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ENDODERMAL SINUS TUMOR : Immunophenotypic expression of a carcinoma

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A series of five endodermal sinus tumors was studied for their cytoskeletal and other phenotypic markers. They included 2 ovarian, 2 testicular, and 1 inguinal tumors. The cytoskeletal expression was also studied by gel electrophoresis and immunoblotting.

Every tumor was diffusely and strongly immunostained for cytokeratin. By SDS-PAGE and immunoblotting, cytokeratins 8 & 18 were detected. Vimentin was focally coexpressed in 4 cases. The stroma was diffusely immunostained for vimentin. None of them expressed desmin, neurofilament, or glial filament protein. Desmoplakin was expressed only in one ovarian tumor. Alpha-fetoprotein and S-100 protein were also diffusely positive among the neoplastic cells ; intracytoplasmic globules were especially strongly immunostained.

These findings suggest that endodermal sinus tumors represent a group of pure malignant epithelial neoplasms, and may be regarded as primitive carcinomas.

Key Words : *Endodermal sinus tumor, Cytokeratin, Immunohistochemistry.*

INTRODUCTION

Endodermal sinus tumor(EST) is a malignant germ cell tumor affecting children and young adults. EST may be reminiscent of yolk sac histologically (Teilum, 1959 ; Nogales-Fernandez et al., 1977) ; however, the precise phenotypic nature of this tumor has been a subject of debate. Generally, intermediate filament(IF) proteins are thought to be cell-type-specific both in normal cells and neoplasms derived therefrom. Therefore antibodies to IF have been widely used in the characterization and differential diagnosis of human tumors (reviewed by Gabbiani et al., 1981 and Moll et al., 1982). The IF profile of various kinds of germ cell tumors including EST has been characterized (Miettinen et al., 1985 ; Michael et al., 1989). It has been reported that both embryonal carcinoma and EST expressed cytokeratin. In contrast, dysgerminomas did not express cytokeratin but express vimentin focally(Miettinen et al., 1985). Neoplastic cells as well as stromal cells have also been reported to express vimentin-(Michael et al., 1989). Teratomas, including both the mature and immature form, showed variable expression of IF proteins in various components of differentiation(Lahdenne et al., 1990). Lahdenne et al.(1990) has also observed that scattered tumor cells in EST and embryonal carcinoma were positive for S-100 protein. In addition to intermediate fila-

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ments, they have been reported to express alpha-fetoprotein, alpha-1-antitrypsin(Prat et al., 1982), type IV collagen, laminin(Barsky and Hannah, 1987), and human chorionic gonadotropin(Cohen et al., 1987). In this paper, we report the cytoskeletal complements as well as the expression of desmoplakin, S-100 protein and alpha-fetoprotein(AFP) in five EST's.

MATERIALS AND METHODS

Five cases of EST were studied by light microscopy, immunohistochemistry, and gel electrophoresis with immunoblotting. They included two ovarian, two testicular, and one inguinal mass. Pertinent clinical and pathologic findings are summarized in Table 1. The tumors were completely excised, in cases 1,2 and 5, whereas in case 3, debulking surgery was performed due to diffuse intraabdominal tumor spreading. Only an incisional biopsy was done in case 4 in which the original tumor measured 10cm in diameter.

For light microscopic examination, representative sections were fixed in 10% buffered formalin, embedded in paraffin and stained with hematoxylin and eosin. For immunohistochemistry, representative sections were snap frozen in isopentane cooled in liquid nitrogen and were stored at –70℃. They were cut into 5 micron sections, air-dried, fixed in cold acetone for 10 minutes, and immunostained by ABC method according to Hsu et al.(1981). The sections were incubated with adequately diluted antibodies in a moist chamber for 40 minutes, followed by biotin-labeled goat anti-mouse IgG and avidin-horseradish peroxidase conjugate. For color-development, 3,3-diamino-benzidine-tetrahydrochloride was used. Antibodies used were as follows ; monoclonal antibodies against cytokeratin 10, 17 and 18 (MNF116 : Dakopatts), anti-cytokeratin 8, 18 (CAM 5.2 : Becton Dikinson), vimentin, neurofilament, desmin, glial filament protein (BioGenex), desmoplakin (Boerhinger Manheim), polyclonal antibodies to alpha-fetoprotein (BioGenex), and S-100 protein (Dakopatts).

