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Hiroaki Hiraga · Takayuki Nojima · Syuiti Abe
Katsushige Yamashiro · Shinya Yamawaki
Kiyoshi Kaneda · Kazuo Nagashima

Establishment of a new continuous clear cell sarcoma cell line

Morphological and cytogenetic characterization and detection of chimaeric *EWS/ATF-1* transcripts

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Abstract Clear cell sarcoma (CCS), a rare tumour of deep soft tissues, often has a t(12; 22) (q13; q12) translocation that induces the formation of a hybrid *EWS/ATF-1* gene. To investigate these alterations further, we established a new continuous cell line directly from a CCS taken from a 9-year-old girl. The cultures were characterized with respect to morphological, ultrastructural, immunohistochemical and karyotypical features and were tested by reverse transcription PCR (RT-PCR) for chimaeric *EWS/ATF-1* transcripts. The continuous cell line, designated KAO, is tumorigenic in nude mice, and the resultant tumours resemble the primary CCS. The tumour cells and the cultured cells have melanosomes in their cytoplasm and are immunoreactive with the melanoma-specific antibody HMB45, but do not express S-100 protein. The cultured CCS cells have the t(12; 22)(q13; q12) translocation and express the hybrid *EWS/ATF-1* gene. No transcripts of the hybrid gene were detected in a malignant cutaneous melanoma tested simultaneously. Although CCS and malignant melanoma are morphologically related, the present results suggest that their geneses differ at the chromosome and molecular levels. They also indicate that chromosome analysis and detection of fusion *EWS/ATF-1* transcripts may be useful adjuvant tools for the diagnosis of CCS.

Key words Clear cell sarcoma · Cytogenetics · *EWS/ATF-1* · Reverse transcription-PCR · Malignant melanoma

H. Hiraga (✉) · K. Nagashima
Department of Pathology, Hokkaido University School of Medicine. N-15, W-7, Kita-ku, Sapporo 060, Japan
Tel: (81) 11-716-1161, ext: 5902, fax: (81) 11-758-4128

T. Nojima
Department of Pathology, Kanazawa Medical University Hospital, Uchinada, Japan

S. Abe
Chromosomal Research Unit, Faculty of Science and Laboratory of Cytogenetics, Division of Biological Sciences, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo, Japan

K. Yamashiro
Division of Clinical Pathology, Department of Clinical Research, Sapporo National Hospital, Sapporo, Japan

S. Yamawaki
Division of Orthopaedic Surgery,
Department of Clinical Research, Sapporo National Hospital, Sapporo, Japan

K. Kaneda
Department of Orthopaedic Surgery, Hakkaido University School of Medicine, Sapporo, Japan

Introduction

Clear cell sarcoma (CCS) is a rare malignant neoplasm that occurs in deep soft tissues, usually near tendons and aponeuroses in the extremities of young adults. Although recognized as a clinicopathological entity more than three decades ago by Enzinger [10] its histogenesis has not been fully established and remains controversial. Thus, some investigators have considered that CCS is of synovial origin because of its histological appearance and ultrastructure, and the intimate anatomical relationship between the tumour and tendons or aponeuroses of synovial tissue [19, 33]. However, the presence of melanosomes within the cytoplasm of CCS cells of some cases [2–4, 9, 17, 18, 24] and the expression of S-100 protein by the tumour cells [3, 7, 17, 24, 34] have led other investigators to believe that CCS is of neural crest origin. Although the results of ultrastructural studies on several CCSs suggested schwannian differentiation [1, 26], most tumours have shown melanocytic differentiation [2, 7, 17, 34]. These findings prompted the designation of malignant melanoma of soft parts rather than that of CCS for the malignancy [7]. Moreover, phenotypic analyses of cell lines derived from CCS have also revealed melanocytic differentiation [12, 30, 35].

The results of recent chromosome analyses indicate that the reciprocal translocation, t(12; 22)(q13–14; q12–13), is a specific primary cytogenetic abnormality in CCS [5, 6, 13, 23, 27–29, 31, 32, 36]. In addition, it was shown by Zucman et al. [40] that this chromosomal translocation fused the *EWS* gene to the gene encoding

the transcription factor ATF-1, thus producing a chimaeric EWS/ATF-1 protein that may be involved in oncogenesis. To investigate these CCS alterations further, we established a new continuous cell line directly from an example of the tumour. We describe the characteristics of the cell line, and present data pertaining to cytogenetic studies and to the detection of hybrid *EWS/ATF-1* transcripts.

Materials and methods

The patient was a 9-year-old Japanese girl, who felt a mass in the lateral side of her left thigh. The T1-weighted magnetic resonance image showed a low intensity mass (size: 3.5×3.5×4.0 cm) located on the deep fascia that invaded the long head of the biceps muscle and subcutaneous tissue. Lymphangiography revealed several enlarged lymph nodes in the left inguinal region. Examination of a needle biopsy revealed that the girl had a CCS. Wide resection of the tumour and inguinal lymphadenectomy were performed. The patient was given adjuvant chemotherapy consisting of five courses of dacarbazine, nimustine hydrochloride and vincristine sulfate (DAV). She has been well, and there has been no evidence of local recurrence or metastasis for the 6.5 years since her hospitalization.

