Histogenesis of Clear Cell Sarcoma of Tendons and Aponeuroses

An Electron-Microscopic, Biochemical, Enzyme Histochemical, and Immunohistochemical Study

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For the purpose of clarifying the histogenesis of clear cell sarcoma of tendons and aponeuroses (CCS) as well as the problem of whether or not CCS is a heterogeneous group of neoplasms, studies based on various methods were performed. Analysis of glycosaminoglycans gave the same results for amelanotic CCS and synovial sarcoma, and the DOPA reaction gave the same negative results for amelanotic CCS and synovial sarcoma. However, the DOPA reaction was also negative in an amelanotic recurrent tumor of a melanotic CCS, and electron-microscopic studies revealed a close resemblance between amelanotic CCS and melanotic CCS. Further, enzyme histochemical studies showed definite differences between synovial sarcoma and amelanotic CCS but gave identical results for amelanotic and melanotic CCS. Immunohistochemical studies revealed the presence of S-100 protein in all CCS cases, both amelanotic and melanotic. These results indicate that CCS is not a heterogeneous group of neoplasms, and that both amelanotic and melanotic CCS are of neural crest origin. (Am J Pathol 1984, 114: 264–272)

SINCE Enzinger[1] suggested clear cell sarcoma of tendons and aponeuroses (CCS) as a new entity in 1965, many cases have been reported,[2,3] and this tumor is becoming firmly established as a specific oncologic entity with a characteristic clinical setting and histologic picture. However, there are still various conflicting theories concerning its histogenesis.

The first electron-microscopic study of CCS was performed by Kubo,[4] who suggested that this tumor should be included in the category of synovial sarcoma on the basis of the existence of biphasic patterns, basement membranes, and pseudoglandular structures with filopodia. On the other hand, cases with melanin production were reported later[5–9] and also shown electron microscopically.[5,7,9] On the basis of these observations, the idea that CCS may be a soft tissue malignant melanoma began to assume greater prominence. Of the cases of this entity, however, some showed no melanin production by either light or electron-microscopic examination. This fact led to a difference in opinion about the histogenesis. Hajdu pointed out that some cases of tendosynovial sarcoma are included in this category, and thus it would be an oversimplification to include all cases of CCS in the category of soft tissue malignant melanoma.[10,11] In fact, there has been no report that identifies amelanotic CCS as an amelanotic melanoma. Tsuneyoshi et al[12] investigated 13 cases and divided them into melanotic type and synovial type. Bearman

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et al[7] reported that leiomyosarcoma and malignant giant cell tumor of the tendon sheath as well as synovial sarcoma show a clear cell pattern and concluded that it is reasonable to think that a number of different neoplasms may present as CCS, and that this is a heterogeneous group of neoplasms with a common clinicopathologic picture.

In particular, the problem of the group without recognizable melanin production has become an issue. According to Enzinger, who is believed to have the greatest experience with this tumor, about 50% of the cases do not show melanin production.[13]

In order to clarify the histogenesis of CCS as well as the problem of whether or not this tumor is indeed a heterogeneous group of neoplasms, we studied cases of CCS by various methods.

Materials and Methods

Eight cases of CCS were collected. The clinical data are summarized in Table 1. Melanin production was observed in 4 of them, which were included in the "melanotic group," and the remaining 4 cases were included in the "amelanotic group."

Light Microscopy

The materials available for this purpose in each case were several formalin-fixed, paraffin-embedded tissue blocks. In addition to hematoxylin and eosin staining, Masson's trichrome staining, alcian blue staining, periodic acid–Schiff staining with and without diastase pretreatment, Fontana staining for melanin with and without bleaching, Pearl staining for iron, and silver impregnation for reticulin were carried out.

Electron Microscopy

In Cases 1, 2, 5, and 6 (recurrent tumor), small pieces of fresh tumor tissue were fixed in buffered 1% osmium tetroxide. Ultrathin sections cut from epon-embedded tissue were stained with uranyl acetate and lead citrate. The sections were examined with a JEOL 100 B electron microscope.

Biochemical Analysis of Glycosaminoglycans

Analysis of glycosaminoglycans was conducted in Cases 1 and 2 and 2 cases of synovial sarcoma. Fresh surgical specimens were minced in acetone. Then the total amounts of uronic acid were measured by the method of Bitter and Muir.[14] Two-dimensional electrophoretic separation and assay of seven subcomponents were accomplished by the method of Hata and Nagai.[15,16]

Enzyme Histochemistry

In Cases 1, 2, and 6 (recurrent tumor), approximately 2-mm-thick blocks were fixed in 10% formol-calcium (pH 7.1–7.2) for 24 hours at 4 C, transferred to cold gum sucrase, and kept for 2 days in the refrigerator. Tissue sections cut 4–6 μ thick in a cryostat were histochemically stained for the following enzymes: alkaline phosphatase (AlPase),[17] acid phosphatase (AcPase),[18] β-glucuronidase (β-Gase),[19] N-acetyl-β-glucosaminidase (N-Gase),[20] and adenosine triphosphatase (ATPase).[21] Enzyme histochemical staining of dopa oxidase (DOPA reaction)[22] was conducted on 4–6-μ sections of fresh frozen, nonfixed, 2-mm-thick blocks. Two cases of biphasic synovial sarcoma and one case of monophasic synovial sarcoma were examined in the same way.