For gel electrophoresis and immunoblotting, cytoskeletal fractions were prepared (Achstaetter et al., 1986). Tissue samples were minced in cold PBS and centrifuged. The pellet was homogenized in low-salt buffer and then high-salt buffer containing 1.5M KCl by Dounce homogenizer. The final pellet was washed and centrifuged with PBS. The washed pellet was used directly for gel electrophoresis and immunoblotting. For electrophoretic separation of cytoskeletal proteins, 10% polyacrylamide gel was used. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis(SDS-PAGE) and protein transfer onto nitrocellulose paper were performed. The membrane was preincubated in the PBS containing 0.05% Tween 20 and then was incubated in the same solution containing the anti-cytokeratin antibodies. After thorough washing and applying alkaline phosphatase-conjugated goat anti-mouse antiserum (Sigma, 1 : 10,000), it was visualized with alkaline phosphatase substrate (Pierce. USA).

Table 1. Clinical summary of 5 EST's

	Age(years)	Sex	Location	Size or Weight
Case 1	29	F	ovary	15X10X8cm
Case 2	2	M	testis	4X3.5X1.5cm
Case 3	22	F	ovary	2250gm
Case 4	1	F	inguinal area	10cm in diameter
Case 5	0.5	M	testis	3X2X1cm

RESULTS

By light microscopic examination, tumors consisted of reticular areas or solid sheets and many rounded papillae of cuboidal epithelial cells with central blood vessels (Schiller-Duval bodies)(Fig. 1). Schiller-Duval bodies were easily seen in every case. Epithelial cells had uniform, rounded nuclei and abundant eosinophilic cytoplasm. Mitotic figures were not frequent. The intermingled stroma consisted of prominent blood vessels and loosely arranged spindle cells. No other mesenchymal elements such as cartilage, bone, muscle, or neural component were seen. Intracytoplasmic hyaline glo-

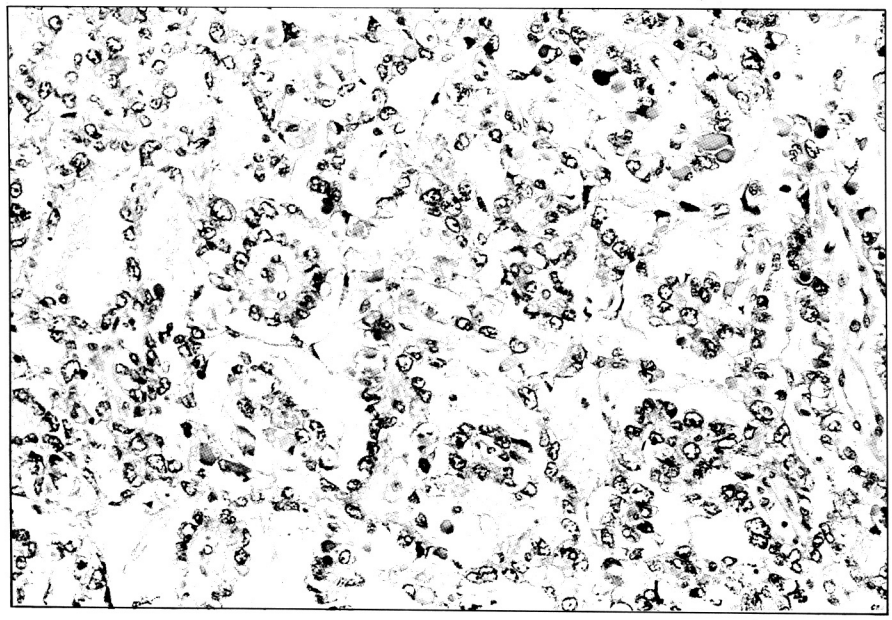

Fig. 1. Endodermal sinus tumor of the ovary (case 1) : Note loose reticular pattern and rounded epithelial papillae with central capillary (Schiller-Duval body) and intracytoplasmic globules are also seen. (H&E, × 100)