A portion of the surgically resected tumour tissue was cut and minced with scissors into small fragments and incubated for 20 min at 37°C in 5 ml of medium RPMI-1640 containing 1.0% collagenase. The supernatant was removed after centrifugation for 5 min at 1500 rpm, the cells suspended in RPMI-1640 containing 10% heat-inactivated fetal bovine serum, penicillin G (100 units/ml) and streptomycin sulfate (100 mg/ml), and inoculated into collagen-coated culture dishes. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Upon reaching confluency, the monolayers were dispersed with trypsin and the cells transferred into new culture dishes. The resulting continuous cell line was designated KAO. The doubling time of the cell population was determined with 1×10^6 cells per 25-cm^2 collagen-coated dish. The number of cells in three dishes was counted at 24-h intervals for up to 120 h and averaged.

For heterotransplantation studies, 2×10^7 KAO cells were suspended in 0.5 ml of potassium phosphate buffer (pH 7.4) and injected s.c. into athymic nude mice (BALB/cAJclnu/nu, Nihon Clea, Tokyo, Japan). The experimental protocol was approved by the Animal Committee of the Institute for Animal Experimentation of Hokkaido University School of Medicine.

Specimens of the primary tumour and of the transplanted tumours formed in nude mice were fixed in 10% neutral, buffered formalin, embedded in paraffin and stained with haematoxylin-eosin (H&E), periodic acid-Schiff, and Fontana-Masson stains. For electron microscopic examination, specimens of the primary and transplanted tumours and of the cultured cells were fixed in 2% glutaraldehyde, post-fixed with 1% osmium tetroxide and embedded in Epon 812. Ultrathin sections were prepared, stained with uranyl acetate and lead citrate and examined with a Hitachi H-800 electron microscope.

Paraffin-embedded sections of the transplanted tumour and of cultured cells grown in chamber/slides (Nunc, Naperville, Ill.) were used for the immunohistochemical assays; the standard three-step indirect streptavidin–biotin–peroxidase method was employed. In addition to the melanoma-specific antibody HMB45 (DAKO; diluted 1:50), antibodies against S-100 protein (Dakopatts, Glostrup, Denmark; 1:10000), vimentin (Bioscience, Emmenbrucke, Switzerland, 1:200), keratin (wide-spectrum screening, DAKO, 1:500), and epithelial membrane antigen (EMA, Dakopatts, 1:100) were used.

For cytogenetic analysis of KAO cells, exponentially growing cultures were incubated for 1 h at 37°C with colcemid (0.2 mg/ml). The cells were then treated for 15 min at 37°C with a hypotonic solution of 0.7% sodium citrate and fixed with acetic acid–methanol (1:3) at 4°C. The cell suspension was centrifuged at 1500 rpm for 5 min and fixed three more times. The cells were then dropped onto cold, wet slides precleaned in ethanol. After 2 weeks, the slides were stained for G-bands using the trypsin-Giemsa banding technique.

A reverse transcription polymerase chain reaction (RT-PCR) was used to assay for the presence of hybrid *EWS/ATF-1* transcripts in KAO cells. Fuji cells, a line derived from a synovial sarcoma [25], and a malignant melanoma resected from the skin of the scrotum were included in the assays and served as control specimens. Total RNA was extracted with ISOGEN, and 1 μg was reverse-transcribed using random hexamers and the GeneAmp RNA-PCR kit (Perkin-Elmer) in accordance with the manufacturer's protocol. The resulting cDNA was amplified by PCR using the oligonucleotide primers 22.1, 5′-CCCACTAGTTACCCACCCCA-3′, and ATF-1.1, 5′-AAAACTCCACTAGGAAATCCATTT-3′. These primers correspond to exon 7 of *EWS* and the 3′ untranslated region of *ATF-1*, respectively [40]. As an internal control, the cDNAs were amplified with primers specific for β-actin. Amplification was carried out in a final volume of 100 μl using AmpliTaq polymerase (Perkin-Elmer) and a Perkin-Elmer thermal cycler. We performed 30 amplification cycles, each consisting of 30 s at 94°C (denaturation), 30 s at 60°C (annealing) and 90 s at 72°C (extension). Tubes with all reagents except template were included in each run. The amplified products were separated by agarose (3%) gel electrophoresis and transferred onto nylon membranes. The membranes were hybridized with the ^{32}P-labelled oligonucleotide probe CCP-1, ATCTATCAGACTAGCAGCGGA, derived from *ATF-1* sequence data [40].

For sequencing, single-strand DNA was generated by asymmetric PCR, using an annealing temperature of 58°C. The resulting products were purified with QIAEX (QIAGEN, Chatsworth, Calif.) and sequenced with the 7-DEAZA sequencing kit.