Table 1—Clear Cell Sarcoma of Tendons and Aponeuroses: Summary of 8 Cases

Case	Age and sex	Anatomic site	Size of tumor (cm)	Treatment	Follow-up (yr)	Number of recurrences	Metastasis	Survival
Amelanotic group								
1	44M	Knee, left	5.0 × 6.0	Excision and irradiation	3	0	–	Living
2	37M	Knee, right	7.5 × 5.0	Excision	3	0	–	Living
3	28F	Patellar tendon, left	3.5 × 2.0	Excision	6	2	–	Living
4	35M	Dorsalis pedis, right	4.0 × 3.5	Excision	3	1	–	Living
Melanotic group								
5	43F	Ankle, left	2.0 × 3.0	Excision and irradiation	4	1	Inguinal (left), lung	Died
6	19F	Buttock, left	5.5 × 4.0	Excision and chemotherapy	1	1	Lung, pancreas, ovary, bone	Died; autopsy
7	36F	Plant pedis, right	2.5 × 2.0	Excision	2	0	–	Living
8	56M	4th toe, left foot (flexor tendon)	1.5 × 1.0	Excision	3	0	–	Living

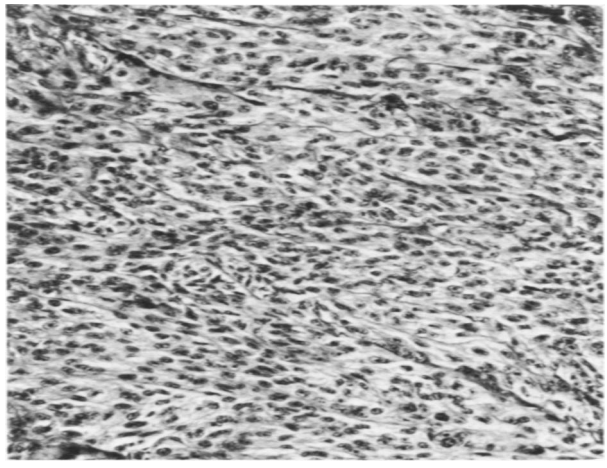

Figure 1 – The tumor consists of compact nests and fascicles of epithelioid or fusiform cells with clear cytoplasm and a prominent nucleolus bordered by delicate septa. (Case 6, H & E, ×160)

Immunohistochemistry

The tissues were examined immunohistochemically for S-100 protein, which is known to be a marker of tumors of neural crest origin such as malignant melanoma, etc., and keratin, whose presence in synovial sarcoma has recently been shown. Rabbit anti-S-100 protein antiserum was supplied through the courtesy of Professor Keiichi Uyemura, Department of Physiology, Saitama Medical School; we had already demonstrated its reliability in immunohistochemical studies of normal nervous tissues and various tumor tissues.[23,24] Rabbit anti-keratin antibody was purchased from DAKO (Denmark). Immunohistochemical staining for these specific antigenic constituents was performed as follows. The 3-μ-thick paraffin sections were deparaffinized and treated with 0.1% trypsin in phosphate-buffered saline (pH 7.4) for 30 minutes. Endogenous peroxidase was blocked by incubation of the slides for 10 minutes in 1% $NaIO_4$. After being washed, the slides were incubated with primary antisera (dilution, 1:500). The slides were then exposed to a biotinylated anti-rabbit immunoglobulin antiserum (dilution, 1:500), avidin (dilution, 1:1000), and biotinylated horseradish peroxidase complex.[25] The reagents were purchased from Vector Laboratories (Vectastain, Burlingame, Calif). We developed the peroxidase reaction by incubating the slides in 0.005% H_2O_2 and 0.02% 3,3'-diaminobenzidine tetrahydrochloride for 10 minutes.