Table 2. Immunohistochemical staining results of 5 EST's

	Cytokeratin		Desmoplakin		Vimentin		Desmin		GFP		NF		S-100		AFP	
	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S
Case 1	+++	−	+++	−	−	+++	−	−	−	−	−	−	+*	−	++	−
Case 2	+++	−	−	−	++*	+++	−	−	−	−	−	−	−	−	+	−
Case 3	+++	−	−	−	++*	+++	−	−	−	−	−	−	+*	−	+++	−
Case 4	+++	−	−	−	++*	+++	−	−	−	−	−	−	+*	−	+	−
Case 5	+++	−	−	−	++*	+++	−	−	−	−	−	−	+*	−	++	−

E : epithelial cells, S : stromal cells
− : negative, + : weakly positive, ++ : moderately positive, +++ : strongly positive
* : focal immunoreactivity
GFP : glial filament protein, NF : neurofilament

bules were consistently found in epithelial cells. They varied in amount, and were exceedingly frequent and large in case 1. Extracellular hyaline globules were rarely seen. One testicular EST (case 2) was extensively necrotic and Schiller-Duval bodies were comparatively rare.

The immunohistochemical results are summarized in Table 2. In all five cases, epithelial components were diffusely and strongly immunostained for cytokeratin (Fig. 2). In four cases, vimentin was focally expressed (Fig. 3). Stromal cells were negative for cytokeratin, but were diffusely immunostained for vimentin in all cases. In case 4, epithelial cells had rather elongated cytoplasm in which vimentin coexpression was especially prominent. Desmoplakin was expressed among the epithelial cells only in one case (Fig. 4). Both epithelial and mesenchymal cells were negative for any other in-

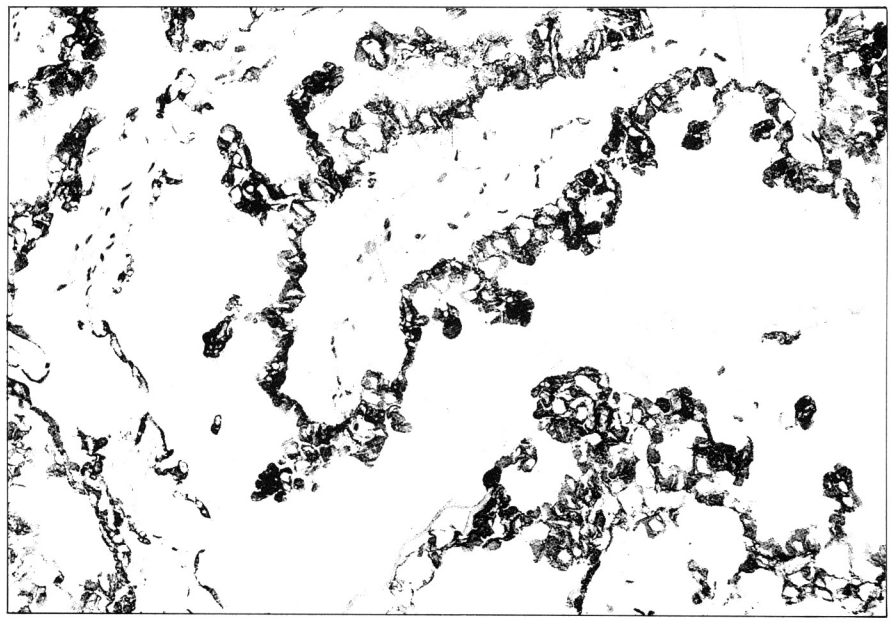

Fig. 2. The distribution of cytokeratin positive cells (case 1) : Cuboidal cells of Schiller-Duval body are strongly immunostained for cytokeratin. (ABC, ×200)