Results

On gross examination, the cut surface of the primary tumour appeared brown in some parts, and white in others. Histologically, the CCS showed a varied architecture, consisting of a diffuse pattern and nests or fasciculi of polygonal or fusiform cells bordered by a delicate framework of fibrous tissue. The individual cells had round to ovoid vesicular nuclei with prominent nucleoli and a finely stippled eosinophilic cytoplasm (Fig. 1). Melanin granules were seen in the cytoplasm; their presence was verified with the Fontana-Masson stain. Ultrastructurally, the tumour was seen to be composed of oval to fusiform cells with round nuclei each containing one or more prominent nucleoli. The cytoplasm contained multiple round mitochondria, little glycogen, and melanosomes at various stages (Fig. 2). One of the inguinal lymph nodes had a metastasis with similar features to those of the primary tumour.

Most cells grew on the surface of the collagen-coated dishes, forming a loosely adherent monolayer. A small proportion of the cells was suspended in the medium. The attached cells proliferated rapidly without exhibiting contact inhibition. On examination with the phase-contrast microscope, the adherent cells appeared spindle-shaped, or tripolar (Fig. 3). The cells showed stable growth and no contamination after more than 50 passages, indicating that we had established a bona fide continuous CCS cell line. This was designated KAO from the patient's first name, Kaori. The doubling time of the cells was approximately 34 h. Electron microscopic ex-

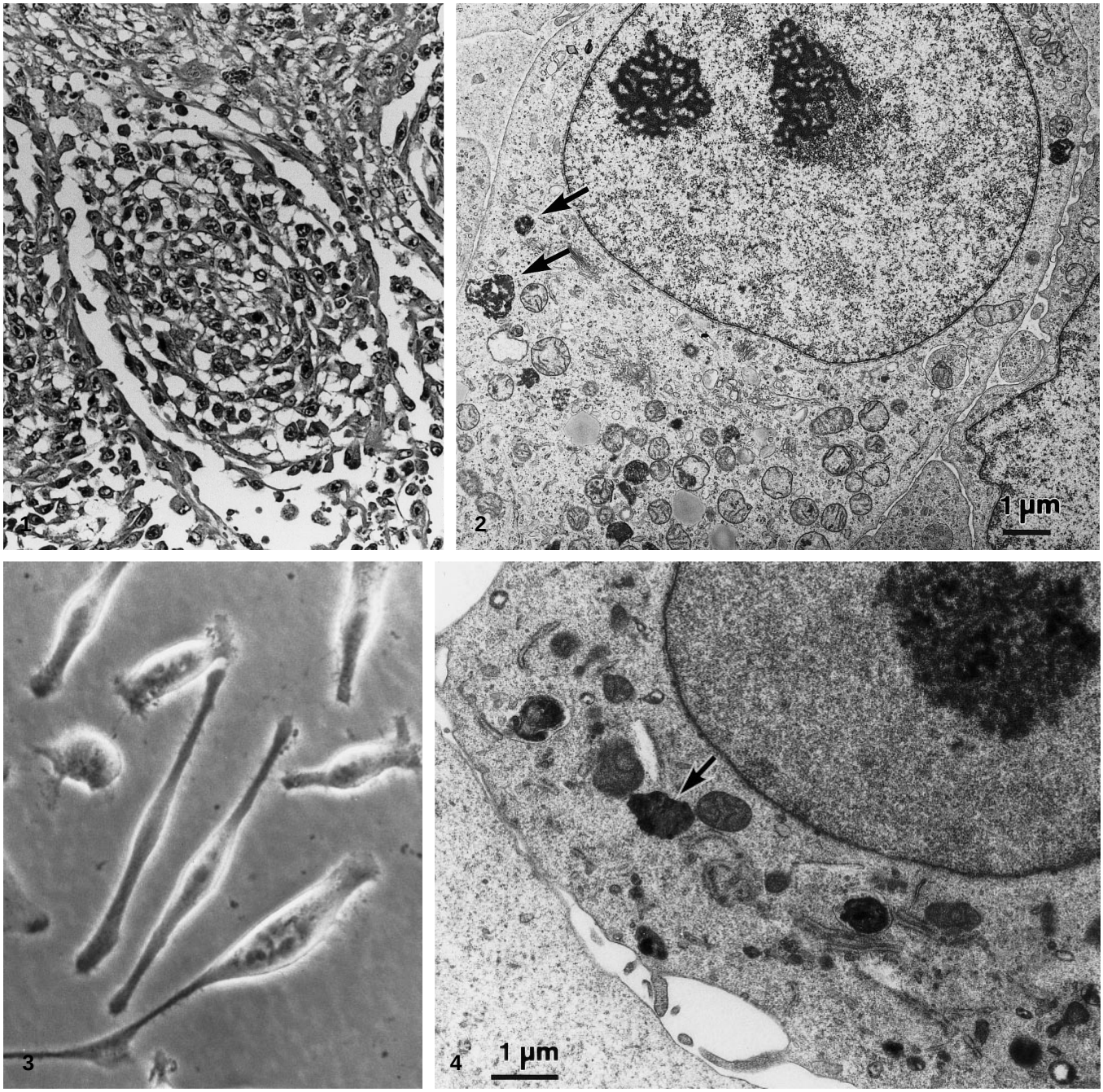

Fig. 1 Histological appearance of the primary tumour. Nests of polygonal and fusiform cells with vesicular nuclei and prominent nucleoli are evident. H&E, ×180