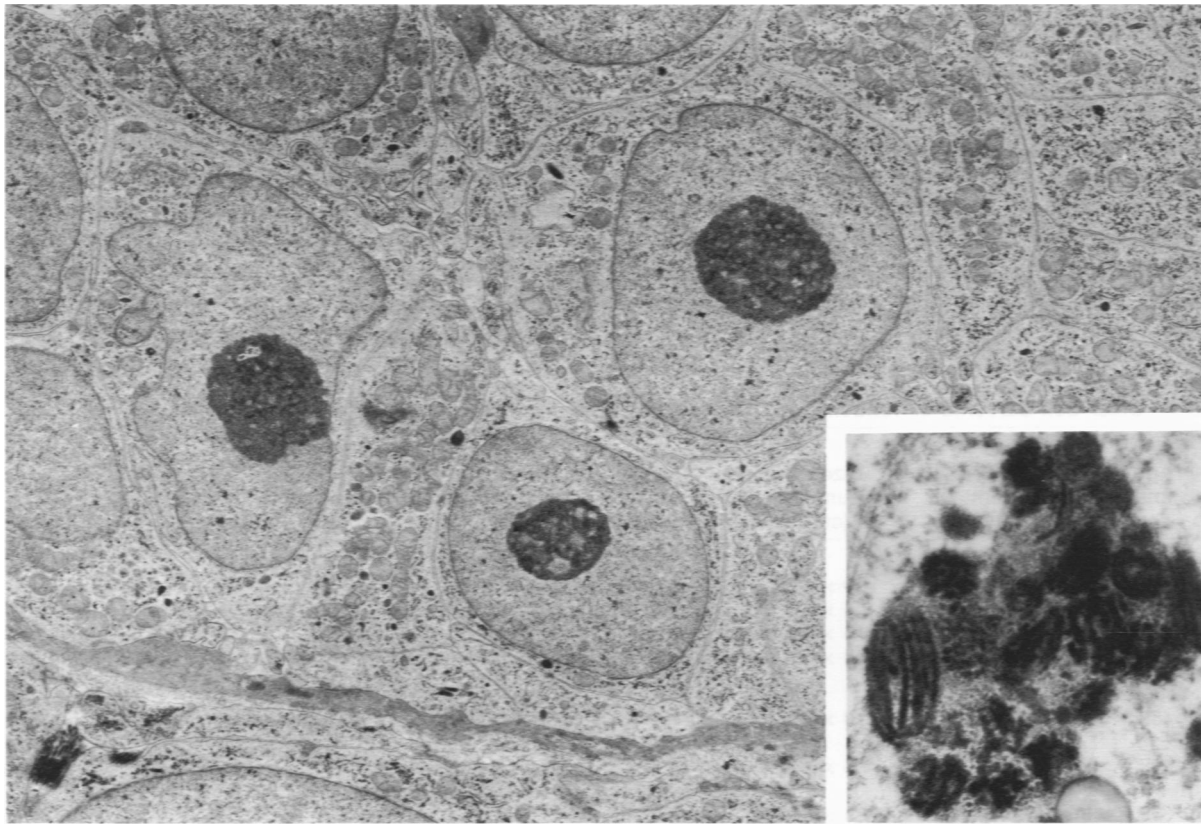

Figure 2 – The oval tumor cells with rounded nuclei and large, centrally located nucleoli are enclosed by the basement membrane. Multiple mitochondria are seen. (Case 6, ×5600) **Inset** – Melanosomes with typical lamellar and "barrel-stave" internal structure. (Case 5, ×50,000)

