Virchows Archiv A Pathol Anat (1992) 421:401–408

Virchows Archiv A
Pathological Anatomy and Histopathology

Epithelioid sarcoma: an electron-microscopic, immunohistochemical and DNA flow cytometric analysis

Tsuyoshi Ishida [1], Teruaki Oka [1], Hiroshi Matsushita [2], and Rikuo Machinami [1]

[1] Department of Pathology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
[2] Department of Pathology, Toranomon Hospital, Okinaka Memorial Institute for Medical Research, Tokyo 105, Japan

Received February 12, 1992 / Received after revision April 27, 1992 / Accepted April 28, 1992

Summary. Eight epithelioid sarcomas (ES) were studied by electron microscopy, immunohistochemistry, and DNA flow cytometry. Ultrastructurally, the tumour cells showed desmosome-like intercellular junctions and numerous microvilli, in addition to whorled arrangements of intermediate filaments. Tumour cells were positive for epithelial membrane antigen, cytokeratin, and vimentin, and negative for carcinoembryonic antigen and desmin. All seven cases examined by flow cytometry showed diploid or hyperploid (near diploid) DNA content. This seems to correspond to the relatively long clinical course and low-grade malignant nature of ES. Although the histogenesis of ES is still uncertain, the results of this study suggest that it is a tumour of primitive mesenchymal cells with the capacity to show epithelial differentiation.

Key words: Epithelioid sarcoma – Immunohistochemistry – Cytokeratin – DNA flow cytometry

Introduction

Epithelioid sarcoma (ES) is a relatively rare, but distinctive, soft tissue neoplasm that has a tendency to occur in the dermis and the fascial planes of the hand, forearm, and pre-tibial region. Since Enzinger's first report of 62 cases in 1970 (Enzinger 1970), many further cases of ES have been reported (Gabbiani et al. 1972; Bryan et al. 1974; Prat et al. 1978; Machinami et al. 1982; Miettinen et al. 1982a; Chase and Enzinger 1985; Mukai et al. 1985; Daimaru et al. 1987; Manivel et al. 1987; Hochstetter et al. 1991). However, the histogenesis of ES is still obscure. Synovial cells (Gabbiani et al. 1972; Machinami et al. 1982; Miettinen et al. 1982a), myofibroblasts (Blewitt et al. 1983), and primitive mesenchymal cells (Manivel et al. 1987; Fischer 1988), have all been considered to be the cells of origin. The histogenesis of ES has also been considered to be that of a variant of malignant fibrous histiocytoma (Soule and Enriquez 1972).

Under light and electron microscopy, ES cells show not only an epithelioid appearance, but also such epithelial characteristics as positive immunoreactivity to several epithelial markers, including cytokeratin, epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA) (Chase et al. 1984; Mukai et al. 1985; Daimaru et al. 1987; Schmidt and Harms 1987; Gerharz et al. 1990). We have examined eight cases of ES immunohistochemically, and ascertained the epithelial nature of ES.

There have recently been a number of reports on DNA flow cytometric analysis, using paraffin-embedded material, of many kinds of tumours, including sarcomas (Kreicbergs et al. 1984, 1987; Mankin et al. 1985). However, reports of quantitative DNA analysis of ES are still few (Molenaar et al. 1988, 1989; Stenman et al. 1990). This paper also reports the nuclear DNA flow cytometric analysis of ES, using paraffin-embedded material.

Materials and methods

Eight cases of ES, taken from the files of the Department of Pathology, University of Tokyo, from 1957 to 1990, were studied. The material was fixed with 10% formalin and embedded in paraffin. Paraffin sections were stained with haematoxylin and eosin, Azan-Mallory, and periodic acid-Schiff (PAS) staining. Colloidal iron stain with or without hyaluronidase pre-treatment was also used for light microscopy.

Two cases (cases 5 and 7) were examined electron microscopically. Small fragments of fresh tumour tissues were fixed in 3% glutaraldehyde in a 0.1 M cacodylate buffer at 4° C; they were then post-fixed in 1% osmium tetroxide and embedded in Epon 812. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined in a Jeol JEM-1200 EX electron microscope.

Immunohistochemical studies were performed on all cases by the avidin-biotin peroxidase complex (ABC) method (Hsu et al. 1981), using paraffin sections. Immunohistochemical reagents were

Correspondence to: T. Ishida

primary mouse anti-human antisera for cytokeratins, vimentin (Dako), desmin (Dako), EMA (Dako), and CEA (Dako). Two kinds of cytokeratin were examined: KL-1 (Immunotech) and CAM5.2 (Becton-Dickinson). The sections were digested with 0.1% trypsin for 15 min at room temperature before incubation with CAM5.2 and anti-desmin antisera. Normal mouse IgG was used for a negative control instead of the primary antibodies. Endogenous peroxidase was blocked by incubation of the slides for 10 min in 3% hydrogenperoxide. After being washed with phosphate-buffered saline, the slides were incubated with primary antibodies overnight at 4° C; they were then exposed to biotinylated anti-mouse immunoglobulins and the ABC complex was applied.

