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Case Report

Epithelioid Sarcoma

Enzyme Histochemical and Ultrastructural Study*

R. Machinami[1], F. Kikuchi[2], and H. Matsushita[3]

[1] Department of Pathology, Faculty of Medicine, Gunma University, Maebashi, Gunma 371
[2] Department of Pathology, Faculty of Medicine, Tokyo University, Tokyo 113
[3] Department of Pathology, Toranomon Hospital, Tokyo 107, Japan

Summary. A case of epithelioid sarcoma was studied by electron microscopy and by light and electron microscopic enzyme histochemistry comparing with several control soft tissues. In addition to previously reported ultrastructural features, such as abundant 10 nm cytoplasmic filaments, desmosome-like cell junctions and small cystic spaces surrounded by filopodia or microvilli of the tumor cells, we encountered 10 nm cytoplasmic filaments showing electron dense condensation with a concentrically oriented or whorled pattern and a finger-print-like arrangement and 5′-nucleotidase activity of tumor cell membrane. Among the control soft tissues, 5′-nucleotidase activity was found only in synovial and endothelial cells. Both tumor and synovial cells showed no activity of adenosine triphosphatase, while marked activity of the enzyme was found in endothelial cells. These results support the concept that epithelioid sarcoma is derived from mesenchymal cells undergoing differentiation toward synovial cells during neoplastic transformation.

Key words: Epithelioid sarcoma – Ultrastructure – 5′-nucleotidase

Since Enzinger's report (Enzinger 1970) of a series of soft tissue sarcomas which he classified as epithelioid sarcomas, several light and electron microscopic studies of these tumors have been reported (Bryan et al. 1971; Mackenzie 1971; Gabbiani et al. 1972; Fisher and Horvat 1972; Santiago et al. 1972; Soule and Enrique 1972; Frable et al. 1973; Patchefsky et al. 1977; Mackay 1977; Prat et al. 1978) and the tumor has become widely recognized. Although epithelioid sarcoma is different from synovial sarcoma

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in histological appearance, the cells of epithelioid sarcoma have been reported to show ultrastructural features similar to those of synovial sarcoma and synovitis, suggesting a synovial origin of epithelioid sarcoma (Gabbiani et al. 1972; Frable et al. 1973; Patchefsky et al. 1977). Recently, we have studied a case of epithelioid sarcoma by enzyme histochemistry and electron microscopy and comparisons with control synovial and other soft tissues have provided results to support the above view. To our knowledge, this is the first report of ultrastructural enzyme histochemistry of an epitheloid sarcoma.

Case Report

A 36-year-old woman noticed a soft tissue tumor with the size of a hen's egg in the antero-lateral part of the right mid-thigh 6 months before the tumor was resected, after a biopsy diagnosis of synovial sarcoma in April 1971. The tumor measured $11 \times 9 \times 7$ cm in size. There was local recurrence 4 years later in spite of Co^{60} irradiation, and the recurrent tumor was removed in July 1975. A second local recurrence appeared in April and a third in August 1976; each of these tumors was removed. Bloody sputum appeared in November 1977. The patient had a fourth local recurrence with removal of the tumor in September 1978. An increase of bloody sputum and "coin lesions" in the radiograph of the chest suggesting pulmonary metastasis were recognized in January 1979. The fifth and sixth local recurrences appeared in June and December 1979, and the tumors were removed again. A right hip joint disarticulation was performed because of the seventh local recurrence with elephantiasis of the right lower extemity in July 1980.

Materials and Methods

All the primary and 7 recurrent tumors were examined routinely in the light microscope. Tissues were fixed in 10% formalin, embedded in paraffin and stained by haematoxylin and eosin, alcian blue, periodic acid-Schiff (PAS), silver impregnation, and Azan-Mallory staining.