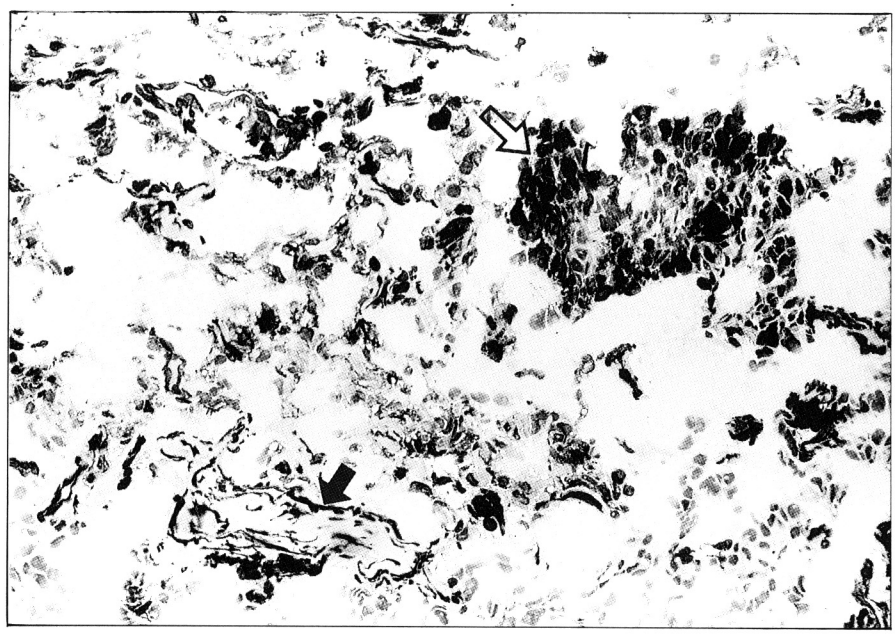

Fig. 3. Immunohistochemistry for vimentin (case 4) : Tumor cells (empty arrow) as well as spindle stromal cells (solid arrow) are strongly positive for vimentin. (ABC, ×200)

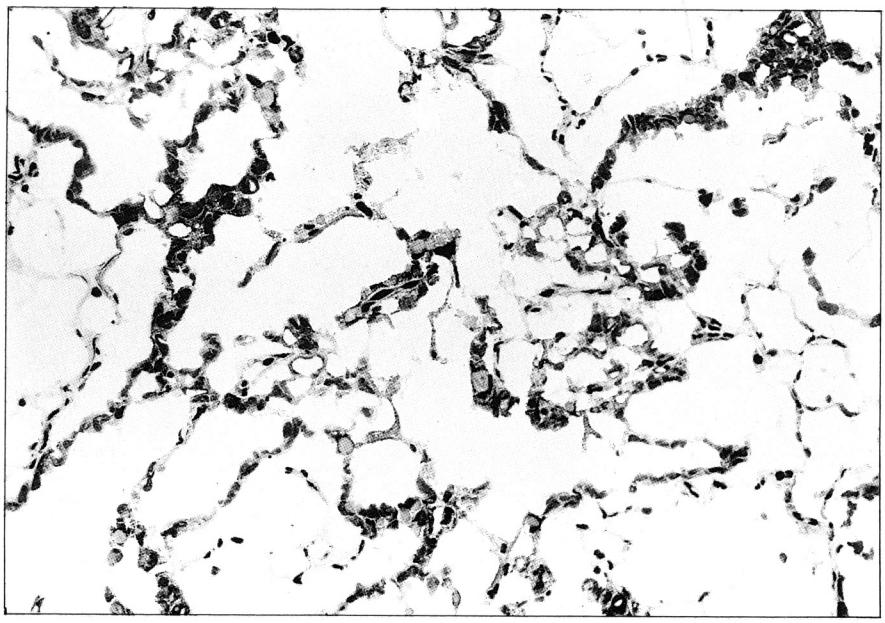

Fig. 4. Immunohistochemistry for desmoplakin (case 1) : Note fine dot-like immunoreactivity pattern on tumor cell surface. (ABC, ×200)