Nuclear DNA content was measured in seven cases by DNA flow cytometry. Human lymphocytes, obtained from a normal lymph node, were fixed in 10% formalin and embedded in paraffin and used as external staining controls of the normal diploid (2C) DNA content. Single cell suspensions of ES were prepared from formalin-fixed and paraffin-embedded material according to the method of Hedley et al. (1983). Sections 50 µm thick were deparaffinized and suspended in 0.5% pepsin (Sigma) in 0.9% sodium chloride, adjusted to pH 1.5 with 2M hydrochloric acid, and incubated for 60 min at 37° C. The supernatants of the free cell suspensions were washed twice in physiological saline solution and in 1.0% sodium citrate at 4° C. The nuclei were stained with a solution of 5 µg/ml propidium iodide (Sigma) in 1.0% sodium citrate, containing 0.2% triton-X, for 30 min at 4° C. Nuclear DNA content was measured in 2000–10000 cells from each tumour, using a flow cytometer (Cytron, Ortho). The DNA index (DI) (Barlogie et al. 1983) was calculated as the ratio of the peak channel of the DNA stem line of the tumour cells to the peak channel of the diploid peak of control lymphocytes. The DNA histograms were divided into three groups: diploid DNA histograms in which one major diploid peak ($0.8 < DI < 1.2$) was observed; hyperploid histograms, which had one major peak showing $DI > 1.2$; and aneuploid histograms, which had more than one G_0/G_1 peak. The percentage of S- and G_2/M-phase cells was calculated as described by Baisch et al. (1975). The mean coefficient of variation of the G_0/G_1 peak for the seven interpretable histograms was 6.9.

Results

The clinical data are summarized in Table 1. The distal extremities were the most common primary sites. Long-standing subcutaneous or deep-seated hard nodules (Fig. 1) or ulcers were the initial symptoms. Wide resection or amputation was carried out after diagnosis in most

Table 1. Clinical data of epithelioid sarcoma patients

Case no.	Age	Sex	Site	Ulcer	Duration of symptoms (years)	Treatment	Local recurrence	Metastasis	Follow-up (years)
1	23	F	Lower leg	–	4	WR	–	–	7 NED
2	25	M	Thigh	–	3	LE	+	–	1 NED
3	29	F	Wrist	+	8	BEA	?	?	?
4	31	M	Forearm	–	9 months	WR	?	?	?
5	31	M	Forearm	–	2	WR	–	–	1 NED
6	40	F	Foot	+	14	BKA	+	+[a]	10 Alive
7	44	F	Forearm	–	20	BEA	+	+[b]	9 Died
8	46	M	Sole	+	6	BKA	+	–	6 NED

WR, Wide resection; BEA, below-elbow amputation; BKA, below-knee amputation; LE, local excision; NED, no evidence of disease

[a] Lung

[b] Breast, oesophagus, lung

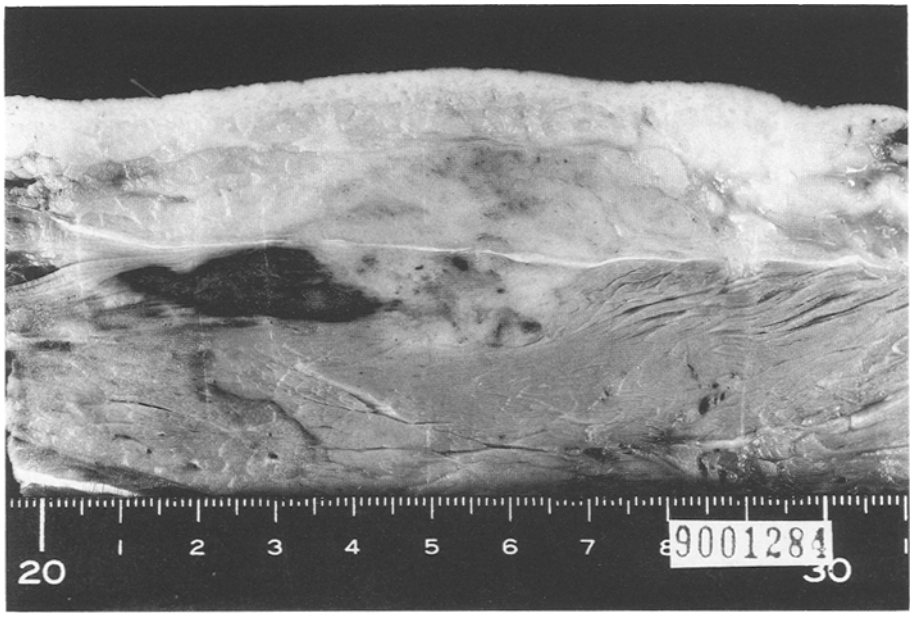

Fig. 1. Grey-white, firm, deeply seated epithelioid sarcoma is tightly attached to the fascial plane and extends to the subcutis and musculature (case 5). A haemorrhage focus was found on the left side of the tumour