Tissues for enzyme histochemistry and electron microscopy were obtained at the sixth recurrence immediately after removal of the tumor. Tissues were fixed in 10% formol calcium at 4° C for 24 h and kept in gum-sucrose (0.88 M) (Holt 1959) at 4° C for a few days before incubation for enzyme histochemistry. Frozen sections were processed for demonstration of alkaline phosphatase, 5′-nucleotidase and adenosine triphosphatase (Burstone 1962; Wachstein and Meisel 1957). Sections incubated in media lacking the substrate were also examined for control studies. Small slices of tissues for electron microscopy were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at 4° C, post-fixed in 1% osmium tetroxide and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead, and examined in a Hitachi HU-12 electron microscope.

For ultrahistochemistry small slices of tissues fixed in 3% glutaraldehyde for 1 h and kept in 0.1 M cacodylate buffer at 4° C for a few days were embedded in agar. The tissues were then sectioned at 50 micron on a Sorvall Tissue Sectioner. The sections were processed for the demonstration of alkaline phosphatase, 5′-nucleotidase and adenosine triphosphatase (Jones and Fox 1976; Wachstein and Meisel 1957). After incubation, the tissues were post-fixed in 1% osmium tetroxide and embedded in Araldite. Ultrathin sections were examined in a JOEL 100 C electron microscope without staining. Sections incubated in media lacking the substrate were examined for control studies of ultrahistochemistry.

Control studies on the three cell membrane associated enzymes of fresh synovial and other soft tissues including the smooth and striated muscles, endothelial cells, fat cells, fibrous tissue, mesenchyme of the umbilical cord were also carried out by the same techniques described above.

Results

Light Microscopy

Nodular aggregates of relatively large and polygonal tumor cells with eosinophilic cytoplasm were surrounded by fibrous connective tissue (Fig. 1). Foci of central necrosis were found in the tumor nodules. The nuclei were round or oval without prominent nucleoli and showing slight pleomorphism. Mitotic figures were not numerous. In some parts of the tumor, the neoplastic cells were spindle in shape with gradual transition between spindle and polygonal cells. No mucin was found in the tumor cells, but only a few alcian blue positive foci were present in the intercellular substance. Fine reticulin fibers were seen among the epithelioid tumor cells.

Of the 3 membrane associated enzymes examined, only 5′-nucleotidase was positive on the tumor cell membranes (Fig. 2). Neither 5′-nucleotidase nor adenosine triphosphatase activity was detectable histologically in the control synovial and other cells except for the endothelial cells and smooth muscle (Table 1). An adenosine triphosphatase reaction was found in the blood vessels and smooth muscle. Slight activity of 5′-nucleotidase was present in the blood vessels. Only the capillaries of granulation tissue were alkaline phosphatase positive. None of the 3 membrane associated enzyme activities could be detected in the striated muscle, fibrous tissue, fat cells, and mesenchyme of the umbilical cord.