Fig. 2 Electron micrograph of the primary tumour. Melanosomes (*arrows*) are seen in the cytoplasm. ×8,900

Fig. 3 Phase-contrast micrograph of the cultured cells photographed at the 50th passage, 24 h after subculture. Spindle-shaped cells are seen

Fig. 4 Electron micrograph of the cultured cells. The cytoplasm contains abundant free ribosomes, some mitochondria, Golgi apparatus, and few melanosomes (*arrow*). ×12,600

amination revealed that most of the cells had some filopodia, abundant cytoplasm and a large round nucleus with a single prominent nucleolus. The cytoplasm of the cultured tumour cells contained numerous free ribosomes, some mitochondria, Golgi apparatus, and a few melanosomes. The latter were atypical in that the membranous filaments failed to align properly (Fig. 4), thus resembling the melanosomes seen in malignant melanoma of the skin [8].

KAO cells inoculated into the subcutaneous space on the back of nude mice formed tumour masses that reached 18 mm in diameter within 2 months. The histological features of these tumours were identical to those of the original CCS. The same was true of their ultra-

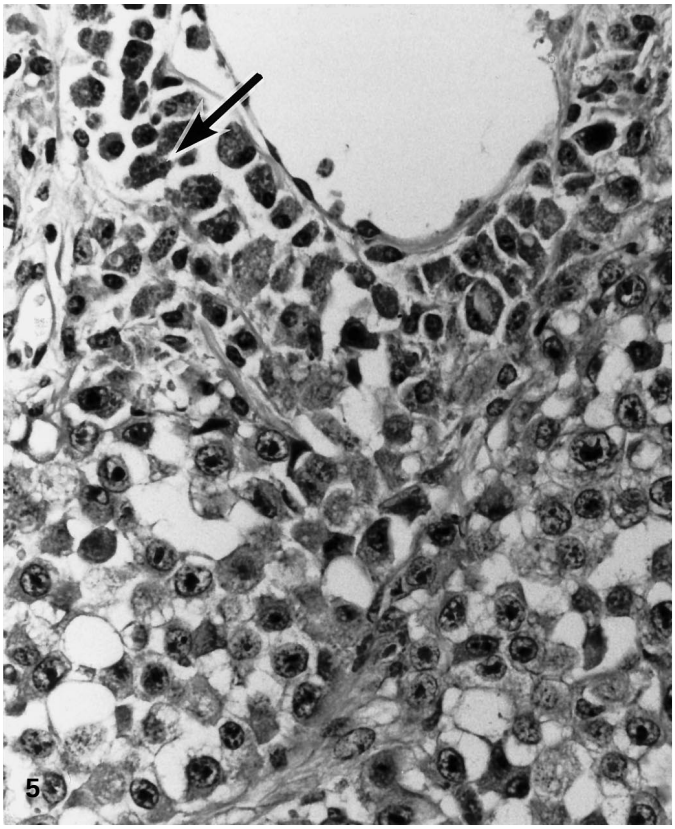

Fig. 5 Histological appearance of the tumours formed in nude mice. Similarity to the primary tumour is evident. Melanin pigment is noted (*arrow*). H&E, ×300

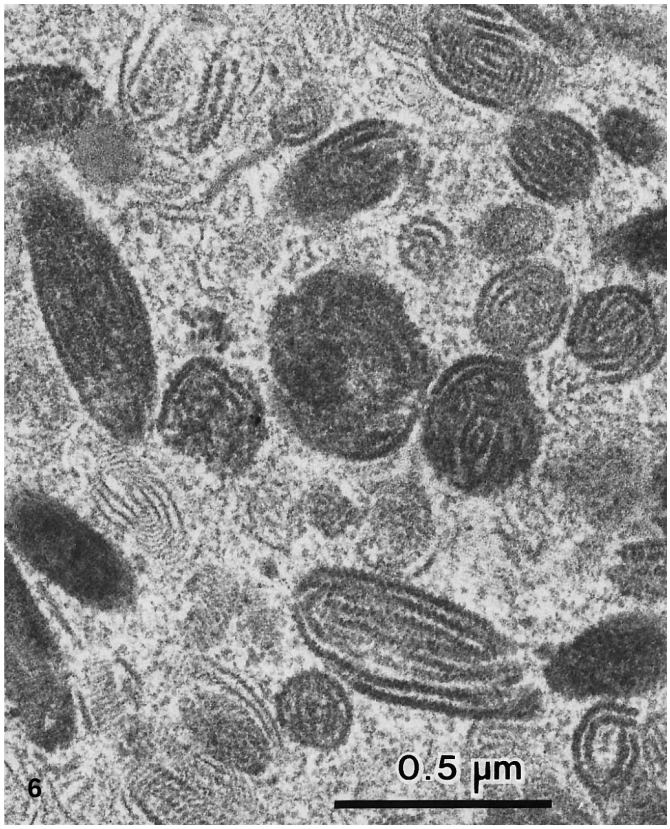

Fig. 6 Electron micrograph of the cells of tumours formed in nude mice. The cytoplasm contains many melanosomes in a variety of stages and some of them are atypical (*arrow*). ×44,200