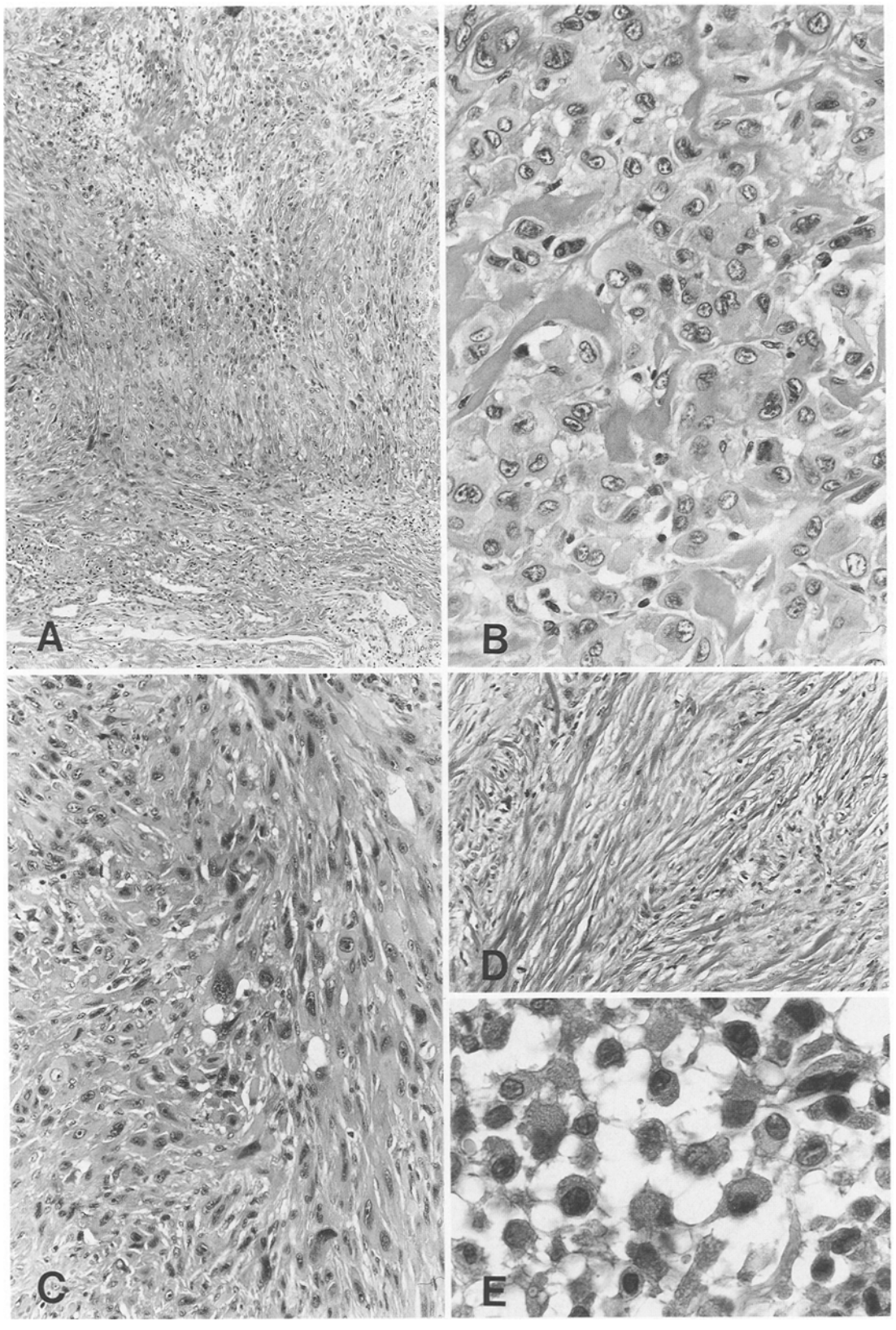

Fig. 2. **A** Granulomatous tumour nodule with central necrosis (case 1); haematoxylin and eosin, ×85. **B** Sheet-like proliferation of polygonal tumour cells showing epithelioid appearance (case 1); haematoxylin and eosin, ×340. **C** Gradual transition between epithelioid cells and spindle cells. A large tumour cell is observed in the centre (case 5); haematoxylin and eosin, ×170. **D** Fibroblast-like spindle-shaped tumour cells (case 2); haematoxylin and eosin, ×170. **E** Non-cohesive tumour cells showing rhabdoid appearance (case 2); haematoxylin and eosin, ×680

cases. In three patients, several local recurrences necessitated the amputation of the involved extremity. Four patients are well, without evidence of tumour, 1–7 years after surgical treatment, and one patient is alive with lung metastasis 10 years after the initial diagnosis. One patient died because of distant metastases 9 years after diagnosis.

On light microscopy the nodular growth pattern of the tumours was conspicuous and degeneration or necrosis was found in the centre of tumour nodules (Fig. 2A). The cells were polygonal or plump with abundant densely eosinophilic cytoplasm showing epithelioid features (Fig. 2B). In one patient who had had pre-operative chemotherapy with *cis*-diamine dichloroplatinum and pirarubicin (case 5), the cells showed moderate pleomorphism (Fig. 2C). Multinucleated giant cells were not observed. Transition between epithelioid cells and spindle-shaped fibroblast-like cells was common (Fig. 2D). Distinctive biphasic patterns, however, were not seen in any of the eight cases. Single or a few mitotic figures were found on high power fields. Intercellular spaces were observed in areas where the tumour cells were loosely arranged (Fig. 2E). Various amounts of hyaluronidase-resistant and colloidal iron-positive material were seen in the intercellular spaces, fibrous stroma, and the surfaces of some tumour cells.

On electron microscopy tumour cells had polygonal or ovoid nuclei, often indented, with small amounts of heterochromatin. Prominent nucleoli were observed in some tumour cells (Fig. 3). The cytoplasm contained moderate amounts of mitochondria, polyribosomes, and rough endoplasmic reticulum. Cytoplasmic filaments (10 nm diameter) with a whorled pattern were abundant in some cells (Fig. 4). Numerous microvilli and desmosome-like intercellular junctions were occasionally observed, together with intercellular gland-like spaces. No basal lamina was seen around the tumour cells.