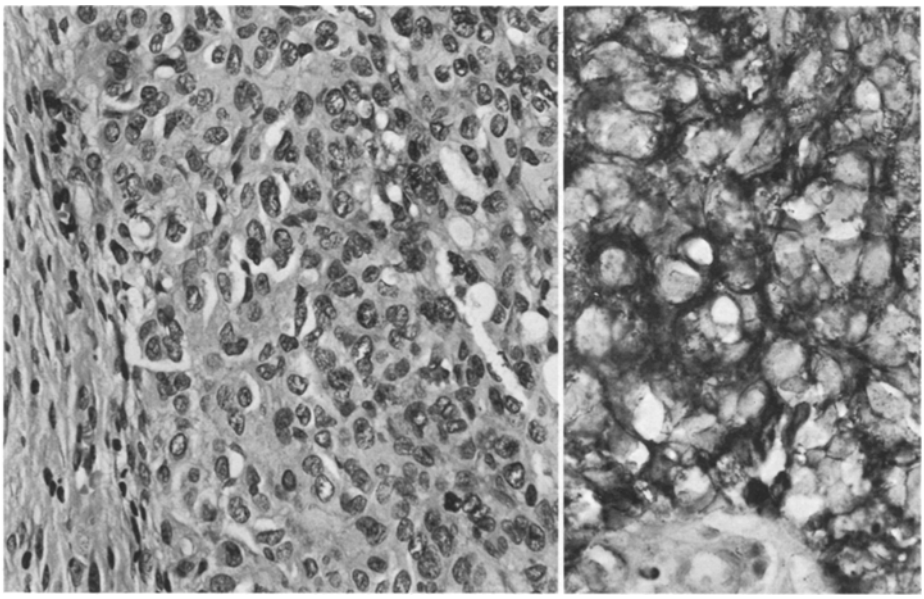

Fig. 1. An epithelioid sarcoma cell nest is surrounded by fibrous connective tissue. H.E. × 280

Fig. 2. Reaction product demonstrating 5′-nucleotidase activity can be seen on the sarcoma cell membrane. × 580

Table 1. Membrane associated enzyme histochemistry of epithelioid sarcoma and control soft tissues

	Al-P	ATP	5′-N
Epithelioid sarcoma	−	−	++
Synovial cells	−	−	+[a]
Smooth muscle	−	+	−~±[b]
Striated muscle	−	−	−
Endothelial cells	−~+[c]	++	+
Fibrous tissue	−	−	−
Fat cells	−	−	−
Mesenchyme	−	−	−

Al-P = alkaline phosphatase
ATP = adenosine triphosphatase
5′-N = 5′-nucleotidase

[a] Only visible by electron microscopy
[b] Smooth muscle of the uterus was negative but that of the stomach showed a very little and almost negligible activity in the electron microscope
[c] Blood vessels of the umbilical cord were negative, whereas capillaries of the granulation tissue were positive

Electron Microscopy

Abundant intracytoplasmic filaments, measuring about 10 nm in diameter, desmosome-like cell junctions or adherent junctions, and filopodia or microvilli were all seen in some of the tumor cells. Most of the tumor cells showed compact arrangement but small cystic spaces surrounded by filopodia or microvilli of tumor cells were occasionally found (Fig. 3). The 10 nm cytoplasmic filaments sometimes showed electron dense condensation forming concentrically oriented or whorled pattern, fingerprint like arrangement, or their mixed pattern (Figs. 4, 5 and 6). Thiner cytoplasmic filaments measuring about 3 nm in diameter were infrequently seen just beneath the cell membrane. No basal lamina was present around the tumor cells. The nuclei were mostly oval with occasional irregular cytoplasmic invagination, small nucleoli and relatively small amount of heterochromatin at the nuclear periphery (Figs. 3 and 4). Mitochondria, rough endoplasmic reticulum, lysosomes and polysomes could be seen in the tumor cells but they were not numerous. A few spindle cells showing abundant rough endoplasmic reticulum and thin cytoplasmic filaments with focal densities were found among the tumor cells. These cells were identical to myofibroblasts (Fig. 4).

Light microscopic findings of the 3 membrane associated enzymes were confirmed in the electron microscope except for the presence of 5′-nucleotidase in the control synovial cells. A reaction product demonstrating 5′-nucleotidase activity could be seen extensively on the cell membrane of tumor cells that were closely packed each other (Fig. 7). Although the enzyme activity of the control synovial cells was negative in the light microscope,

