Virchows Archiv A Pathol Anat (1989) 415:51–60

Virchows Archiv A
Pathological Anatomy and Histopathology

Neural crest origin of clear cell sarcoma of tendons and aponeuroses

Ultrastructural and enzyme cytochemical study of human and nude mouse-transplanted tumours

Yoshio Mii[1], Yoshizumi Miyauchi[1], Kanya Hohnoki[1], Hiroshi Maruyama[2], Masahiro Tsutsumi[2], Kayoko Dohmae[3], Susumu Tamai[1], Yoichi Konishi[2], and Takahisa Yamanouchi[3]

[1] Department of Orthopedic Surgery, Nara Medical University,
[2] Department of Oncological Pathology, Cancer Center, Nara Medical University, Kashihara, Nara 634, Japan
[3] Quarters for Experimentally Infected Animals, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan

Summary. In order to clarify the histogenesis of clear cell sarcoma of tendons and aponeuroses (CCS), two cases of human and one nude mouse-transplanted CCS line were studied using an ultrastructural and enzyme cytochemical approach. Most of the tumour cells obtained from the primary and transplanted CCS demonstrated melanosomes in various stages of development within the cytoplasm, whereas no melanosomes could be identified in the metastatic CCS. However, cholinesterase and tyrosinase activities could be demonstrated not only in the melanotic primary and transplanted CCS but also in the amelanotic metastatic CCS. The results therefore support the hypothesis that CCS is a soft tissue tumour derived from the neural crest.

Key words: Clear cell sarcoma – Melanosomes – Cytochemistry – Electron microscopy – Nude mouse

Introduction

Since Enzinger (1965) first described clear cell sarcoma of tendons and aponeuroses (CCS) as a distinct soft tissue sarcoma entity, many additional cases have been reported in the literature. Some authors have considered that CCS is of synovial origin, based on its histological appearance and the apparent intimate anatomical relationship between the tumours and tendon or aponeurosis synovial tissue (Enzinger 1965; Kubo 1969; Hajdu et al. 1977). However, electron microscopic investigations have revealed the presence of melanosomes within the cytoplasm of CCS cells in some cases (Dutra 1970; Hoffman and Carter 1973; Mackenzie 1974; Bearman et al. 1975; Boudreaux et al. 1978; Ekfors and Rantakokko 1979; Chung and Enzinger 1983; Raynor et al. 1979; Parker et al. 1980; Eckardt et al. 1983; Kindblom et al. 1983; Mukai et al. 1984; Benson et al. 1985; Ohno et al. 1986). Furthermore, recent immunohistochemical studies demonstrated S-100 protein positivity for this neoplasm (Kindblom et al. 1983; Mukai et al. 1984; Benson et al. 1985). These observations have therefore lead some investigators to conclude that CCS might be a variant of malignant melanoma (Ekfors and Rantakokko 1979; Chung and Enzinger 1983; Benson et al. 1985) or malignant schwannoma (Azumi and Turner 1983; Ohno et al. 1986). Although some cytochemical studies of this neoplasm have been performed, there is only limited information available regarding enzymatic evidence of melanogenesis in CCS cells at the electron microscopic level.

In order to clarify the histogenetic origin of CCS, ultrastructural and enzyme cytochemical characteristics including the expression of tyrosinase and cholinesterase activity, were studied in two primary cases of CCS, a metastatic lesion and in one transplantable CCS line derived from one of the two cases and established in nude mice.

Materials and methods

Two cases of CCS (case 1 and 2) were selected for electron microscopic investigation. Relevant clinical data are summarized in Table 1. Specimens were obtained from the primary

Offprint requests to: Y. Mii, Department of Orthopedic Surgery, Nara Medical University, 840 Shijo-Cho, Kashihara, Nara 634, Japan

Table 1. Clinical data for CCS patients

Case	Age/Sex	Location	Therapy	Metastasis	Follow up
1	51/M	1. Buttock*	Resection	Lungs	Died, 2 years postoperativ.
2	50/M	1. Foot*	Thigh amputation Chemotherapy	Back*, Thighs, Leg, Lungs	Died, 1.5 years postoperativ.

* Specimens obtained from these tumours were processed for this study. CCS: Clear cell sarcoma of tendons and aponeuroses

Table 2. Enzymes, substrates, capturing agents and inhibitors used for the cytochemical studies of CCS

Enzyme	Substrate	Capturing agent	Inhibitor
5′-Nucleotidase	AMP	Cerium	Levamisole
Alkaline phosphatase	β-Glycerophosphate	Lead	Levamisole
Acid phosphatase	β-Glycerophosphate	Cerium	–
Thiamine pyrophosphatase	Thiamine pyrophosphate	Lead	–
Acetyl cholinesterase	Acetylthiocholine	Copper	iso-OMPA, Eserine
Tyrosinase	L-DOPA	–	–

AMP: Adenosine 5′-monophosphoric acid; iso-OMPA: Tetraisopropylpyrophosphoramide; L-DOPA: L-3,4-dihydroxyphenylalanine

tumour in each of two cases, a metastatic tumour of case 2, and from transplantable tumours established in nude mice. For the enzyme cytochemical study, specimens from the metastatic tumour and transplantable tumours were used. Transplantation was carried out by the following method: tissue fragments of about 1 mm in diameter which were obtained from the case 2 primary were subcutaneously transplanted with a trocar into two areas in the back of BALB/c nu/nu mice under aseptic conditions. In each generation, five animals were used as recipients. When the transplanted tumours reached about 10 mm in diameter, the next transplantation was performed. Transplanted tumours of the 5th and the 6th generations were used in this study. Small blocks were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 4° C for 2 h and then washed with 0.1 M cacodylate buffer containing 0.25 M sucrose at 4° C for 12 h. The specimens were postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer at 4° C for 1 h, dehydrated in a graded series of ethanols, and embedded in Epon 812. Thick sections cut at 0.5 μm were stained with toluidine blue to allow selection of representative areas from which to prepare ultrathin sections for electron microscopic observations. These sections were cut with an LKB-8800 Ultrotome and stained with uranyl acetate and lead citrate. They were examined under a JEOL 1200-EX electron microscope.