The results of the immunohistochemical study are summarized in Table 2. All cases showed positive immunoreactivity for KL-1 and vimentin. Four cases were also positive for CAM5.2. Immunoreactivity for cytokeratins and vimentin was found in the cytoplasm (Fig. 5A, B). All cases were also positive for EMA, and its immunoreactivity was noted exclusively on the cell membrane (Fig. 5C). These antigens were expressed not only in the epithelioid, but also in the spindle-shaped tumour cells (Fig. 5D, E). All cases were negative for CEA and desmin.

The results of flow cytometric DNA measurements are presented in Table 3. The DNA index of the tumour cells ranged from 0.91 to 1.37 (mean: 1.18). It was shown that the tumour cells had diploid or hyperploid (near

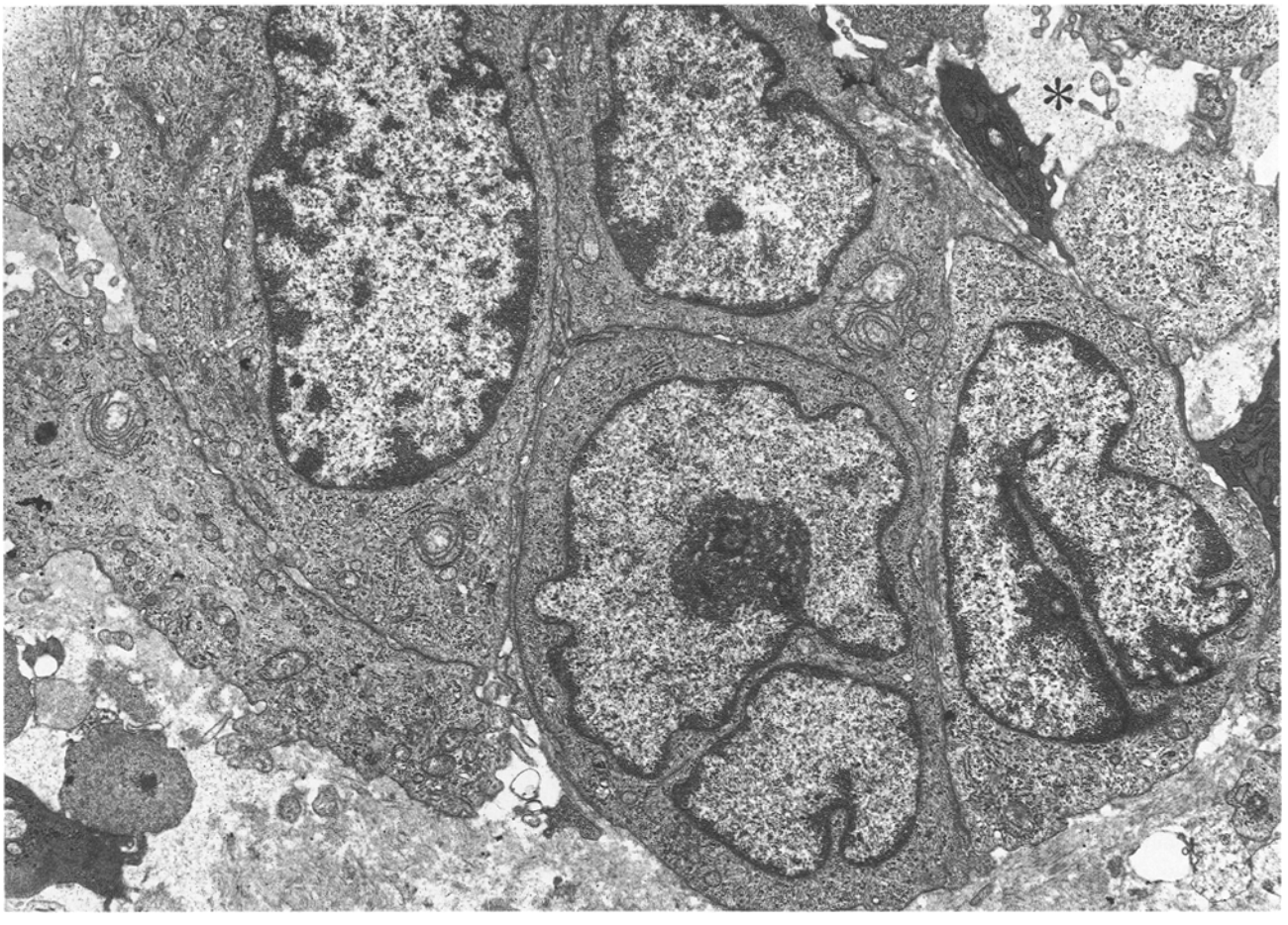

Fig. 3. Compactly packed ovoid tumour cells with gland-like space (*asterisk*) (case 7). ×10000