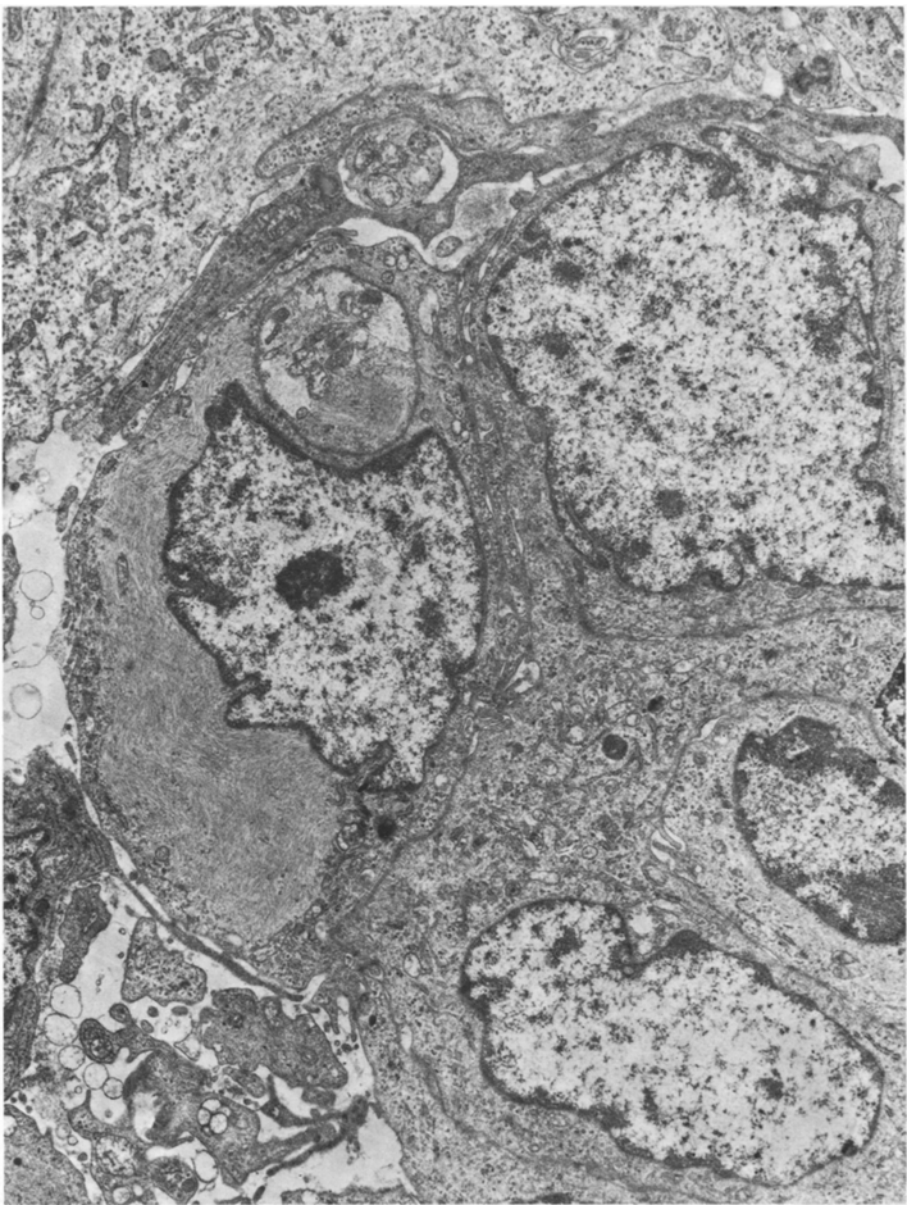

Fig. 3. Epithelioid sarcoma cells are forming a cleft-like space. Abundant intracytoplasmic filaments and desmosome-like cell junctions are characteristic of this neoplasm. × 7,200

slight activity was seen on the cell membrane in the electron microscope (Fig. 8). A very slight, almost negligible activity of 5′-nucleotidase was found in the smooth muscle of the stomach. Tissues incubated in media lacking the substrate showed consistently negative results in the light and electron microscopes.