For demonstration of enzyme activities at the electron microscope level, small blocks of tumour tissue were fixed in 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M cacodylate buffer at 4° C for 30 min; sections about 40–60 μm thick were cut on a model G Vibratome (Oxford Instruments Inc.). These sections were incubated in the various reaction media prepared for demonstrating activities of the following enzymes: 5′-nucleotidase (Robinson and Karnovsky 1983a), alkaline phosphatase (AlPase, after Mayahara et al. 1967), acid phosphatase (AcPase, after Robinson and Karnovsky 1983b), thiamine pyrophosphatase (TPPase, after Novikoff and Goldfischer 1961), acetylcholinesterase (AchE, after Karnovsky 1964), and tyrosinase (after Hunter et al. 1970). For controls, substrate free media were used for all the reactions. In addition, the following inhibitors were applied to determine reaction specificity: 0.5 mM levamisole (an inhibitor of nonspecific AlPase), 0.1 mM tetraisopropylpyrophosphoramide (iso-OMPA, an inhibitor of nonspecific cholinesterase), and 0.2 mM eserine (an inhibitor of esterase). Enzymes, substrates, capturing agents and inhibitors used in the cytochemistry are summarized in Table 2. Following the incubation, the sections were processed for electron microscopy as detailed above, and ultrathin sections were contrasted only with lead citrate.

Results

Light microscopy: The primary tumours in both of the cases show similar histological characteristics. They are composed of round or oval, packed cells with clear cytoplasm, arranged in small nests separated by collagenous stromal elements (Fig. 1). The histological appearance of the metastatic tumour from case 2 is essentially similar to that of the primary. In nude mouse-transplanted tumours, the major histological features of the original tumour from case 2 are retained, the lesions presenting as relatively uniform populations, mostly composed of small round cells arranged in sheets. However, slight changes occur during serial transplantation, and the fibrous septa separating each cell nest are observed to be thinner and less pronounced than those evident in the original tumour (Fig. 2). Mitotic figures are infrequent in all except the transplanted lesions. Melanin pigments can be detected only in limited areas of the two primary tumours in sections stained with haematoxylin and eosin for light microscopy.