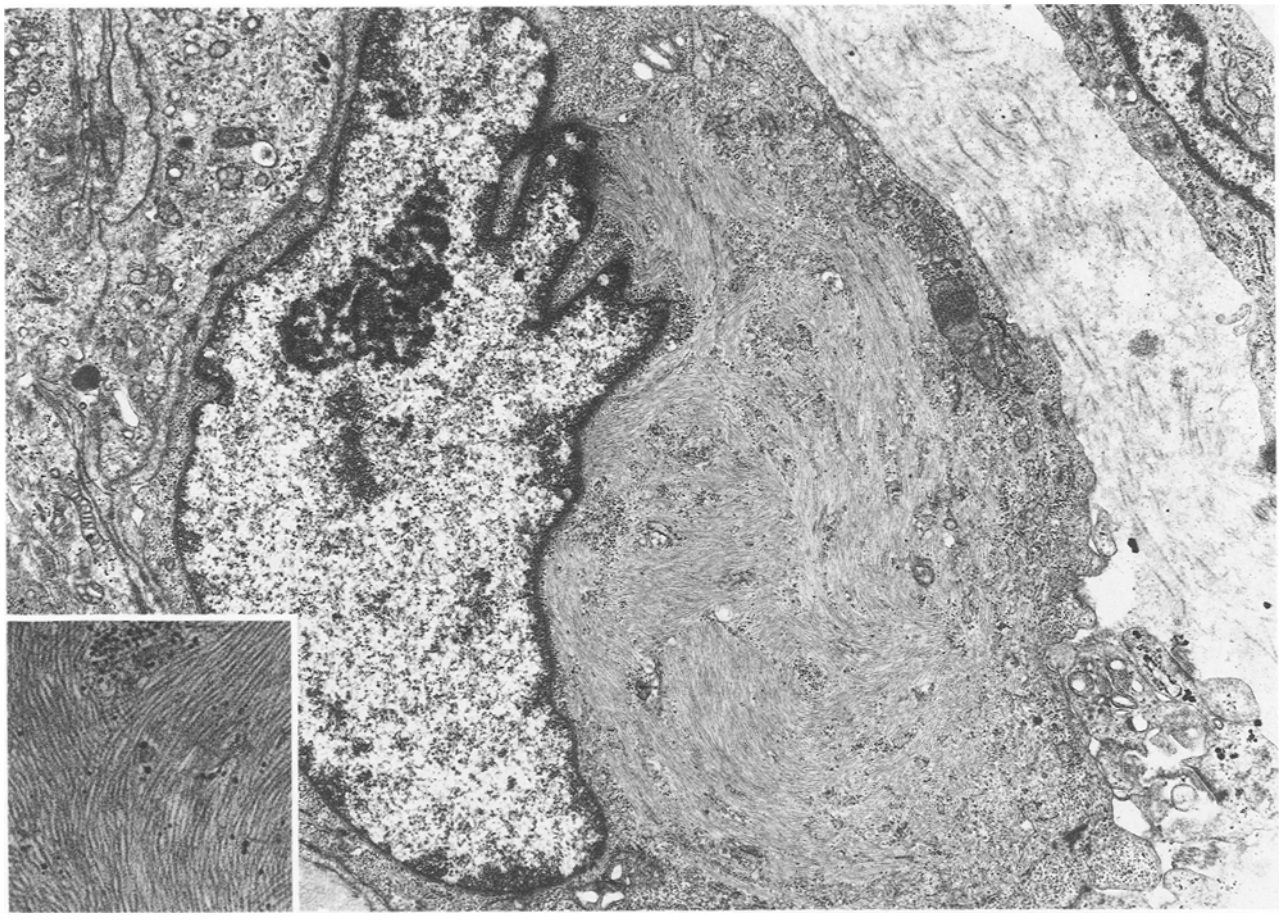

Fig. 4. Abundant intermediate filaments, showing whorled arrangement, in the cytoplasm (case 7), ×12500. *Inset*: High power view of bundles of intermediate filaments. ×40000

Table 2. Immunohistochemical data of epithelioid sarcomas

Case no.	KL-1	CAM5.2[a]	EMA	CEA	Vimentin	Desmin[a]
1	+	+	+	−	+	−
2	+	−	+	−	+	−
3	+	+	+	−	+	−
4	+	−	+	−	+	−
5	+	−	+	−	+	−
6	+	+	+	−	+	−
7	+	−	+	−	+	−
8	+	+[b]	+	−	+	−

[a] With trypsin pretreatment
[b] In recurrent tumour

Table 3. Flow cytometric DNA analysis of epithelioid sarcomas

Case no.	Ploidy	DNA index	G_0+G_1 (%)	S (%)	G_2+M (%)
1	Hyperploid	1.29	59.90	27.52	12.58
2	Hyperploid	1.37	63.34	29.69	6.97
3	Diploid	0.98	64.99	23.31	11.70
4	Diploid	0.91	49.43	28.82	21.75
5	Hyperploid	1.25	64.87	23.69	11.64
6	Diploid	1.19	65.64	20.58	13.78
7	NI	−	−	−	−
8	Hyperploid	1.27	66.30	17.78	15.91

NI, Not interpretable

diploid) stem lines (Fig. 6). No aneuploid peaks were found. The DNA content in case 7 could not be measured because of the large amount of cell debris.