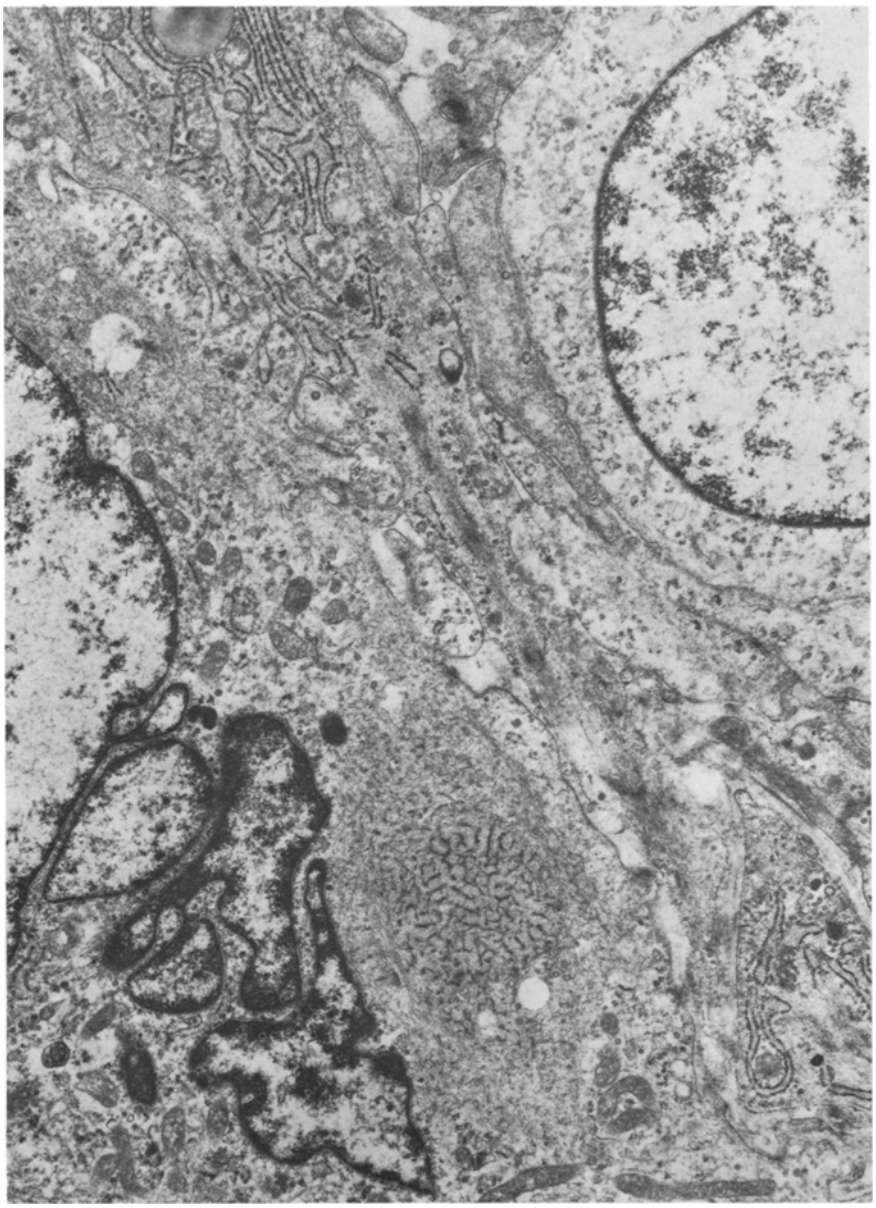

Fig. 4. An epithelioid sarcoma cell with markedly lobulated nucleus and fingerprint-like condensation of 10 nm cytoplasmic filaments can be seen. Part of a myofibroblast is present in the middle of the figure. ×11,520

Discussion

The clinical and histopathological features of this case coincide well with those of Enzinger's report (Enzinger 1970) as well as of other subsequent studies (Bryan et al. 1971; Mackenzie 1971; Gabbiani et al. 1972; Fisher

