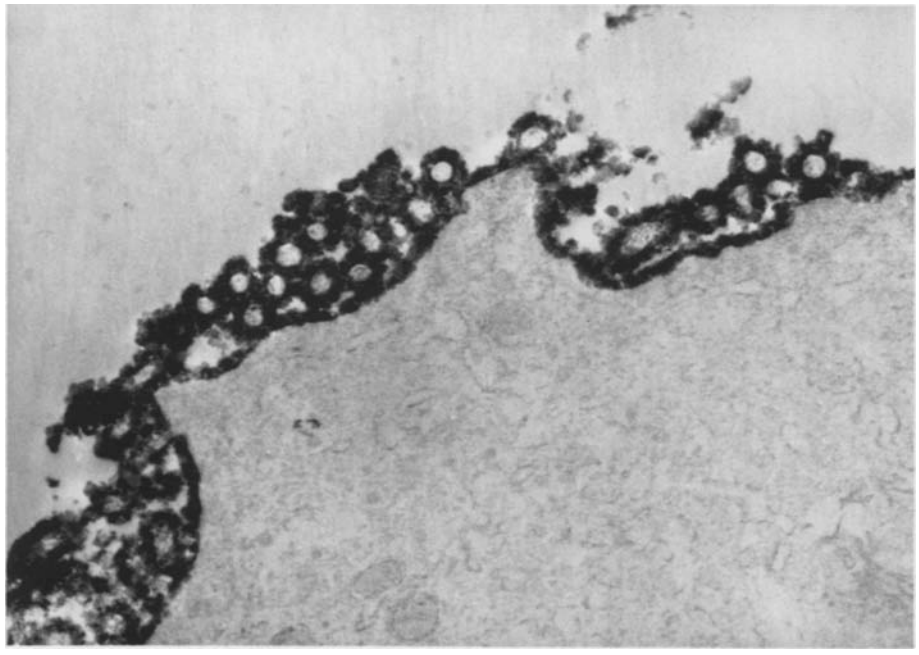

Fig. 4. Electron micrograph of an ovarian cystadenocarcinoma shows electron-dense reaction products for TPA at the cell surface and microvillous surfaces with a particulate character. (Immunoperoxidase stain × 40000)

Very little is known about the exact site of secretion and synthesis of TPA at the cellular level. The present light microscopic observation showed the staining for TPA to be confined to the cell surfaces. The ultrastructural localization of TPA confirmed this finding in that the electron-dense reaction products were exclusively located on the cell surfaces including microvillous surfaces. No other site of definite immune reaction for TPA could be observed in the cells. This finding was suggestive of secretion and probably synthesis of TPA at the cell membrane.

Continued study of the expression of different tumour markers in histological material may result in greater precision in diagnosis and prognosis as well as opening new ways for therapy.

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