Discussion

Ultrastructurally, several characteristics of ES cells have been described, including their abundant intracytoplasmic intermediate filaments, their junctional structure, filopodial or microvillous cytoplasmic processes with intercellular spaces, and the absence of basal lamina (Gabbiani et al. 1972; Machinami et al. 1982; Miettinen et al. 1982a; Mukai et al. 1985; Fischer 1988). In the two cases that were studied electron microscopically, these characteristics were also found. The abundant intermediate filaments in the cytoplasm of ES cells corresponded to the eosinophilic cytoplasm that showed an epithelioid appearance under the light microscope. Although the presence of tonofilaments in ES cells has been described

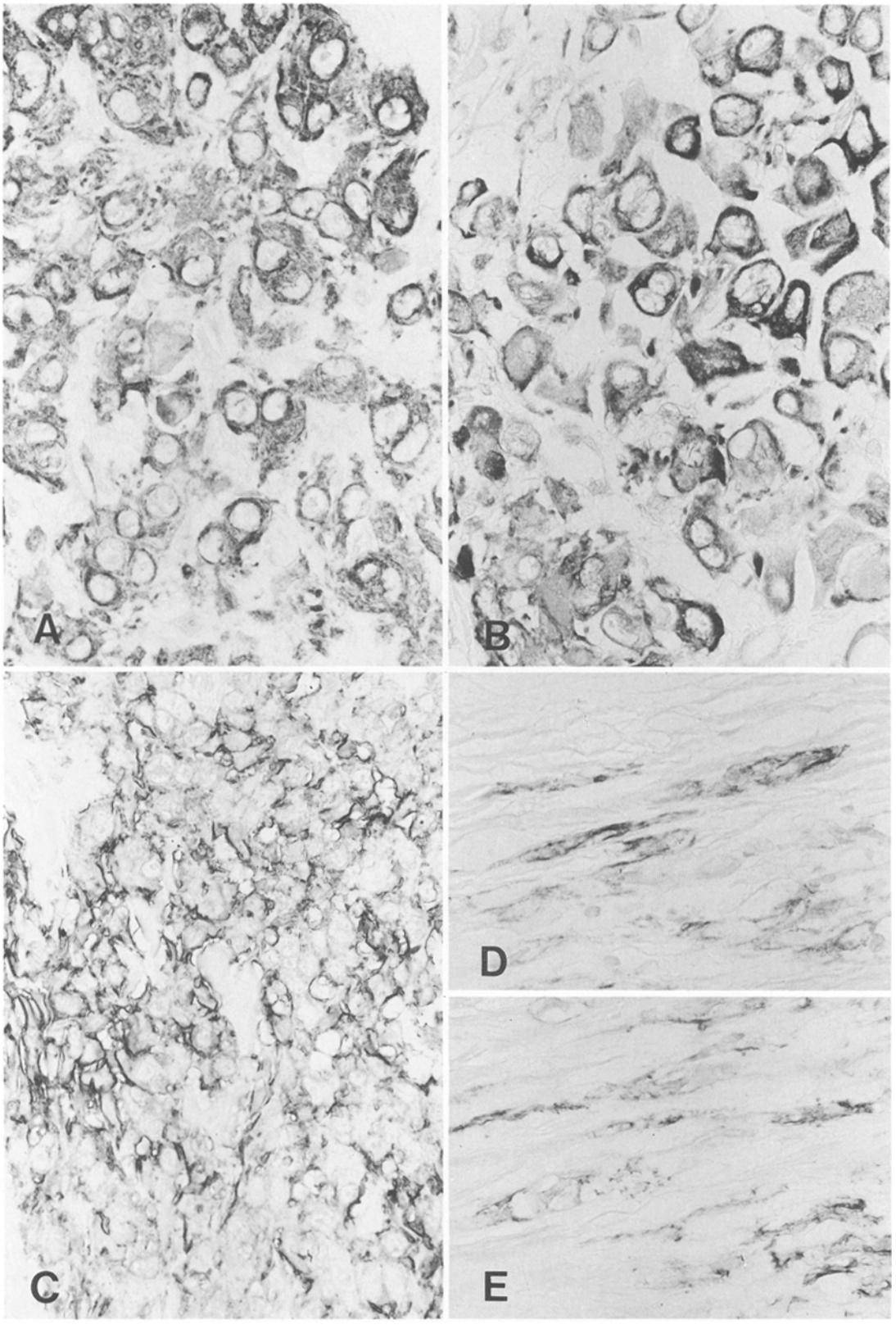

Fig. 5A–E. Immunohistochemistry of epithelioid sarcoma. **A** Epithelioid tumour cells are positive for cytokeratin (KL-1), ×450. **B** Epithelioid tumour cells are positive for vimentin, ×450. **C** Immunoreactivity for epithelial membrane antigen is shown on the surface of epithelioid tumour cells, ×450. **D** Spindle-shaped tumour cells are positive for cytokeratin (KL-1), ×450. **E** Spindle-shaped tumour cells are also positive for epithelial membrane antigen, ×450