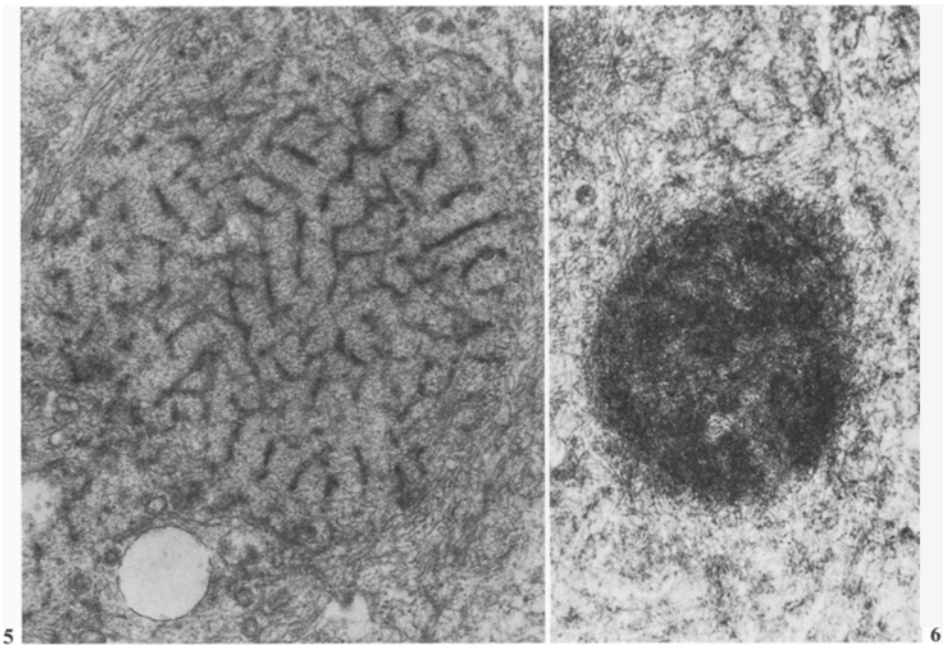

Fig. 5. Higher magnification of the fingerprint-like figure found in Fig. 4. × 36,000

Fig. 6. Cytoplasmic filaments of tumor cells, measuring about 10 nm in diameter, showing condensation with concentrically oriented or whorled pattern. × 36,000

and Horvat 1972; Santiago et al. 1972; Soule and Enriquez 1972; Frable et al. 1973; Patchefsky et al. 1977; Mackay 1977; Prat et al. 1978) describing the following characteristics. Epithelioid sarcoma is most often found in adult males as a subfascial or subcutaneous lesion of the limbs. Microscopically the tumor has a characteristic nodular appearance and is composed of both epithelioid cells with acidophilic cytoplasm and spindle cells resembling fibroblasts. Areas of focal necrosis are common, and there may be more or less abundant collagen surrounding the nodular mass of tumor cells. The tumor grows slowly, often as multiple nodules, frequently along fibrous structure such as tendons and fascia. The clinical course is relatively long over periods of several years. In spite of apparently adequate local removal, the tumor recurs frequently and may metastasize widely.

Several electron microscopic characteristics of epithelioid sarcoma cells have been reported so far describing thin and thick intracytoplasmic filaments measuring 3 to 10 nm in diameter, cell junctional apparatus such as maculae adherentes and desmosomes, and many distinct spaces surrounded by filopodia or microvilli; no basal lamina has been identified (Enzinger 1970; Gabbiani et al. 1972; Fisher and Horvat 1972; Frable et al. 1973; Patchefsky et al. 1977; Mackay 1977). These ultrastructural features of epithelioid sarcoma are similar to those of synovial sarcoma except the basal lamina, which has been observed at the base of epithelioid