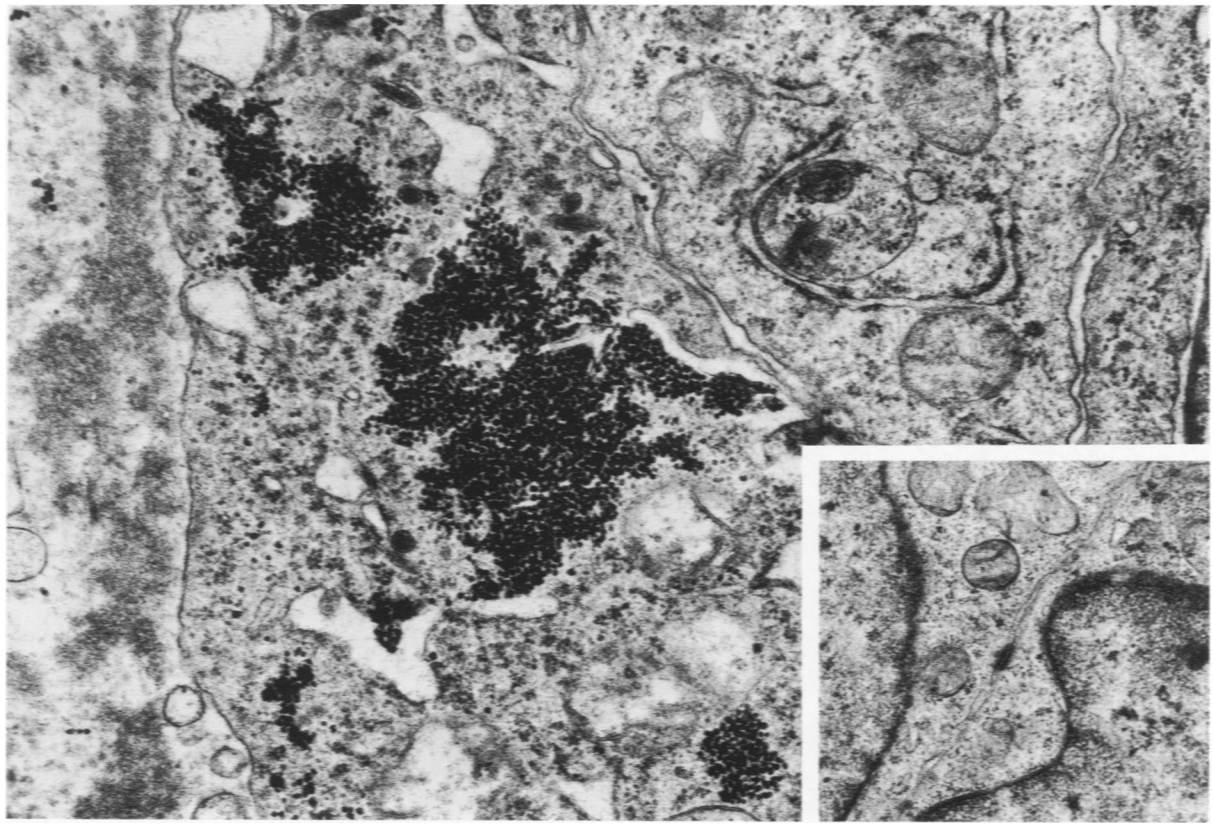

Figure 3 – The tumor cell with intracytoplasmic glycogen rests on the basement membrane. (Case 2, ×48,000) **Inset** – A junctional complex is observed. (Case 1, ×13,000)

The same reaction was conducted for synovial sarcoma, malignant melanoma, and leiomyosarcoma, as the occasion demanded.

Results

Light Microscopy

All 8 cases showed the characteristic histologic picture reported by Enzinger,[1] i.e., a rather uniform pattern composed of compact nests or fascicles of rounded or fusiform cells with clear cytoplasm bordered and defined by a delicate framework of fibrocollagenous tissue (Figure 1). The individual cells possessed round to ovoid vesicular nuclei with prominent basophilic nucleoli and clear or pale-staining cytoplasm (Figure 1). Mitotic figures were very scarce. In all cases except Case 2, multinucleated giant cells having 10–15 peripherally located nuclei were present. Various amounts of intracellular glycogen were observed according to the case. Fontana-stain-positive brown pigment that disappeared upon bleaching – melanin – was observed in Cases 5–8. In Case 6, a large amount of melanin was observed in the primary tumor, but no melanin was present in the recurrent tumor or metastatic lesions. On the other hand, in Case 5, a large amount of melanin was seen in all the primary and metastatic lesions. Although iron-stain-positive brown pigment was observed in Cases 1–4, no melanin was observed.

Electron Microscopy

Findings observed in 2 cases of the amelanotic group resembled those of 2 cases of the melanotic group. That is, the tumor tissue consisted of oval or fusiform cells with rounded or irregularly shaped nuclei and a biphasic pattern such as observed in synovial sarcoma was not definitely recognizable (Figure 2). A large, centrally located, single nucleolus was prominent in almost all cells in all 4 cases (Figures 2 and 4). The cytoplasm contained multiple mitochondria (Figures 2 and 4) and aggregates of glycogen (Figure 3). Basement membrane (Figures 2 and 3), junctional complex (Figure 3, inset), electron-dense bodies that were hard to differentiate from lysosomes, etc. (Figure 4), and the interdigitation between neighboring cells (Figure 4) were observed in all 4 cases. The only significant difference between the