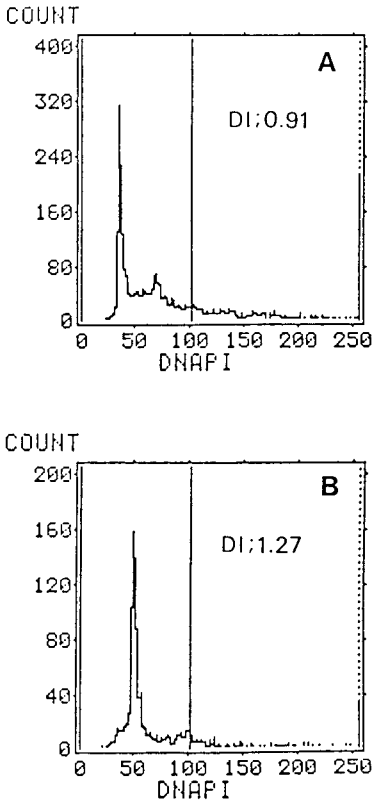

Fig. 6A, B. DNA ploidy pattern obtained by flow cytometry. **A** Diploid stem line is shown. A relatively large population of S-phase cells is seen (case 4). **B** A hyperdiploid stem line is shown (case 8). The *ordinate* shows cell number and the *abscissa* shows relative DNA content. *DI*, DNA index

by Fisher (1988), no tonofilaments were observed in our study. Desmosome-like intercellular junctions may indicate an epithelial nature for the tumour cells.

Several immunohistochemical studies of ES (Chase et al. 1984; Mukai et al. 1985; Daimaru et al. 1987; Schmidt and Harms 1987; Gerharz et al. 1990) have demonstrated immunoreactivity for EMA, cytokeratin, and vimentin. This was confirmed in our study. The presence of EMA and cytokeratin has usually been regarded as evidence of the epithelial nature of tumour cells (Mukai et al. 1985; Daimaru et al. 1987; Manivel et al. 1987). Like a carcinoma with some mesenchymal tissue, so a sarcoma might have tissue of an epithelial nature. Synovial sarcoma has been reported to show a distinctive biphasic pattern and immunohistochemically demonstrable epithelial characteristics (Miettinen et al. 1982b; Corson et al. 1983; Ordonez et al. 1990). ES, like synovial sarcoma, has a peculiar differentiation potential; the tumour cells can apparently differentiate into both mesenchymal and epithelial forms. It is understandable that Manivel et al. (1987) recommended the use of the paradoxical term "epithelial"sarcoma instead of "epithelioid" sarcoma. Cytokeratin is an intermediate filament protein that functions as a cytoskeleton forming the structural framework in epithelial cells and the presence of cytokeratin in ES and synovial sarcoma cells thus seems to be responsible for the epithelioid histological appearance. Since poorly differentiated carcinomas may often express not only epithelial but also mesenchymal markers, such as vimentin, there are thus no immunohistochemical differences between poorly differentiated carcinoma and ES (Daimaru et al. 1987; Manivel et al. 1987).

Malignant rhabdoid tumours, which consist of round or polygonal tumour cells containing filament-rich acidophilic inclusions (so-called rhabdoid cells), also express cytokeratin, vimentin, and EMA (Tsuneyoshi et al. 1985). It is sometimes difficult to differentiate malignant rhabdoid tumour from ES both histologically and electron microscopically, because rhabdoid cells are also observed in ES (Tsuneyoshi et al. 1987; Molenaar et al. 1989; Perrone et al. 1989; Chase 1990). Clinical findings are important in differentiating these two tumours.

There are few data on DNA content in ES available in the literature, but previous reports have shown that ES cells are diploid (Molenaar et al. 1988, 1989; Stenman et al. 1990). Our study also showed a diploid or hyperploid DNA content in ES. The single G_0/G_1 stem line of ES is compatible with such histological characteristics as little pleomorphism and the rarity of multinucleated cells.

Several prognostic factors have been described for ES, including location of the tumour, mitotic figures, haemorrhage, necrosis, vascular invasion, and regional lymph node metastasis (Prat et al. 1978; Chase and Enzinger 1985). Although DNA ploidy is a useful prognostic factor in many malignant tumours, all the cases of ES in this study showed a diploid or hyperploid (near diploid) pattern. It appears from this result that flow cytometric DNA analysis is not useful for predicting the prognosis of ES.

ES often recurs and sometimes metastasizes. Its clinical course, however, is relatively long (Enzinger 1970; Chase and Enzinger 1985). The DNA ploidy pattern of ES seems to correspond to the relatively low grade of malignancy and long clinical course of this disease.

References

Baisch H, Gohde W, Linden WA (1975) Analysis of PCP-data to determine the fraction of cells in the various phases of cell cycle. Radiat Environ Biophys 12:31–39

Barlogie B, Raber MN, Schumann J, Johnson TS, Drewinko B, Schwartzendruber DE, Gohde W, Andreeff M, Freireich EJ (1983) Flow cytometry in clinical cancer research. Cancer Res 43:3982–3997

Blewitt RW, Aparicio SGR, Bird CC (1983) Epithelioid sarcoma: a tumour of myofibroblasts. Histopathology 7:573–584

Bryan RS, Soule EH, Dobyns JH, Prichard DJ, Linscheid RL (1974) Primary epithelioid sarcoma of the hand and forearm. A review of thirteen cases. J Bone Joint Surg [Am] 56:458–465

Chase DR (1990) Rhabdoid versus epithelioid sarcoma (letter). Am J Surg Pathol 14:792–794

Chase DR, Enzinger FM (1985) Epithelioid sarcoma. Diagnosis, prognostic indicators, and treatment. Am J Surg Pathol 9:241–263

Chase DR, Weiss SW, Enzinger FM, Langloss JM (1984) Keratin in epithelioid sarcoma. An immunohistochemical study. Am J Surg Pathol 8:435–441