Table 1 The number of chromosome copies/cell in the KAO cell line[a]

No. of copies/ cell	Normal chromosome																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
0									1								1						
1	18	18		18				1	17			18	18		1		17						
2			18		18	18		7		18	18			18	17	18		3	18	18		18	18
3								10										15			18		
4							18																

No.of copies/ cell	Marker chromosome[b]																		
	der (12)	der (22)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	NCA[c]
0								1			6	1	10	3	7	7	15	13
1	18	18	18	18	18	18	18	17	18	18	12	17	8	15	11	11	3	5

[a] Data based on the analysis of 18 G-banded cells.
[b] Marker chromosome: der(12); der(12)t(11;22)(q24;q12), der(22); der(22)t(11;22)(q24;q12)
[c] NCA; noclonal abnormality

structure. Thus, the tumour was composed of nests of polygonal cells defined by a delicate framework of fibrocollagenous tissue. The individual tumour cells possessed round to ovoid vesicular nuclei with prominent nucleoli and finely stippled eosinophilic cytoplasm (Fig. 5). Intracellular melanin was seen in H&E-stained sections. This was verified with the Fontana-Masson stain. Electron microscope examination revealed that the cells of the tumours produced in nude mice were ovoid or short and spindle shaped, and that they had numerous filopodia. Their nuclei were round with an irregular nuclear membrane and had one or two prominent nucleoli.

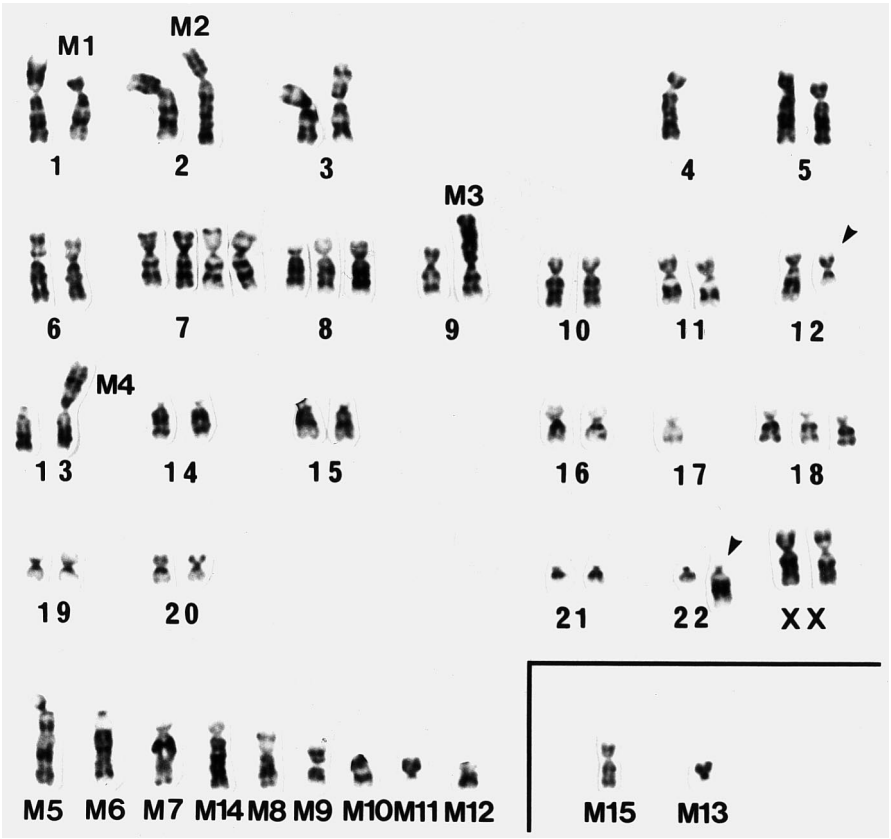

Fig. 8 Karyotype of a cultured CCS cell. *Arrow heads* point to the chromosome 12; 22 translocation. *M* denotes marker chromosomes. Two types of marker chromosomes of the other metaphases (*inset*). G-banding

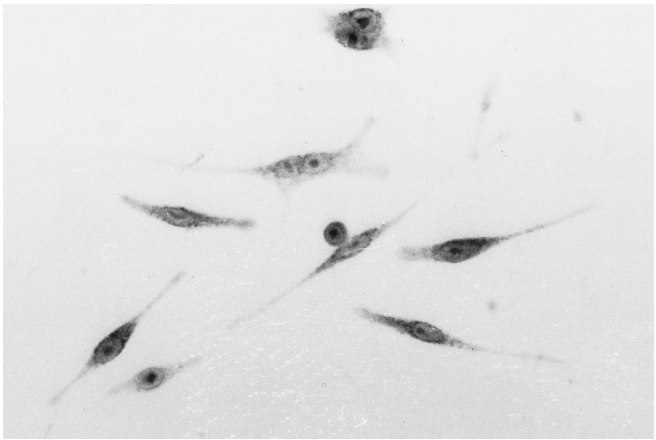

Fig. 7 Immunostaining of the cultured cells for HMB-45 showing granular staining in most cells