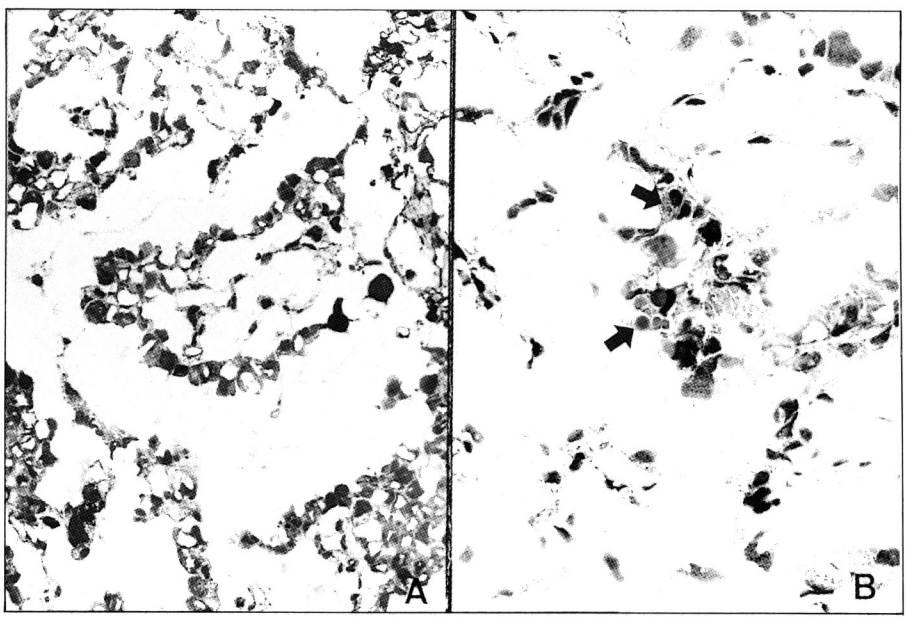

Fig. 5. Immunohistochemistry for AFP (A) and S-100 protein (B) A : Tumor cells are diffusely immunostained for AFP with variable intensity. (ABC, ×200) B : Intracytoplasmic globules as well as tumor cells (solid arrows) are weakly positive for S-100 protein. (ABC, ×200)

termediate filaments such as desmin, neurofilament, or glial filament protein. Anti-AFP antibody was diffusely immunostained epithelial cells with variable intensity (Fig. 5a). Intracytoplasmic hyaline globules were strongly stained by antibodies against both AFP and S-100 protein (Fig. 5b). Although extracytoplasmic globules were rarely seen, they were also positive for both AFP and S-100 protein.

By gel electrophoresis and immunoblotting, cytokeratin peptides 8 and 18 were detected from the samples(Fig. 6).