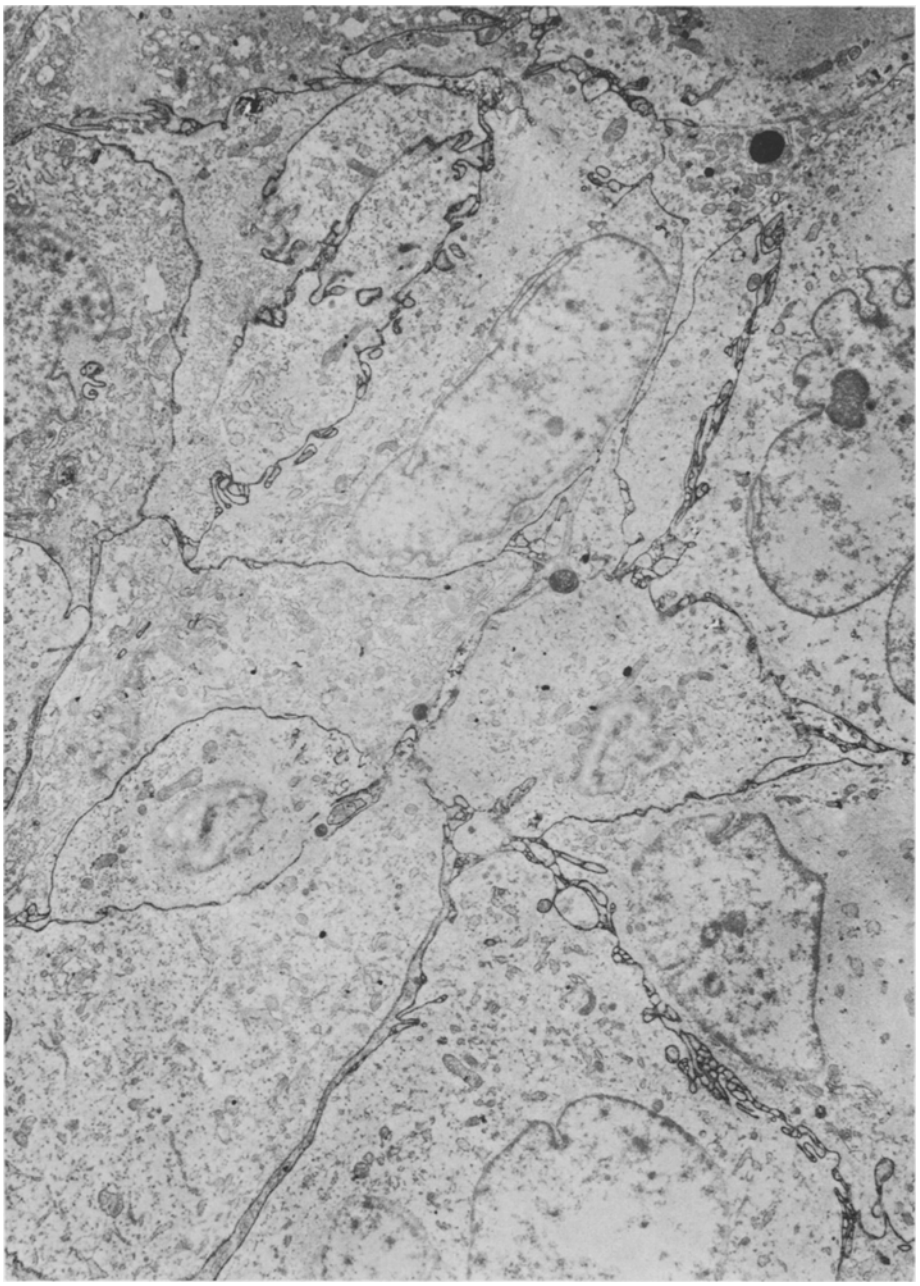

Fig. 7. Electron dense reaction product of 5′-nucleotidase activity can be seen on epithelioid sarcoma cell membrane. Unstained section. ×3,700