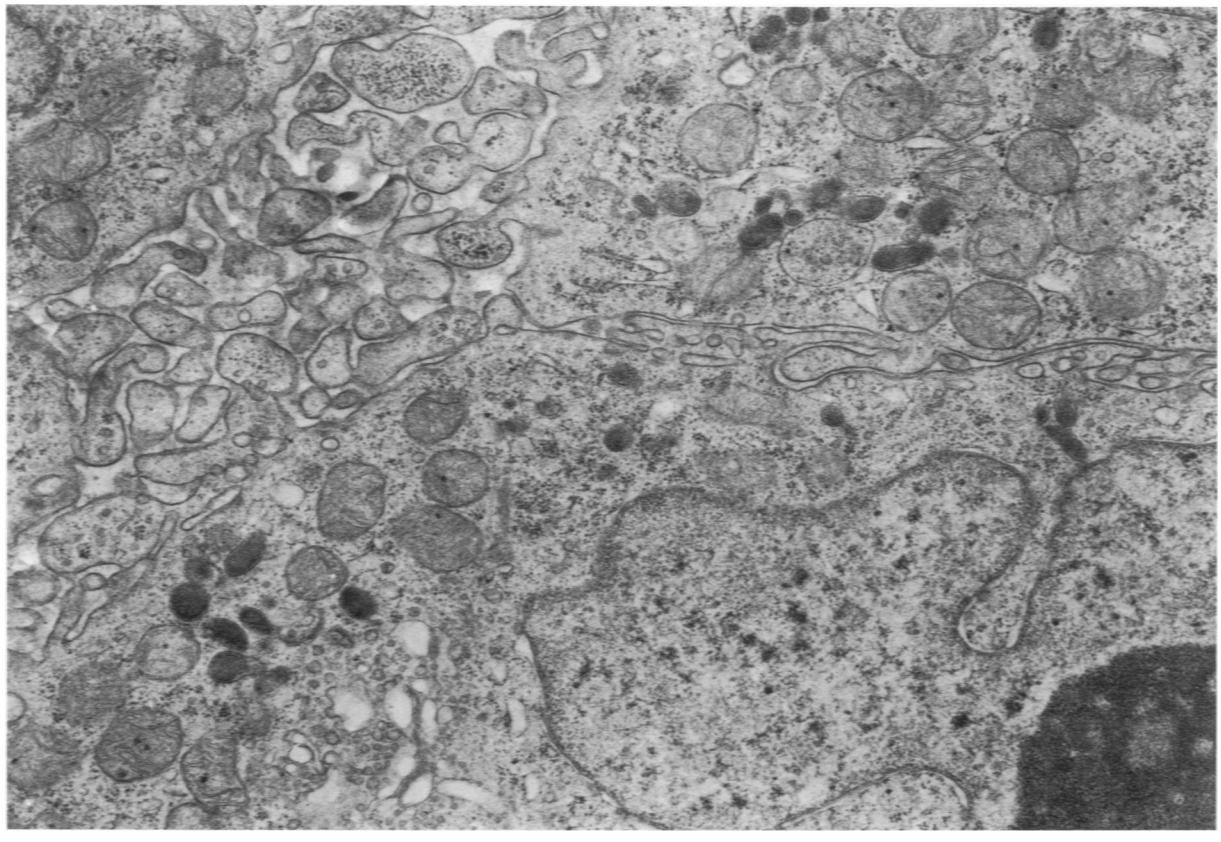

Figure 4—Interdigitation between neighboring cells and an irregularly shaped nucleus with a large nucleolus are seen. Multiple mitochondria and dense bodies are also seen. (Case 2, ×17,000)

amelanotic group and the melanotic group was the presence or absence of melanosomes. Melanosomes in various stages of development were present in Case 5 of the melanotic group (Figure 2, inset). Although a large amount of melanin was observed in the primary tumor of Case 6 by light microscopy, no melanin was present in the recurrent tumor. By electron microscopy, also, no melanosomes were revealed.

Biochemical Analysis of Glycosaminoglycans

As shown in Table 2, there were no significant differences between amelanotic CCS and synovial sarcoma cases in terms of the total amounts of glycosaminoglycans (uronic acid) and the ratios of subcomponents.

Enzyme Histochemistry

As shown in Table 3, 3 cases of CCS examined (2 of the amelanotic group and 1 case of recurrent amelanotic tumor in the melanotic group) showed a similar staining pattern, and all enzyme activities except AlPase were observed to the same degree in almost all tumor cells (Figure 5B). In synovial sarcomas, on the other hand, stronger enzyme activity was observed in the epithelial cells or large plump cells than in fibroblastic cells (Figure 5A and 6A). In other words, unlike synovial sarcoma, no biphasic pattern was observed in CCS by enzyme histochemistry. In synovial sarcomas examined, AlPase activity was observed in the epithelial cells or the large plump cells, whereas there was no AlPase activity in cases of

Table 2—Fractionation of Glycosaminoglycans

Case	Uronic acid (μg/g dry tissue)	HA (%)	HS (%)	DS (%)	Ch-S (%)
Case 1	2041	21	23	33	23
Case 2	2674	5	30	40	25
Synovial sarcoma	2257	27	24	27	22
Synovial sarcoma	2048	10	35	29	26

HA, hyaluronic acid; HS, heparan sulfate; DS, dermatan sulfate; Ch-S, chondroitin sulfate.

Table 3—Summary of Enzyme Histochemistry

				Biphasic synovial sarcoma (2 cases)			Monophasic synovial sarcoma (1 case)	
	Case 1	Case 2	Case 6	Epithelial cells	Large plump cells	Fibroblastic cells	Large plump cells	Fibroblastic cells
AlPase	–	–	–	+	+ ~ –	–	+ ~ –	–
AcPase	+	+	+	+	+ ~ –	–	+ ~ –	–
β-Gase	+	+	+	+	+ ~ –	–	+ ~ –	–
N-Gase	+	+	+	+	+ ~ –	–	+ ~ –	–
ATPase	+	+	+	+	+ ~ –	–	+ ~ –	–
DOPA reaction	–	–	–	–	–	–	–	–

CCS examined (Figure 6). The DOPA reaction was positive in a pigmented nevus of a control (Figure 7A) but was negative in synovial sarcoma and CCS cases (Figure 7B).