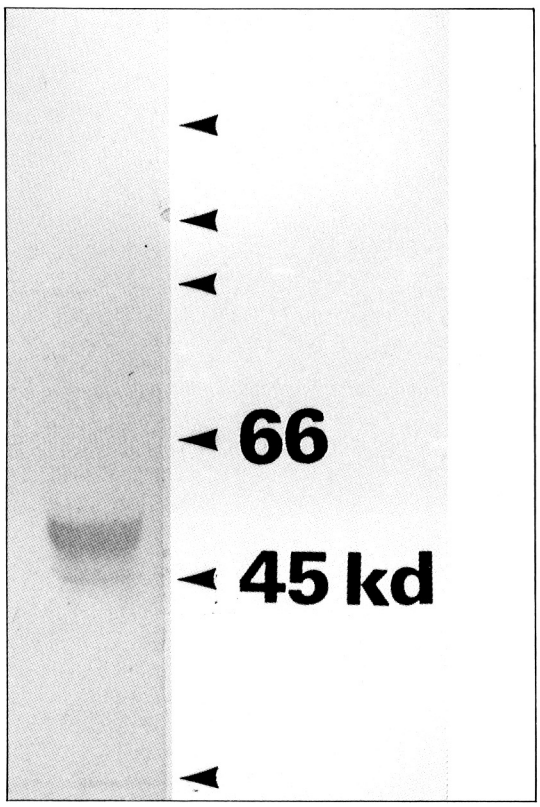

Fig. 6. Immunoblotting detection for cytokeratin proteins using monoclonal anticytokeratin 8 and 18 antibody. Both cytokeratin 8 (50Kd) and 18 (45Kd) were detected. (Arrow heads indicate molecular weight markers ; 205, 116, 97, 66, 45 and 29Kd, downward)

DISCUSSION

EST is the one of the most aggressive tumors in the ovary, testis, and many other organs occuring in a young age group. The three year-survival has been reported to be only 13% and subclinical metastasis was usual (Kurman et al., 1976). While the serum AFP level is invariably elevated, the chorionic gonadotropin level is within normal limits. According to Teilum's hypothesis, EST is generally regarded to be of germ cell origin and recapitulates normal yolk sac elements in contrast to embryonal carcinoma (Teilum, 1959).

The expression of cytokeratin in EST has been observed in previous reports (Miettinen et al., 1985 ; Michael et al., 1989 ; Lahdenne et al., 1990). Michael et al., (1989) have reported that cytokeratin was expressed in spindle cells of the "mesenchyme-like" area as well as in epithelial components. However, in our study such "stromal" cells did not express cytokeratin while epithelial cells were strongly and diffusely positive. Stromal cells were diffusely immunostained for vimentin. Epithelial components focally coexpressed vimentin as well as cytokeratin. The coexpression of cytokeratin and vimentin is observed in various kinds of epithelial cells and tumors (Holthofer et al., 1983 ; Czernobilsky et al., 1985 ; Moll et al., 1987), and may still represent an epithelial differentiation. In addition to cytokeratin, desmoplakin, which is one of dense plaque proteins in desmosomes serving as an anchoring site for IF (Cowin et al., 1985), is a marker of epithelial differentiation. Therefore epthelial cells generally express both cytokeratins and desmoplakins. Most EST's expressed only cytokeratin, which represents the phenotypic heterogeneity in EST's.

AFP and S-100 protein were diffusely immunostained, especially in intracytoplasmic eosinophilic globules. It has been reported that intracytoplasmic globules are usually stained for AFP, but may be also positive for alpha-1-antitrypsin, albumin, transferrin, fibronectin, laminin, type IV collagen and large proteoglycan having chondroitin sulfate side chain (Nakashima et al., 1990 ; Joseph et al., 1990 ; Harms et al., 1986 ; Barsky et al., 1987 ; Jacobsen, 1990). As was reported by Lahdenne et al.(1990), we also observed S-100 protein positivity in epihtelial cells. S-100 proteins are known to be present in the nucleus and cytoplasm of variable cells such as

melanocytes, chondrocytes, adipocytes, as well as neural cells (Nakajima et al., 1982 ; Perentes et al., 1987). However it is not clear whether the presence of S-100 protein represents any specific form of differentiation in EST.

The expression pattern of IF strongly supported the view that EST was a pure malignant epithelial neoplasm. In distinction from teratomas, EST's expressed cytokeratin diffusely. They also expressed various markers of differentiation concomitantly. Therefore, based on the phenotypic expression, EST may be regarded as a special type of primitive carcinoma.

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