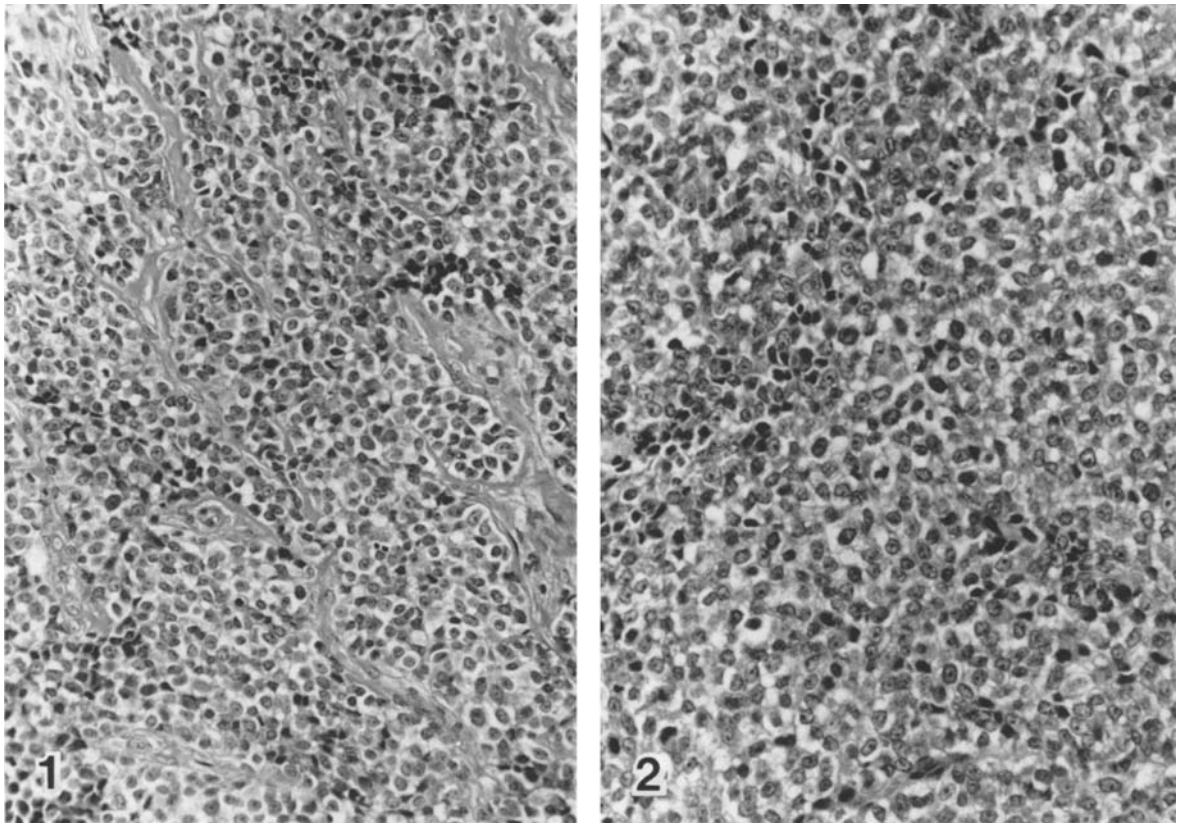


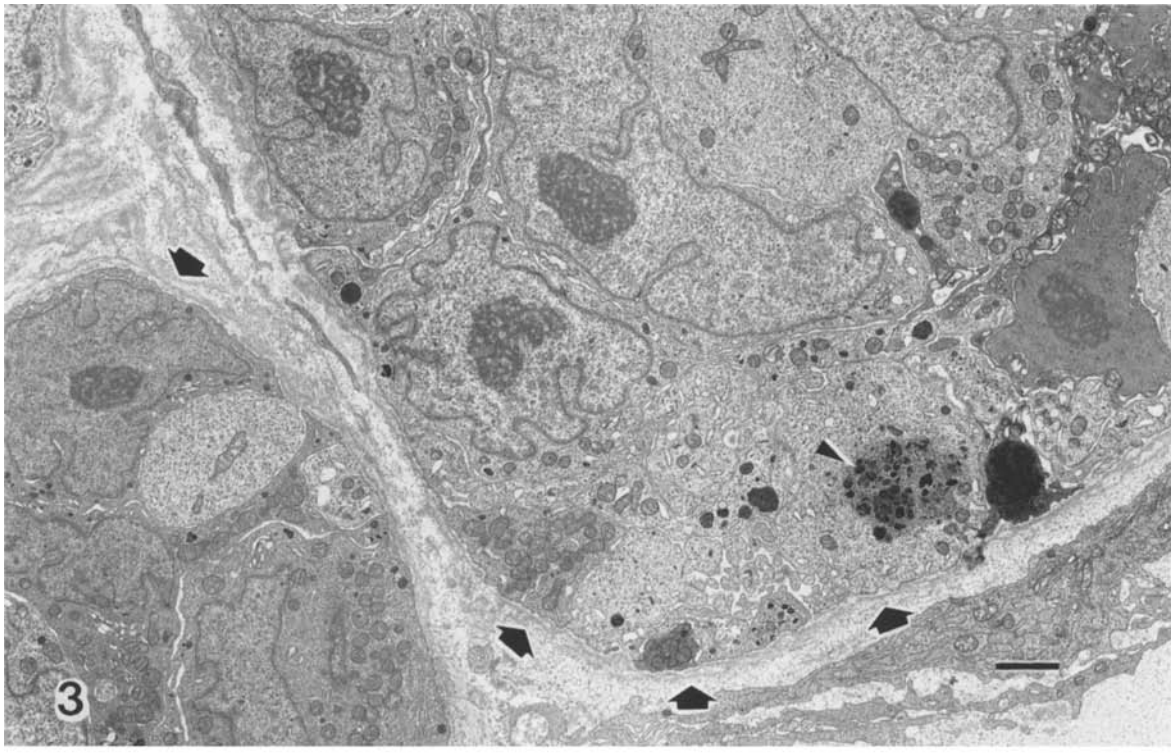
Fig. 1. Histology of the primary tumour from case 1. Tumor cells with clear cytoplasm are observed arranged in small nests separated by fibrous stromal elements. Haematoxylin and eosin stain, × 200

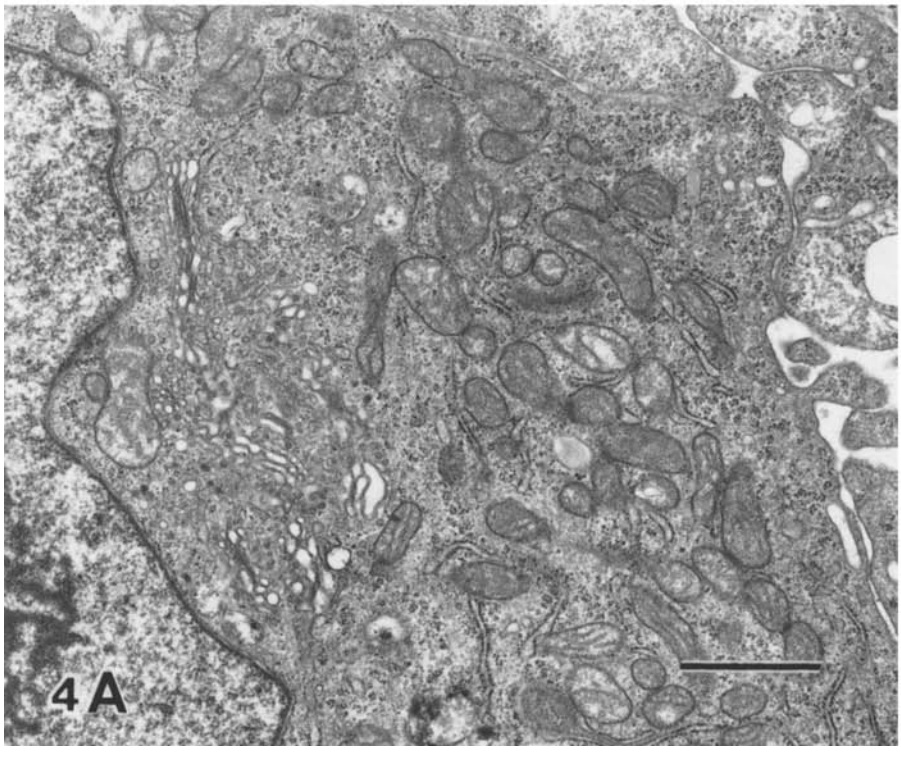
Fig. 2. Histology of the transplanted tumour at 6th generation. Note the uniform population of small round cells without cell nest formation. Haematoxylin and eosin stain, × 250

Immunostaining for S-100 protein in paraffin embedded sections is positive in most of the tumour cells from both metastatic and transplanted lesions.

On electron microscopy the ultrastructural appearance of the primary tumours in the two cases is also similar. Tumour cells are arranged in compact sheets, separated into nests by collagenous stroma, each group of closely apposed cells being surrounded by a continuous basal lamina (Fig. 3). The cells are oval or round with abundant cytoplasm and the nuclei are irregular in outline with chromatin granules generally being dispersed and only thin peripheral condensation being evident. Nucleoli are prominent in most of the cells. Occasionally, the tumour cells have cytoplasmic processes and the intermediate type of intercellular junction, but interdigitation of cytoplasmic processes is not observed. The cytoplasm contains moderate amounts of organelles including numerous mitochondria, free ribosomes, single elements of rough endoplasmic reticulum with thin cisternae, and well-developed Golgi areas (Fig. 4A). In many tumour cells, abundant cytoplasmic glycogen is observed. Most of these particles are negative in routinely stained sections, but can be clearly stained by alkaline bismuth (Fig. 4B). Many tumour cells contain abundant melanosomes in various stages of development (Fig. 5). The ultrastructural characteristics of the metastatic tumour from case 2 are essentially similar to those of the primary tumour with the exception that no melanosomes can be detected in the cytoplasm, even after extensive searching. In the transplanted lesions, the cells are small and their nuclear-cytoplasmic ratio is high. These tumour cells are closely apposed and arranged in sheets without forming cell nests. Basal laminae are inconspicuous and absent in some areas. The organelles are generally well developed but only few, immature melanosomes are observed.