Corson JM, Weiss LM, Banks-Schlegel SP, Pinkus GS (1983) Kera-

tin proteins in synovial sarcoma (letter). Am J Surg Pathol 7:107–109

Daimaru Y, Hashimoto H, Tsuneyoshi M, Enjoji M (1987) Epithelial profile of epithelioid sarcoma. An immunohistochemical analysis of eight cases. Cancer 59:134–141

Enzinger FM (1970) Epithelioid sarcoma. A sarcoma simulating a granuloma or a carcinoma. Cancer 26:1029–1041

Fisher C (1988) Epithelioid sarcoma: the spectrum of ultrastructural differentiation in seven immunohistochemically defined cases. Hum Pathol 19:265–275

Gabbiani G, Fu YS, Kaye GI, Lattes R, Majno G (1972) Epithelioid sarcoma. A light and electron microscopic study suggesting a synovial origin. Cancer 30:486–499

Gerharz CD, Moll R, Meister P, Knuth A, Gabbert H (1990) Cytoskeletal heterogeneity of an epithelioid sarcoma with expression of vimentin, cytokeratins, and neurofilaments. Am J Surg Pathol 14:274–283

Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA (1983) Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 31:1333–1335

Hochstetter AR, Meyer VE, Grant JW, Honegger HP, Schreiber A (1991) Epithelioid sarcoma mimicking angiosarcoma: the value of immunohistochemistry in the differential diagnosis. Virchows Arch [A] 418:271–278

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Kreicbergs A, Silvfersward C, Tribukait B (1984) Flow DNA analysis of primary bone tumors. Relationship between cellular DNA content and histopathologic classification. Cancer 53:129–136

Kreicbergs A, Tribukait B, Willems J, Bauer HCF (1987) DNA flow analysis of soft tissue tumors. Cancer 59:128–133

Machinami R, Kikuchi F, Matsushita H (1982) Epithelioid sarcoma. Enzyme histochemical and ultrastructural study. Virchows Arch [A] 397:109–120

Manivel JC, Wick MR, Dehner LP, Sibley RK (1987) Epithelioid sarcoma. An immunohistochemical study. Am J Clin Pathol 87:319–326

Mankin HJ, Connor JF, Schiller AL, Perlmutter N, Alho A, McGuire M (1985) Grading of bone tumors by analysis of nuclear DNA content using flow cytometry. J Bone Joint Surg [Am] 67:404–413

Miettinen M, Lehto VP, Vartio T, Virtanen I (1982a) Epithelioid sarcoma. Ultrastructural and immunohistologic features suggesting a synovial origin. Arch Pathol Lab Med 106:620–623

Miettinen M, Lehto VP, Virtanen I (1982b) Keratin in the epithelial-like cells of classical biphasic synovial sarcoma. Virchows Arch [B] 40:157–161

Molenaar WM, Dam-Meiring A, Kamps WA, Cornelisse CJ (1988) DNA-aneuploidy in rhabdomyosarcomas as compared with other sarcomas of childhood and adolescence. Hum Pathol 19:573–579

Molenaar WM, Dejong B, Dam-Meiring A, Postima A, Devries J, Hoekstra HJ (1989) Epithelioid sarcoma or malignant rhabdoid tumor of soft tissue? Epithelioid immunophenotype and rhabdoid karyotype. Hum Pathol 20:347–351

Mukai M, Torikata C, Iri H, Hanaoka H, Kawai T, Yakumaru K, Shimoda T, Mikata A, Kageyama K (1985) Cellular differentiation of epithelioid sarcoma. An electron-microscopic, enzyme-histochemical, and immunohistochemical study. Am J Pathol 119:44–56

Ordonez NG, Mahfouz SM, Mackay B (1990) Synovial sarcoma: an immunohistochemical and ultrastructural study. Hum Pathol 21:733–749

Perrone T, Swanson PE, Twiggs L, Ulbright TM, Dehner LP (1989) Malignant rhabdoid tumor of the vulva: is distinction from epithelioid sarcoma possible? A pathologic and immunohistochemical study. Am J Surg Pathol 13:845–858

Prat J, Woodruff JM, Marcove RC (1978) Epithelioid sarcoma. An analysis of 22 cases indicating the prognostic significance of vascular invasion and regional lymph node metastasis. Cancer 41:1472–1487

Schmidt D, Harms D (1987) Epithelioid sarcoma in children and adolescents. An immunohistochemical study. Virchows Arch [A] 410:423–431

Soule EH, Enriquez P (1972) Atypical fibrous histiocytoma, malignant fibrous histiocytoma, malignant histiocytoma, and epithelioid sarcoma. A comparative study of 65 tumors. Cancer 30:128–143

Stenman G, Kindblom LG, Willems J, Angervall L (1990) A cell culture, chromosomal and quantitative DNA analysis of a metastatic epithelioid sarcoma. Deletion 1p, a possible primary chromosomal abnormality in epithelioid sarcoma. Cancer 65:2006–2013

Tsuneyoshi M, Daimaru Y, Hashimoto H, Enjoji M (1985) Malignant soft tissue neoplasmas with the histologic features of renal rhabdoid tumors: an ultrastructural and immunohistochemical study. Hum Pathol 16:1235–1242

Tsuneyoshi M, Daimaru Y, Hashimoto H, Enjoji M (1987) The existence of rhabdoid cells in specified soft tissue sarcomas. Virchows Arch [A] 411:509–514