Immunohistochemistry

As shown in Table 4, S-100 protein was demonstrated immunohistochemically in all cases of malignant melanoma and all cases of both melanotic and amelanotic CCS. The number of tumor cells containing S-100 protein varied with each case and also varied greatly according to site even in the same case (Figure 8). In general, positive findings were observed very frequently in spindle tumor cells (Figure 8A), whereas only a very few of the cells showed positive findings in the areas with melanin (Figure 8C). Control studies substituting anti-S-100 protein antiserum absorbed with S-100 protein showed complete ab-

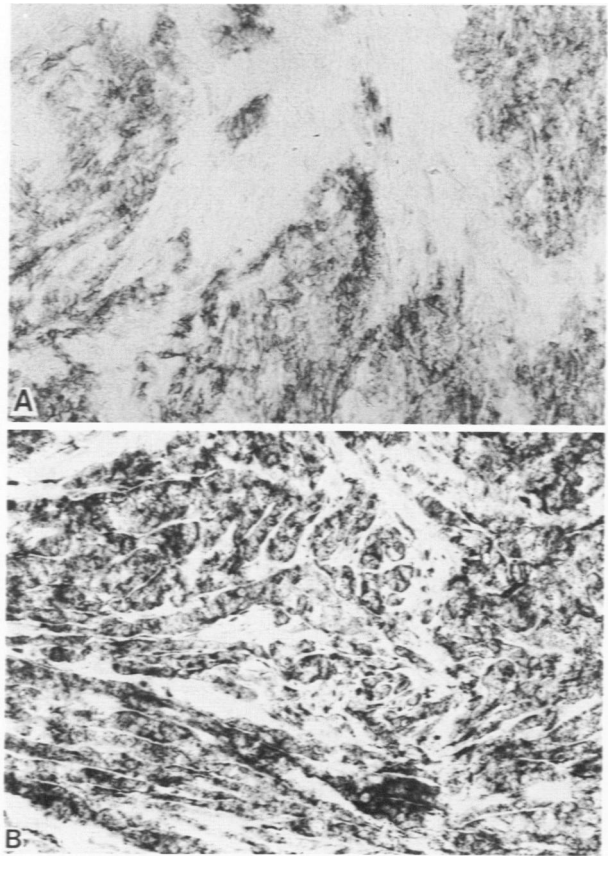

Figure 5—AcPase. **(A)**—In monophasic synovial sarcoma, only large, plump cells show positive staining. (Counterstained with methyl green, ×160) **(B)**—In CCS (Case 5), all round and spindle cells show positive staining. (Counterstained with methyl green, ×160) (With a photographic reduction of 8%)

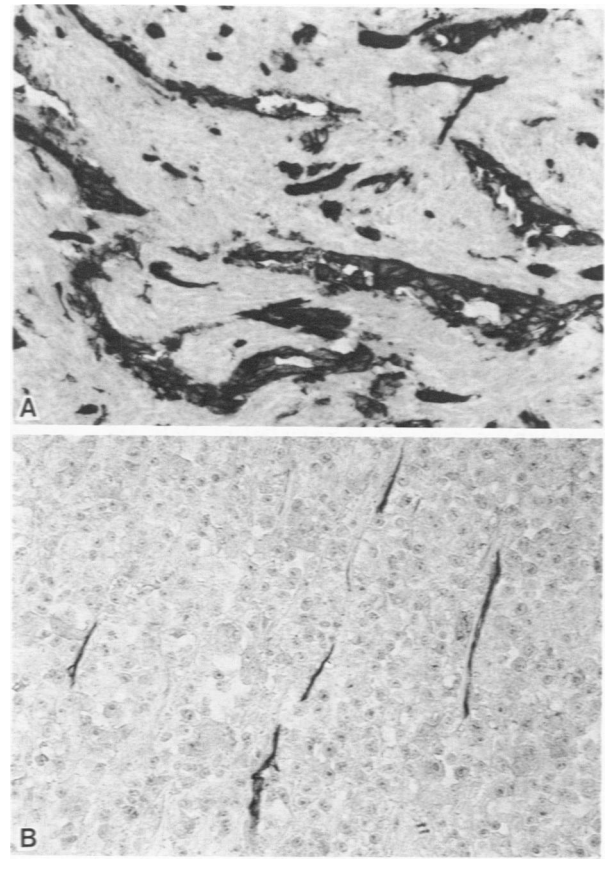

Figure 6—AlPase. **(A)**—In biphasic synovial sarcoma, epithelial cells of glandular structures show strong positive staining. (Counterstained with methyl green, ×160). **(B)**—In CCS (Case 1), all tumor cells show negative staining. Only capillaries show positive staining. (Counterstained with methyl green, ×160) (With a photographic reduction of 8%)