One metastatic tumour and the nude mouse-transplanted tumours were examined for cytochemistry in this study, the results being similar





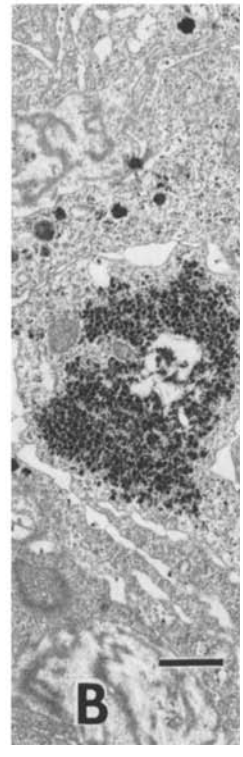


Fig. 3. Electron micrograph of primary tumour cells from case 2. Note the irregularly shaped nuclei and prominent nucleoli, and an aggregate of melanin pigments (*arrow head*) in the cytoplasm. Each cell nest is surrounded by a continuous basal lamina (*arrows*). Bar = 2 μm. × 4400

Fig. 4. Electron micrographs of primary tumour cells from case 2. Note the presence of short cell processes, numerous mitochondria, free ribosomes, irregular rough endoplasmic reticulum, and well-developed Golgi areas (**A**), and glycogen particles (**B**). Bar = 1 μm. × 16600 (**A**), × 7500 (**B**)

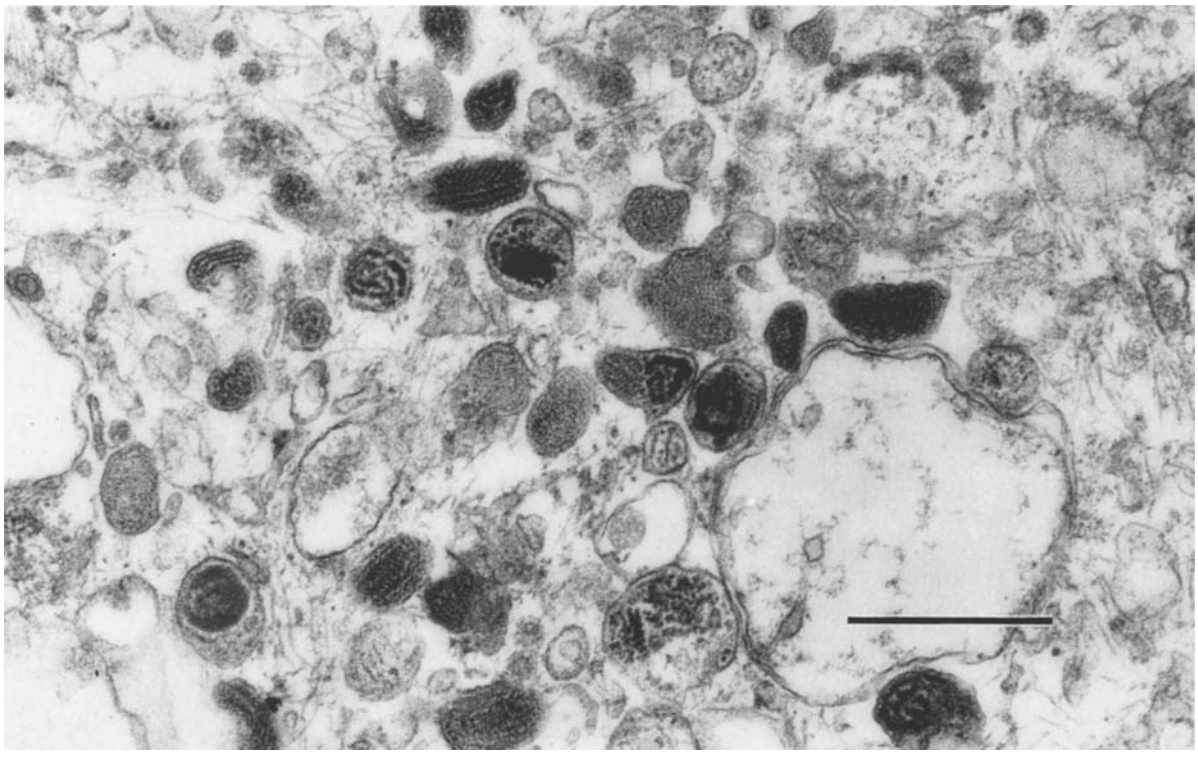


Fig. 5. Cytoplasmic detail from the case 1 primary tumour. Note abundant melanosomes in various stages of development. Bar = 0.5 μm. × 52000

in both cases. Under the electron microscope the 5′-nucleotidase and AlPase activities are positive on the cell surfaces of most tumour cells (Fig. 6A and 6B). The reaction for AlPase is completely inhibited by adding of levamisole to the medium, this latter not affecting the reaction for 5′-nucleotidase. AcPase activity is localized in lysosome-like dense bodies which are rarely observed in the tumour cells. TPPase activity is found in the transface of the tumour cell Golgi complexes. AchE activity is localized in the cell membranes, perinuclear cisternae, and small vesicles in the cytoplasm (Fig. 7A). Iso-OMPA almost completely inhibits the reaction in the cisternae of the perinuclear spaces and the vesicles in the cytoplasms (Fig. 7B), whereas eserine completely inhibits at all sites (Fig. 7C). These results indicate that the activities demonstrated in the cisternae of the perinuclear spaces and the vesicles are probably derived from non-specific cholinesterase, and those in the plasma membranes are from AchE. Tyrosinase-reaction product is observed within the cytoplasm of some tumour cells, localized in Golgi lamellae and vacuoles, and premelanosomes. The activity of this enzyme can be demonstrated not only in the transplanted cells but also in the metastatic tumour in which no melanosomes are detectable by routine electron microscopy (Fig. 8). Using substrate-free control media, no reaction products are observed with any of the cytochemical procedures. Findings for subcellular localization of reaction products of all enzymes investigated this study are summarized in Table 3.