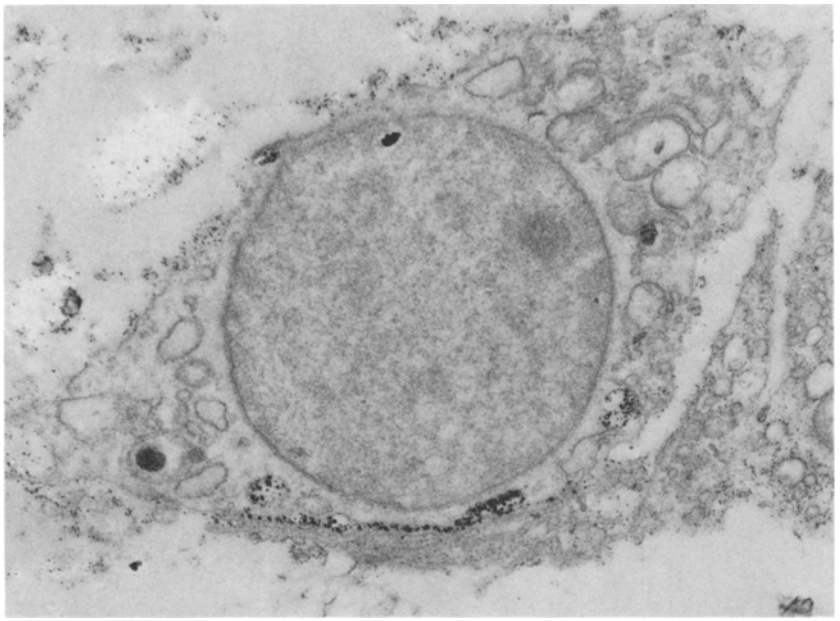

Fig. 8. Control synovial cells showing slight membrane reaction of 5′-nucleotidase at the site of close contact each other. Unstained section. × 12,500

cells of biphasic synovial sarcoma (Mickelson et al. 1980). Although all of these electron microscopic findings of epithelioid sarcoma were confirmed in this study, a peculiar finding of 10 nm cytoplasmic filaments showing condensation with concentrically oriented or whorled appearance and fingerprint like arrangement was observed in the present case. This seems to be a new ultrastructural finding of epithelioid sarcoma, but the nature of this peculiar structure is unknown. A few myofibroblasts were present in a cell nest of epithelioid sarcoma in this case. Myofibroblasts have been reported to appear in malignant fibrous histiocytoma, pleomorphic liposarcoma and rhabdomyosarcoma (Reddick et al. 1979).

The neoplastic cells of epithelioid sarcoma and the control synovial cells were similar in enzyme histochemical appearance showing positive activity of 5′-nucleotidase but without any activity of adenosine triphosphatase and alkaline phosphatase in the plasma membrane. Although 5′-nucleotidase activity was so weak in the control synovial cells that the activity could only be demonstrated in the electron microscope in a few cells in this study, marked increase of the enzyme activity has been demonstrated histochemically in the synovial membrane of rheumatoid arthritis (Henderson et al. 1980). 5′-nucleotidase is a widely distributed enzyme of the cell membrane (Hardonk 1968; Oscar and Schwartz 1968) and may be associated with a transport mechanism. It is interesting that the activity of this enzyme may fluctuate under various physiological conditions (Vorbrodt and Borun 1979) during embryonic development (Damjanov et al. 1977) and difference

of species (Hardonk 1968; Oscar and Schwartz 1968). There was a difference of the membrane associated enzyme activities between the synovial cells and other control soft tissues. Although the activity of 5′-nucleotidase was found not only in the synovial cells but also in the blood vessels and to a slight extent in the smooth muscle, the latter two tissues showed positive activity of adenosine triphosphatase which was negative in the synovial cells.

Thus it has been shown here that epithelioid sarcoma cells have several ultrastructural and histochemical findings similar to those of synovial cells and synovial sarcoma. These results would support the concept that uncommited mesenchymal cells may undergo differentiation toward synovial structure with neoplastic transformation and may show histological appearance of epithelioid sarcoma (Patchefsky et al. 1977).

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