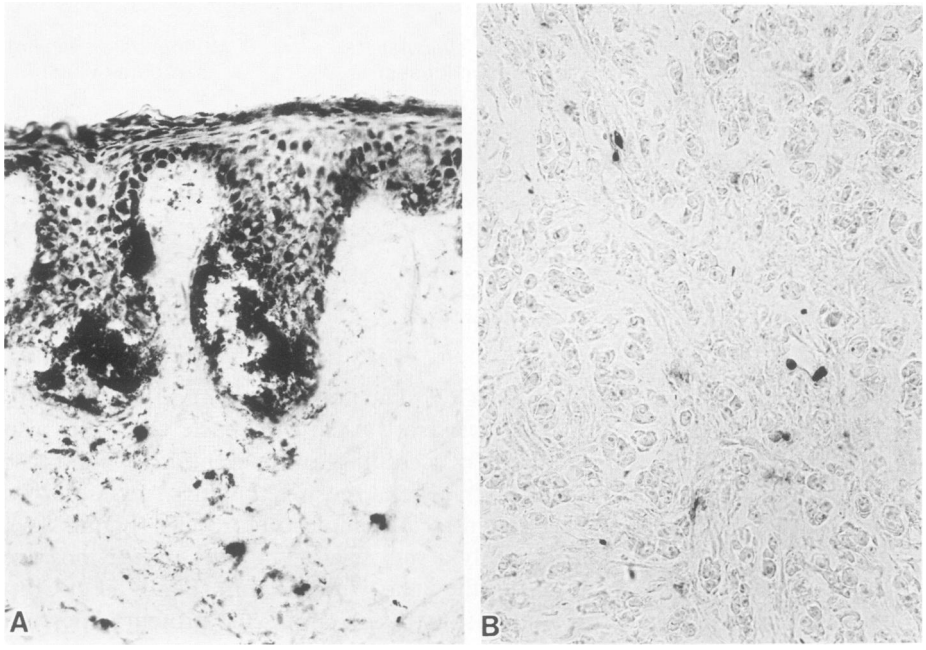

Figure 7—DOPA reaction. **(A)**—In a pigmented nevus of a control, nevus cells show positive staining. (Counterstained with methyl green, ×160). **(B)**—In CCS (Case 2), all tumor cells show negative staining. The small cells with positive staining are leukocytes.[22] (Without counterstain, ×160)

sence of staining. Keratin was not observed in CCS but was very frequently found in synovial sarcoma (Table 4).

Discussion

The fact that the total amounts as well as the ratios of subcomponents of uronic acid in amelanotic CCS and in synovial sarcoma were approximately the same suggests that amelanotic CCS might be included in the category of synovial sarcoma. In addition, the results of the DOPA reaction, which is considered to be the best method for evaluating melanin productivity at present, suggest that, like synovial sarcoma, CCS in which no melanin is revealed by light microscopy might be unrelated to melanin production. However, it must be kept in mind that the DOPA reaction was also negative in the recurrent tumor without melanin in CCS of the melanotic group, and that, in addition, the DOPA reaction is negative even in amelanotic malignant melanoma.[22]

Enzyme histochemical studies other than the DOPA reaction also suggest characteristics common to amelanotic and melanotic CCS. It was also clearly revealed that the biphasic pattern observed in synovial sarcoma is not present in CCS. The fact that AlPase-positive cells were not observed in CCS, in spite of the fact that they were observed in synovial sarcoma, indicates that there is a difference in character between synovial sarcoma and CCS.

Kubo,[4] on the basis of the existence of the basement membrane, a biphasic pattern, and pseudo-

Table 4—Summary of Immunohistochemistry

Case	S-100 protein	Keratin
Amelanotic group		
1 (3 paraffin blocks)	+(3/3)	−(0/3)
2 (5 paraffin blocks)	+(4/5)	−(0/5)
3 (2 paraffin blocks)	+(1/2)	−(0/2)
4 (3 paraffin blocks)	+(2/3)	−(0/3)
Melanotic group		
5 (2 paraffin blocks)	+(1/2)	−(0/2)
6 (4 paraffin blocks)	+(1/4)	−(0/4)
7 (2 paraffin blocks)	+(2/2)	−(0/2)
8 (1 paraffin block)	+(1/1)	−(0/1)
Synovial sarcoma		
Biphasic (6 cases)	−(0/6)	+(6/6)
Monophasic (4 cases)	−(0/4)	+(2/4)
Malignant melanoma (10 cases)	+(10/10)	
Leiomyosarcoma (7 cases)	−(0/7)	