Discussion

The ultrastructural characteristics of the two CCS cases examined in the present study were similar to those previously reported in the literature (Kubo 1969; Tsuneyoshi et al. 1978; Kindblom et al. 1983; Epstein et al. 1984; Mukai et al. 1984; Benson et al. 1985). The tumours were composed of closely apposed cells forming cell nests surrounded by continuous basal laminae. The irregularly-shaped nuclei with prominent nucleoli, well-developed cytoplasmic organelles, and abundant glycogen particles observed in most of these tumour cells have all been described as characteristic. In addition, the observation that primary tumour cells contained melanosomes in various stages of development, while transplanted tumours contained immature melanosomes is also in line with previous reports.

The histogenesis of CCS was initially considered to be tenosynovial because the tumours fre-

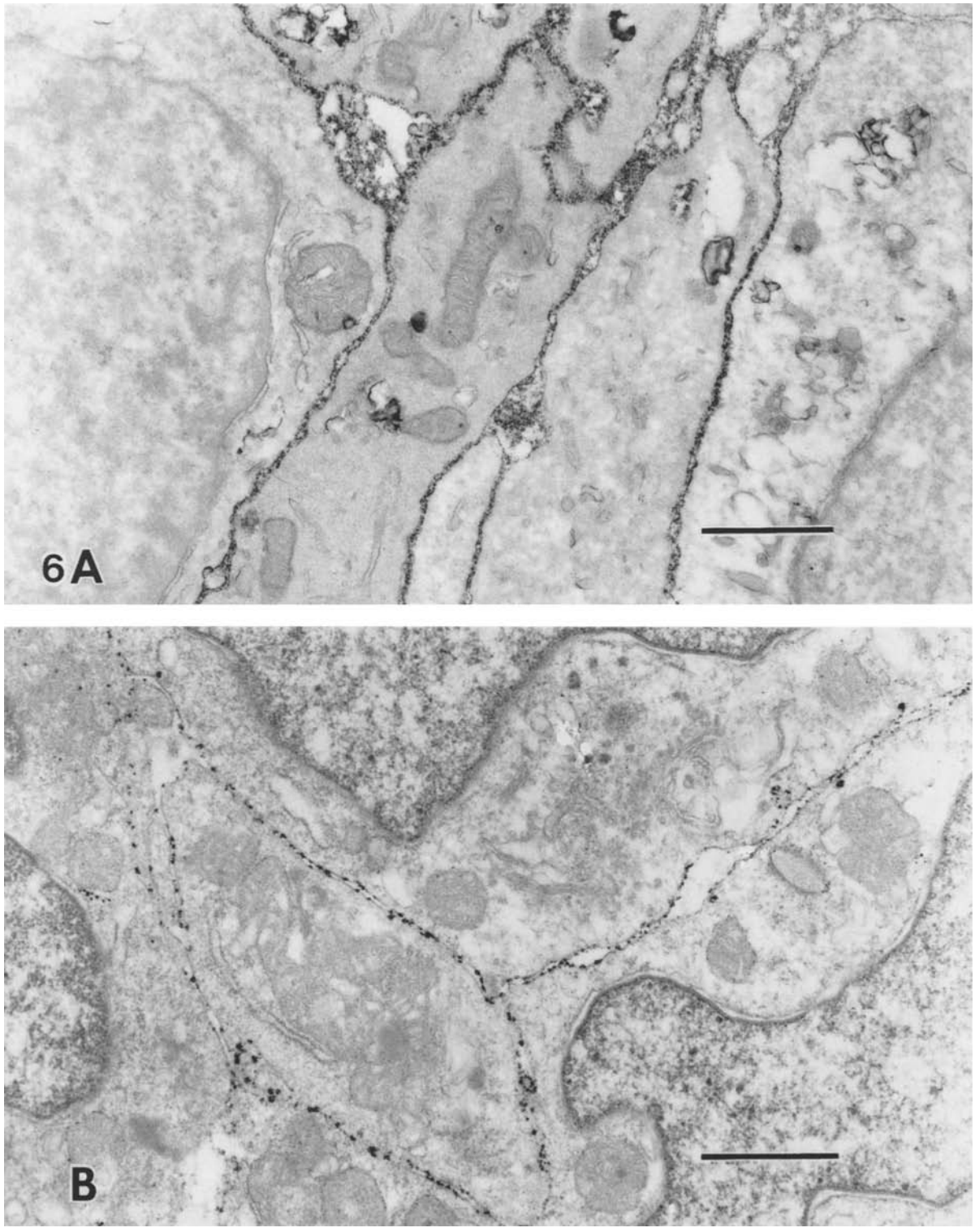


Fig. 6. Cytochemistry of 5′-nucleotidase (**A**) and AlPase (**B**) in the metastatic tumour. Note localization of reaction products of these enzymes on the cell surfaces. Bar = 1 μm. × 22000 (**A**), × 22500 (**B**)