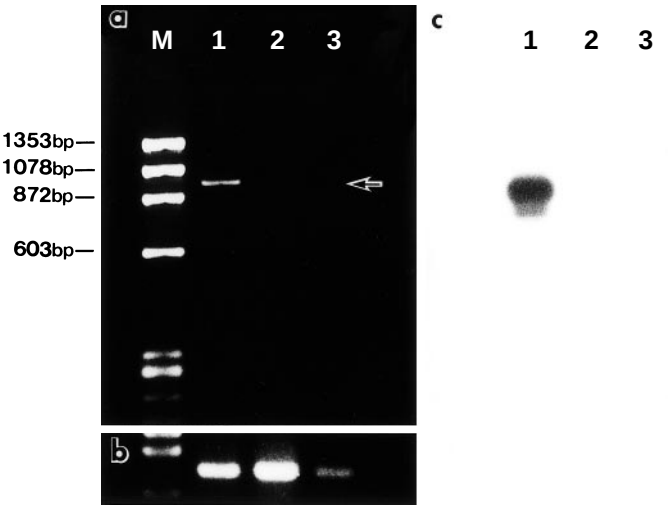

Fig. 9a–c Detection of fusion *EWS/ATF-1* transcripts in KAO cells by reverse transcription-PCR (RT-PCR). Total RNA extracted from KAO cells, Fuji cells and an excised cutaneous malignant melanoma was subjected to RT-PCR using *EWS*- and *ATF-1*-specific primers. The amplified DNA was separated by electrophoresis using 3% agarose gels. **a** Ethidium bromide-stained gel showing the approximate 950 bp PCR product (*arrow*) in lane 1. **b** Control for RNA integrity: RT-generated β-actin cDNA amplified by PCR. **c** Amplified DNA, transferred from the gel to nylon membrane, and probed with the ^{32}P-labelled *ATF-1*-specific oligonucleotide probe CCP-1. *M* = ϕ X174DNA-Hae III digest (size marker). *Lanes 1* KAO cells; *2* malignant melanoma of the skin; *3* synovial sarcoma cell line Fuji

The cytoplasm had numerous free ribosomes, mitochondria, Golgi apparatus, and arrays of rough endoplasmic reticulum. The cytoplasm of these tumour cells had more melanosomes than that of the cultured cells from which the xenografts were derived. The melanosomes were in various stages, and some of them were atypical (Fig. 6).

Most CCS cells of the primary tumour, of the cultures and of the tumours formed in nude mice were stained by the vimentin and melanoma-specific antibody HMB45

(Fig. 7). In contrast, they did not react with the antibodies to S-100 protein, keratin, or EMA.

The chromosome number of KAO cells varied from 31 to 103, with a mode of 58. The karyotypes of 18 G-banded cells, an example of which is shown in Fig. 8, are summarized in Table 1. The KAO cells usually had the reciprocal translocation involving chromosomes 12 and 22, t(12; 22)(q13; q12), as well as various numerical and structural chromosome abnormalities, including tetrasomy of chromosome 7, monosomy of chromosome 17, and 15 consistent markers.

The RNA from KAO cells yielded a major, approximately 950-bp DNA fragment by RT-PCR. The fragment's identity as a portion of the cDNA of the chimaeric *EWS/ATF-1* transcript was verified by hybridization with the *ATF-1*-specific oligonucleotide probe CCP-1 (Fig. 9). Sequencing of the amplified DNA revealed the same in-frame junction as was previously described by Zucman et al. [40]. In contrast, the RT of the RNA from the synovial sarcoma cell line and from the malignant cutaneous melanoma tumour did not promote DNA amplification with the *EWS*- and *ATF-1*-specific primers. Nevertheless, amplification of b-actin DNA was seen in all instances (Fig. 9).

Discussion

There are relatively few reports on the establishment of continuous CCS cell lines [12, 30, 35]. As shown here, we initiated a new cell line directly from the surgically resected CCS of a 9-year-old girl. The cells grow in monolayers, and upon injection into nude mice produce tumours that display ultrastructural and immunohistochemical features similar to those of the primary human sarcoma. It is of particular interest that melanosomes were identified in the cytoplasm of the primary tumour cells, KAO cells and cells in the tumours generated by heterotransplantation. It is evident from these sets of observations that KAO cells have retained characteristics of the original CCS. Moreover, the continuous cell line, like the primary tumour, showed positive immunoreactivity with HMB45, but did not express S-100 protein, which was different from the reported CCS [24, 30]. The reason for the S-100-non-reactivity of our CCS is unknown.

Recent studies revealed a similar or identical translocation, t(12; 22) (q13-14; q12-13), in 11 of 18 CCSs [5, 6, 12, 13, 20, 22, 23, 27–32, 35, 36], and it has been suggested that this translocation represents a primary cytogenetic abnormality. As our data indicate, KAO cells have the t(12; 22) (q13; q12) translocation. As shown by Zucman et al., the genes involved in the translocation form hybrid transcripts of the N-terminal domain of EWS and the bZIP domain of ATF-1 [40]. We found that KAO cells had the fused gene that encodes the chimaeric EWS/ATF-1 protein, and we verified, by sequencing the PCR product, that the breakpoint of the translocation was the same as that previously reported [40]. These findings are the first confirmatory results in CCS cell lines that the t(12; 22) translocation in CCS induces fusion of portions of the *EWS* and *ATF-1* genes.