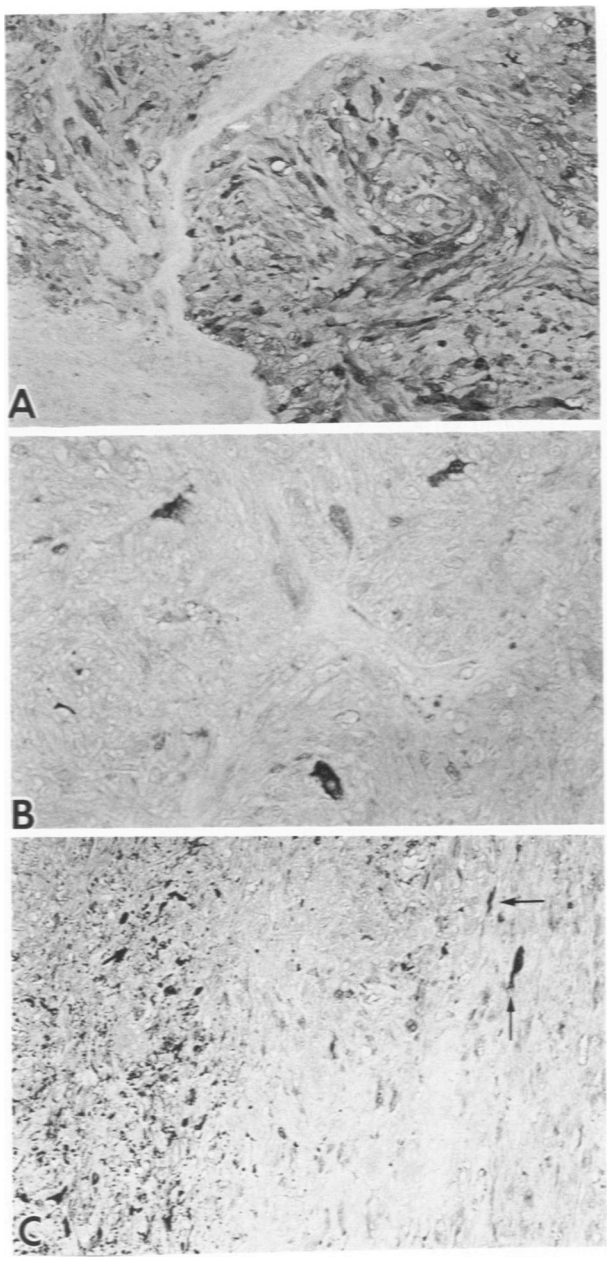

Figure 8—Immunoperoxidase stain for S-100 protein. **(A)**—Many spindle tumor cells show strong positive staining. (Case 4, without counterstain, ×160) **(B)**—Some tumor cells show positive staining. (Case 8, without counterstain, ×320) **(C)**—In the melanotic area, only a small number of tumor cells show positive staining (*arrow*). (Case 5, without counterstain, ×160) (With a photographic reduction of 28%)

glandular structures with filopodia, suggested that CCS should be included in the category of synovial sarcoma. In our electron-microscopic studies, the basement membrane was seen also in melanotic CCS, and no biophasic pattern was observed in amelanotic CCS. In this study, interdigitation between neighboring cells was observed in both the amelanotic and melanotic groups. This interdigitation is without doubt one of the features of synovial sarcoma also,[26] but it is hard to think that this interdigitation is the same as the pseudoglandular structure with filopodia which is a commonly reported diagnostic feature of synovial sarcoma.[26-28] Furthermore, it must be kept in mind that basement membrane, junctional complex, multiple mitochondria, etc., as well as this interdigitation are also observed in malignant melanoma.[29]

Tsuneyoshi et al.[12] divided CCS into synovial type and melanotic type and enumerated the cytologic differences between the two types. However, we did not find such differences. According to Tsuneyoshi et al., for example, multinucleated giant cells were observed only in the synovial type. We found them in all four cases of melanotic CCS.

Previously, no immunohistochemical study of CCS has been reported, nor have biochemical and enzyme histochemical studies. Keratin has conventionally been considered to be the intermediate filament specific for the epithelial cell,[30,31] but it has recently been shown that keratin is very frequently present only in synovial sarcoma among the soft tissue tumors.[32,33] In the present study also, keratin was very frequently demonstrated immunohistochemically in synovial sarcoma, but no keratin was observed in CCS. On the other hand, S-100 protein is known to be an excellent marker of tumors of neural crest origin, and its presence in the soft tissue tumors derived from Schwann cells and melanocytes has been reported.[23,24] It is very important that, in the present study, S-100 protein was demonstrated immunohistochemically in all cases of malignant melanoma and CCS (both the melanotic and amelanotic) but not in synovial sarcoma or leiomyosarcoma.

The results of these studies suggest that CCS is not a heterogeneous group of neoplasms, and there are many characteristics common to the melanotic and amelanotic groups, both of which are of neural crest origin—melanocyte or Schwann cell. In other words, CCS is a soft tissue malignant melanoma or Schwann cell tumor with melanin (malignant melanocytic schwannoma). Because the fact that some investigators suggest a close relationship between Schwann cells and dermal melanocytes,[35] soft tissue malignant melanoma is considered to be closely related to malignant melanocytic schwannoma. However, we wish to consider CCS as a soft tissue malignant melanoma, because a large amount of glycogen has almost never been observed in malignant schwannoma, and no case of CCS that had large nerve bundles attached or that was combined with neurofibromatosis has ever been reported. In addition, no characteristic whorl-like or lamellar structures of cell membranes, prominent myelin figures, or pinocytotic vesicles, which are electron-microscopically observed in malignant schwannoma,[36] were observed in CCS in this study.

We believe that, in studying the histogenesis of CCS in the future, the focus should be placed on the point of how melanocytes embryologically stray into the areas of tendons and aponeuroses when they migrate from the neural crest to the dermal areas.[37]

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Note Added in Proof

The S-100 protein has recently also been demonstrated immunohistochemically in CCS by L. G. Kindblom, P. Lodding, and L. Angervall (Virchows Archiv[A] 1983, 401:109–128) and E. B. Chung and F. M. Enzinger (Am J Surg Pathol 1983, 7:405–413).

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