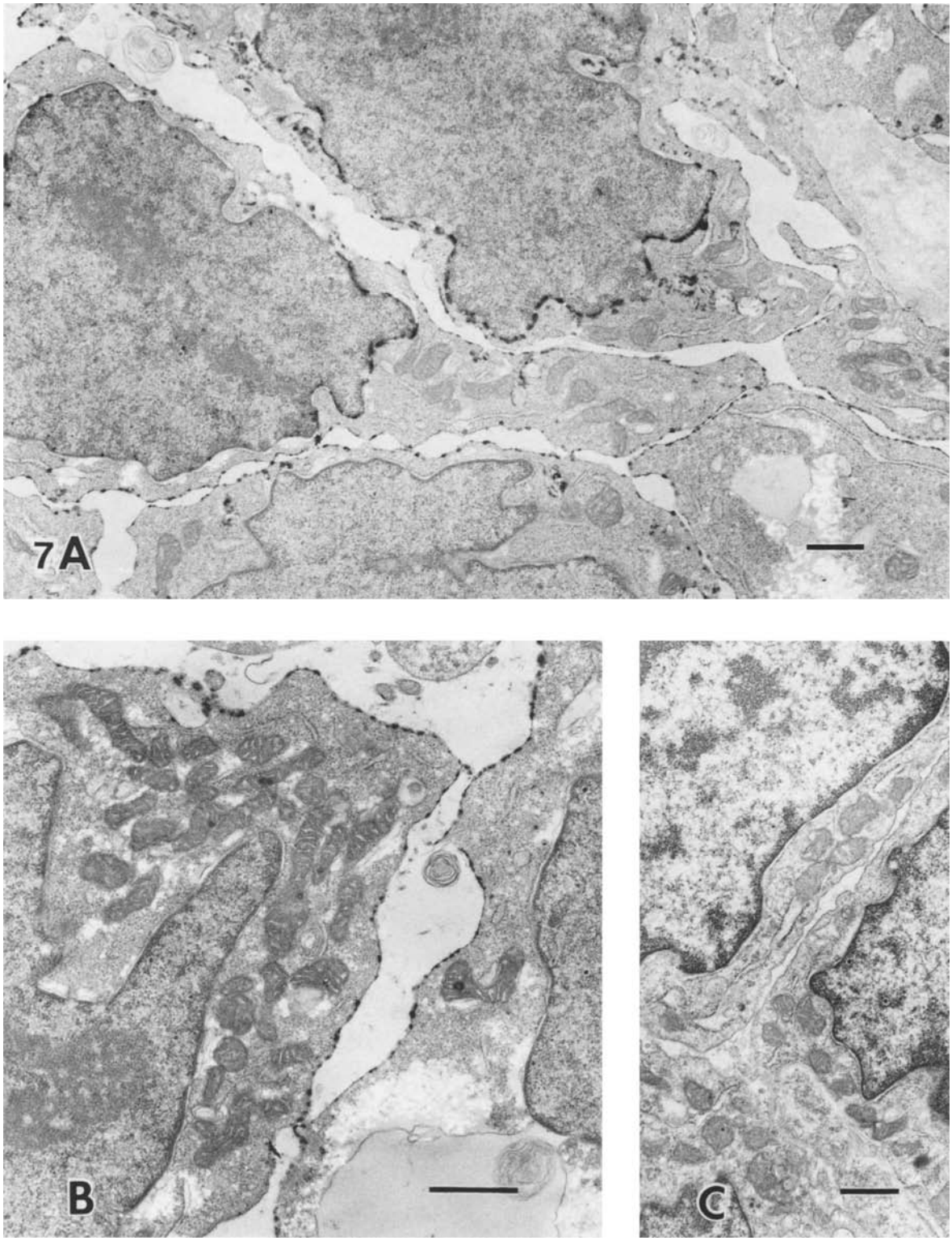


Fig. 7. Cytochemistry of AchE in the metastatic tumour. Reaction product is observed in the cell membranes, perinuclear cisternae and cytoplasmic small vesicles (**A**). Iso-OMPA inhibits non-specific cholinesterase activity in the perinuclear cisternae and the vesicles (**B**), whereas eserine completely inhibits the activity of esterase at all sites (**C**). Bar = 1 μm. ×11250 (**A** and **C**), ×15000 (**B**)

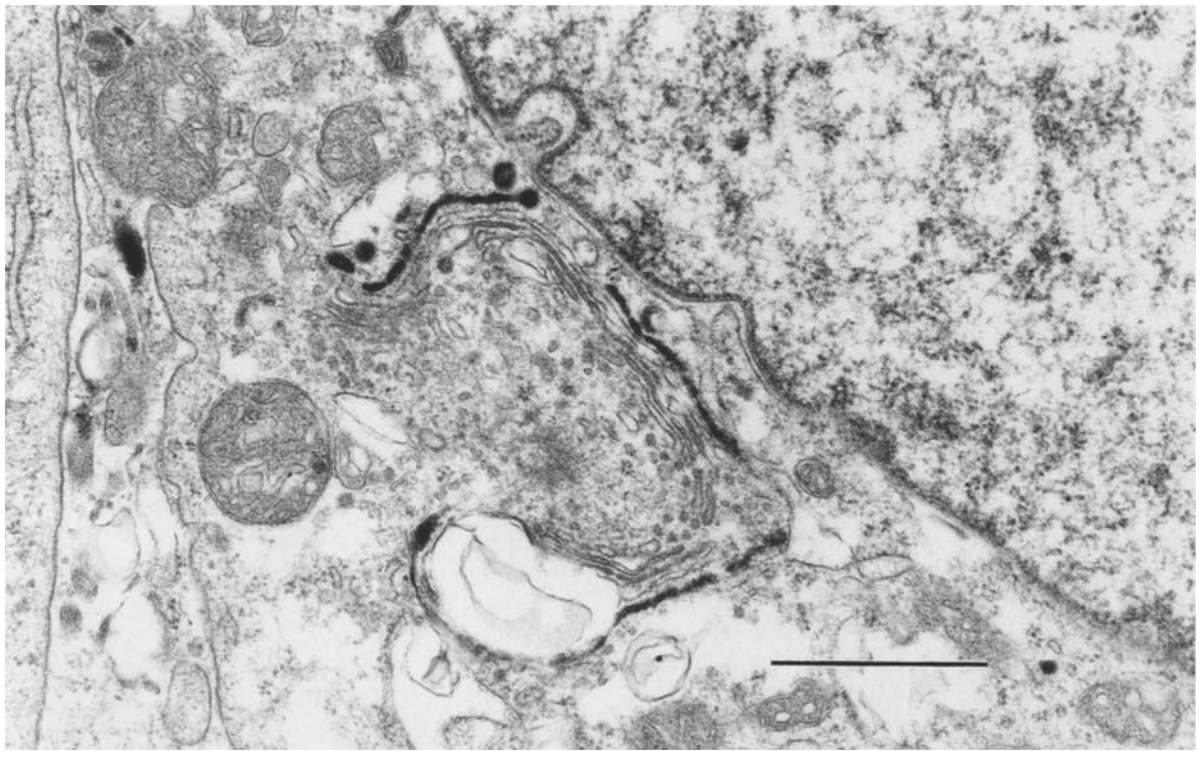


Fig. 8. Cytochemistry of tyrosinase in the amelanotic metastatic tumour. Note the reaction product localized in Golgi lamellae and vacuoles. Bar = 1 μm. × 30000