The protein encoded by the *ATF-1* gene is a transcription factor that may normally be regulated by cAMP [14, 38]. However, in CCS, the chimaeric protein lacks a consensus protein kinase A phosphorylation site which might contribute to the regulation of the transcription activity of *AFT-1* by cAMP, but retains the bZIP domain necessary for heterodimerization and DNA binding [16,38]. Fujimura demonstrated that EWS-ATF-1 binds weakly to DNA in vitro but functions as an efficient constitutive transcriptional activator, unlike the normal ATF-1, which needs to be induced with cAMP [15]. Consequently it is possible that the fused *EWS/ATF-1* gene also acts as a potent oncogene that may be involved in the genesis of CCS. This hypothesis may be verified by experiments using this cell line in addition to the previous ones. They include transfection of the antisense *EWS/ATF-1* expression plasmids to the cultured cells or microinjection of anti-fusion protein antibody to them.

Although there is still some controversy regarding a synovial or neuroectodermal histogenesis [37], the idea that CCS is a homogeneous clinicopathological entity of neural crest origin has found increasing support [7, 11, 24]. Our finding of numerous melanosomes in the tumour cells and the positive immunoreactivity with HMB45 favours the latter notion. However, it is not clear whether CCS is a subtype of malignant cutaneous melanoma, since there are several ultrastructural [2], immunohistochemical [7, 17, 34], and phenotypical [12, 30, 35] similarities. However, it is difficult to explain differences regarding the biological behaviour and clinical features of CCS and malignant melanoma of the skin. Moreover, since the t(12; 22) (q13; q12) translocation and chimaeric *EWS/ATF-1* transcripts are not found in the latter condition, we postulate that the mechanisms involved in the oncogenesis of CCS and malignant cutaneous melanoma are dissimilar. Therefore, we agree with the suggestion of Enzinger and Weiss that CCS should not be considered to be a malignant melanoma, but rather segregated as a unique tumour of the soft parts [11].

In the differential diagnosis of CCS synovial sarcoma, fibrosarcoma, malignant peripheral nerve sheath tumour, malignant melanoma, and metastasis from renal cell carcinoma, all have to be considered, as well as metastases of malignant melanoma simulating soft tissue sarcoma [21]. Although chromosome analysis is useful for differentiating CCS from other tumours, it is inconvenient in clinical use and not always sensitive enough. For example, a rearrangement of the *EWS* gene was detected by Southern blotting and hybridization in a case of Ewing's sarcoma, yet the characteristic t(11; 22) translocation was not found by chromosome analysis [39]. However, RT-PCR is a highly sensitive and feasible method, which yields results in 2 days.