Table 3. Subcellular localization of enzyme activities and effects of enzyme inhibitors

Enzyme	Localization of reaction product	Effect of inhibitor
5′-Nucleotidase	Cell membrane	−(Levamisole)
Alkaline phosphatase	Cell membrane	+(Levamisole)
Acid phosphatase	Lysosome	N.T.
Thiamine pyrophosphatase	Trans-face of Golgi complex	N.T.
Acetyl cholinesterase	Cell membrane Perinuclear cisterna Small vesicle	−(iso-OMPA), +(Eserine) +(iso-OMPA), +(Eserine) +(iso-OMPA), +(Eserine)
Tyrosinase	Golgi lamella Golgi vacuole Premelanosome	N.T.

N.T.: Not tested, +: Completely inhibited; −: Not affected; iso-OMPA: Tetraisopropylpyrophosphoramide

quently develop within tendons or aponeuroses (Enzinger 1965; Kubo 1969; Hajdu et al. 1977). Recently, however, most investigators have considered the lesion to be of neural crest origin, on the basis of melanin identification in a large number of CCS cases. Some authors have therefore regarded this neoplasm as a variant of malignant melanoma, or schwannoma (Azumi and Turner 1983; Ohno et al. 1984). Production of melanin has been reported in normal dermal Schwann cells (Garcia and Szabo 1979) as well as in experimental melanotic tumour cells (Nakai and Rappaport 1963). Azumi and Turner (1983) described a case of CCS with an characteristic appearance which they interpreted as indicating melanotic Schwann cell differentiation, observation of basement membranes, interdigitation of neighboring cells and intracytoplasmic microfilaments being cited as evi-

dence. In the present study, although we frequently observed basal laminae surrounding each cell nest, at least in the primary tumours we could not identify any other features supportive of Schwann cell differentiation.

To the authors' knowledge, this is the first ultrastructural study on tyrosinase activity of CCS cells. This enzyme is of direct relevance since it is well known that identification of tyrosinase activity is evidence of melanin production, according to investigations of melanogenesis of normal pigment cells or malignant melanoma cells (Seiji and Iwashita 1965; Mishima 1966; Novikoff et al. 1968; Hunter et al. 1970; Maul and Romsdahl 1970; Hunter et al. 1978). In the present study, tyrosinase activity could be clearly demonstrated in cells from both primary tumours under the electron microscope. Furthermore, this activity was also confirmed in metastatic tumour cells in which melanosomes could not be found even after extensive seaching. The finding of tyrosinase activity within amelanotic CCS cells is in line with the earlier demonstration of this enzyme in amelanotic melanoma cells reported by Hunter et al. (1978). Recently, Mukai et al. (1984) suggested that the DOPA reaction is negative in both melanotic as well as amelanotic CCS on the basis of light microscope findings. As shown in the present study, however, reaction product may be difficult to detect under light microscope because of its localization within the Golgi areas.

Another striking new observation in this study is the demonstration of cholinesterase activity within the tumour cells. According to Mishima (1966), this enzyme can be expressed concomitantly with tyrosinase in the cytoplasm of melanosome-producing melanoma cells. The present enzyme cytochemical study therefore supplies further evidence of their twin involvement in melanin production by tumour cells in CCS.

The significance of the positive enzyme histochemical results for 5′-nucleotidase, AlPase, AcPase and TPPase is obscure. It is likely, however, that expression of these enzymes is not specific for this neoplasm since they have been demonstrated in many other types of neoplastic cells (Mayahara et al. 1967; Tokumitsu et al. 1981; Robinson and Karnovsky 1983b).

The present immunohistochemical results concerning S-100 protein positivity in metastatic and the nude mouse transplanted CCS are in agreement with the findings of other investigators (Chung and Enzinger 1983; Kindblom et al. 1983; Mukai et al. 1984; Benson et al. 1985). According to Nakajima et al. (1982a, 1982b), this protein is a highly selective marker of Schwann cells and melanocytes. However, its expression is not specific and similarities between melanocytic and peripheral nerve sheath tumours are considered to be only reflections of the same embryological derivation from the neural crest.

In conclusion, the findings for tyrosinase and cholinesterase activities in CCS cells documented in the present report taken together with the other evidence available in the literature are strongly indicative of a neural crest origin for CCS. Why this soft tissue tumour develops frequently in tendons or aponeuroses is unclear, but a derivation from deeply migrating melanocytes or melanotic Schwann cells as suggested by Azumi and Turner (1983) is a clear possibility which warrants further investigation.

Acknowledgements. These studies were supported in part by Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture and Ministry of Health and Welfare of Japan.

References

Azumi N, Turner RR (1983) Clear cell sarcoma of tendons and aponeuroses: Electron microscopic findings suggesting Schwann cell differentiation. Hum Pathol 14:1084–1089

Bearman RM, Noe J, Kempson RL (1975) Clear cell sarcoma with melanin pigment. Cancer 36:977–984

Benson JD, Kraemer BB, Mackay B (1985) Malignant melanoma of soft parts: An ultrastructural study of four cases. Ultrastruct Pathol 8:57–70

Boudreaux D, Waisman J (1978) Clear cell sarcoma with melanogenesis. Cancer 41:1387–1394

Chung EB, Enzinger FM (1983) Malignant melanoma of soft parts. A reassessment of clear cell sarcoma. Am J Surg Pathol 7:405–413

Dutra FM (1970) Clear cell sarcoma of tendons and aponeuroses: Three additional cases. Cancer 25:942–946

Eckardt JJ, Pritchard DJ, Soule EH (1983) Clear cell sarcoma. Clinicopathologic study of 27 cases. Cancer 52:1482–1488

Ekfors TO, Rantakokko V (1979) Clear cell sarcoma of tendons and aponeuroses: Malignant melanoma of soft tissue? Report of four cases. Pathol Res Pract 165:422–428

Enzinger FM (1965) Clear-cell sarcoma of tendons and aponeuroses. Cancer 18:1163–1174

Epstein AL, Martin AO, Kempson R (1984) Use of a newly established human cell line (SU-CCS-1) to demonstrate the relationship of clear cell sarcoma to malignant melanoma. Cancer Res 44:1265–1274

Garcia RI, Szabo G (1979) Melanosomes in dermal Schwann cells of human and rodent skin. Arch Dermatol Res 264:83–87

Hajdu SI, Shiu MH, Fortner JG (1977) Tenosynovial sarcoma. A clinicopathological study of 136 cases. Cancer 39:1201–1217

Hoffman GJ, Carter D (1973) Clear cell sarcoma of tendons and aponeuroses with melanin. Arch Pathol 95:22–25

Hunter JAA, Mottaz JH, Zelickson AS (1970) Melanogenesis: Ultrastructural histochemical observations on ultraviolet irradiated human melanocytes. J Invest Derm 54:213–221

Hunter JAA, Paterson WD, Fairley D (1978) Human malignant melanoma. Melanosomal polymorphism and the ultrastructural DOPA reaction. Brit J Derm 98:381–390

Karnovsky MJ (1964) The localization of cholinesterase activity in rat cardiac muscle by electron microscopy. J Cell Biol 23:217–232

Kindblom LG, Lodding P, Angervall L (1983) Clear-cell sarcoma of tendons and aponeuroses. Virchows Arch Pathol Anat 401:109–128

Kubo T (1969) Clear-cell sarcoma of patellar tendon studied by electron microscopy. Cancer 24:984–953

Mackenzie DH (1974) Clear cell sarcoma of tendons and aponeuroses with melanin production. J Pathol 114:231–237

Maul GG, Romsdahl MM (1970) Ultrastructural comparison of two human malignant cell lines. Cancer Res 30:2782–2790

Mayahara H, Hirano H, Saito T, Ogawa K (1967) The new lead citrate method for the ultracytochemcial demonstration of activity of non-specific alkaline phosphatase (Orthophosphoric monoester phosphohydrase). Histochemie 11:88–96

Mishima Y (1966) Cholinesterase and tyrosinase activity in malignant melanoma. Cancer 19:665–673

Mukai M, Torikata C, Iri H, Mikata A, Kawai T, Hanaoka H, Yakumaru K, Kageyama K (1984) Histogenesis of clear cell sarcoma of tendons and aponeuroses. An electronmicroscopic, biochemical, enzyme histochemical, and immunohistochemical study. Am J Pathol 114:264–272

Nakai T, Rappaport H (1963) A study of the histogenesis of experimental melanotic tumors resembling blue nevi: The evidence in support of their neurogenic origin. Am J Pathol 43:175–199

Nakajima T, Watanabe S, Sato Y, Kameya T, Hirota T, Shimosato Y (1982a) An immunoperoxidase study of S-100 protein distribution in normal and neoplastic tissues. Am J Surg Pathol 6:715–727

Nakajima T, Watnabe S, Sato Y, Kameya T, Shimosato Y, Ishihara K (1982b) Immunohistochemical demonstration of S-100 protein in malignant melanoma and pigment nevus, and its diagnostic application. Cancer 50:912–918

Novikoff AB, Goldfischer S (1961) Nucleoside diphosphatase activity in the Golgi apparatus and its usefulness for cytologic studies. Proc Natl Acad Sci USA 47:802–810

Novikoff AB, Albala A, Biempica L (1968) Ultrastructural and cytochemical observations on B-16 and Harding-Passay mouse melanomas. The origin of premelanosomes and compound melanosomes. J Histochem Cytochem 16:299–316

Ohno T, Park P, Utsunomiya Y, Hirahata H, Inoue K (1986) Ultrastructural study of a clear cell sarcoma suggesting Schwannian differentiation. Ultrastruct Pathol 10:39–48

Parker JB, Marcus PB, Martin JH (1980) Spinal melanotic clear-cell sarcoma: A light and electron microscopic study. Cancer 46:718–724

Raynor AC, Vergas-cortes F, Alexander RW (1979) Clear-cell sarcoma with melanin pigment: A possible soft-tissue variant of malignant melanoma. Case report. J Bone Joint Surg 61-A:276–280

Robinson JM, Karnovsky MJ (1983a) Ultrastructural localization of 5′-nucleotidase in guinea pig neutrophils based upon the use of cerium as capturing agent. J Histochem Cytochem 31:1190–1196

Robinson JM, Karnovsky MJ (1983b) Ultrastructural localization of several phosphatases with cerium. J Histochem Cytochem 31:1197–1208

Schmidt D, Mackay B (1982) Ultrastructure of human tendon sheath and synovium: Implications for tumor histogenesis. Ultrastruct Pathol 3:269–283

Seiji M, Iwashita S (1965) Intracellular localization of tyrosinase and site of melanin formation in melanocytes. J Invest Derm 45:305–314

Tokumitsu S, Tokumitsu K, Fishman WH (1981) Intracellular alkaline phosphatase activity in cultured human cancer cells. Histochemistry 73:1–13

Tsuneyoshi M, Enjoji M, Kubo T (1978) Clear cell sarcoma of tendons and aponeuroses: A comparative study of 13 cases with a provisional subgrouping into the melanotic and synovial types. Cancer 42:243–252

Received December 8, 1988/Accepted February 23, 1989