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References

1. Azumi N,Turner RR (1983) Clear cell sarcoma of tendons and aponeuroses: electron microscopic findings suggesting Schwann cell differentiation. Hum Pathol 14:1084–1089
2. Bearman RM, Noe J,Kempson RL (1975) Clear cell sarcoma with melanin pigment. Cancer 36:977–984
3. Benson JD, Kraemer BB, Mackay B (1985) Malignant melanoma of soft parts. An ultrastructural study of four cases. Ultrastruct Pathol 8:57–70
4. Boudreaux D, Waisman J (1978) Clear cell sarcoma with melanogenesis. Cancer 41:1387–1394
5. Bridge JA, Borek DA, Neff JR, Huntrakoon M (1990) Chromosomal abnormalities in clear cell sarcoma. Implications for histogenesis. Am J Clin Pathol 93:26–31
6. Bridge JA, Sreekantaiah C, Neff JR, Sandberg AA (1991) Cytogenetic findings in clear cell sarcoma of tendons and aponeuroses. Malignant melanoma of soft parts. Cancer Genet Cytogenet 52:101–106
7. Chung EB, Enzinger FM (1983) Malignant melanoma of soft parts. A reassessment of clear cell sarcoma. Am J Surg Pathol 7:405–413
8. Curran RC, McCann BG (1976) The ultrastructure of benign pigmented naevi and melanocarcinomas in man. J Pathol (Lond) 119:135–146
9. Ekfors TO, Rantakokko V (1979) Clear cell sarcoma of tendons and aponeuroses: malignant melanoma of soft tissues? Report of four cases. Pathol Res Pract 165:422–428
10. Enzinger FM (1965) Clear-cell sarcoma of tendons and aponeuroses. An analysis of 21 cases. Cancer 18:1163–1174
11. Enzinger FM, Weiss SW (1995) Malignant tumors of the peripheral nerves. In: Enzinger FM, Weiss SW (eds) Soft tissue tumors. Mosby Year Book, St Louis, pp 913–919
12. 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
13. Fletcher JA (1992) Translocation (12;22)(q13-14;q12) is a nonrandom aberration in soft-tissue clear-cell sarcoma. Genes Chromosom Cancer 5:184
14. Flint KJ, Jones NC (1991) Differential regulation of three members of the ATF/CREB family of DNA-binding proteins. Oncogene 6:2019–2026
15. Fujimura Y, Ohno T, Siddique H, Lee L, Rao VN, Reddy ES (1996) The EWS-ATF-1 gene involved in malignant melanoma of soft parts with t(12;22) chromosome translocation, encodes a constitutive transcriptional activator. Oncogene 12:159–167
16. Hai TW, Liu F, Coukos WJ, Green MR (1989) Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. Genes Dev 3:2083–2090
17. Hasegawa T, Hirose T, Kudo E, Hizawa K (1989) Clear cell sarcoma. An immunohistochemical and ultrastructural study. Acta Pathol Jpn 39:321–327
18. Hoffman GJ, Carter D (1973) Clear cell sarcoma of tendons and aponeuroses with melanin. Arch Pathol 95:22–25
19. Kubo T (1969) Clear-cell sarcoma of patellar tendon studied by electron microscopy. Cancer 24:948–953
20. Limon J, Debiec RM, Nedoszytko B, Liberski PP, Babinska M, Szadowska A (1994) Aberrations of chromosome 22 and polysomy of chromosome 8 as non-random changes in clear cell sarcoma. Cancer Genet Cytogenet 72:141–145
21. Lodding P, Kindblom LG, Angervall L (1990) Metastases of malignant melanoma simulating soft tissue sarcoma. A clinico-pathological, light- and electron microscopic and immunohistochemical study of 21 cases. Virchows Arch [A] 417:377–388
22. Molenaar WM, DeJong B, Buist J, Idenburg VJ, Seruca R, Vos AM, Hoekstra HJ (1989) Chromosomal analysis and the classification of soft tissue sarcomas. Lab Invest 60:266–274
23. Mrozek K, Karakousis CP, Perez MC, Bloomfield CD (1993) Translocation t(12;22)(q13;q12.2–12.3) in a clear cell sarcoma of tendons and aponeuroses. Genes Chromosom Cancer 6:249–252
24. 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 electron-microscopic, biochemical, enzyme histochemical, and immunohistochemical study. Am J Pathol 114:264–272
25. Nojima T, Wang YS, Abe S, Matsuno T, Yamawaki S, Nagashima K (1990) Morphological and cytogenetic studies of a human synovial sarcoma xenotransplanted into nude mice. Acta Pathol Jpn 40:486–493
26. 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
27. Peulve P, Michot C, Vannier JP, Tron P, Hemet J (1991) Clear cell sarcoma with t(12;22) (q13-14;q12). Genes Chromosom Cancer 3:400–402
28. Reeves BR, Fletcher CD,Gusterson BA (1992) Translocation t(12;22)(q13;q13) is a nonrandom rearrangement in clear cell sarcoma. Cancer Genet Cytogenet 64:101–103
29. Rodriguez E, Sreekantaiah C, Reuter VE, Motzer RJ, Chaganti RS (1992) t(12;22)(q13;q13) and trisomy 8 are nonrandom aberrations in clear-cell sarcoma. Cancer Genet Cytogenet 64:107–110
30. Sonobe H, Furihata M, Iwata J, Ohtsuki Y, Mizobuchi H, Yamamoto H, Kumano O (1993) Establishment and characterization of a new human clear-cell sarcoma cell-line, HS-MM. J Pathol (Lond) 169:317–322
31. Speleman F, Colpaert C, Goovaerts G, Leroy JG, Van ME (1992) Malignant melanoma of soft parts. Further cytogenetic characterization. Cancer Genet Cytogenet 60:176–179
32. Stenman G, Kindblom LG, Angervall L (1992) Reciprocal translocation t(12;22)(q13;q13) in clear-cell sarcoma of tendons and aponeuroses. Genes Chromosom Cancer 4:122–127
33. Steven I. Hajdu MHS, Fortner JG (1977) Tendosynovial sarcoma. A clinicopathological syudy of 136 cases. Cancer 39:1201–1217
34. Swanson PE, Wick MR (1989) Clear cell sarcoma. An immunohistochemical analysis of six cases and comparison with other epithelioid neoplasms of soft tissue. Arch Pathol Lab Med 113:55–60
35. Takenouchi T, Ito K, Kazama T, Ito M (1994) Establishment and characterization of a clear-cell sarcoma (malignant melanoma of soft parts) cell line. Arch Dermatol Res 286:254–260
36. Travis JA, Bridge JA (1992) Significance of both numerical and structural chromosomal abnormalities in clear cell sarcoma. Cancer Genet Cytogenet 64:104–106
37. 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
38. Yoshimura T, Fujisawa J, Yoshida M (1990) Multiple cDNA clones encoding nuclear proteins that bind to the tax-dependent enhancer of HTLV-1: all contain a leucine zipper structure and basic amino acid domain. EMBO J 9:2537–2542
39. Zucman J, Delattre O, Desmaze C, Plougastel B, Joubert I, Melot T, Peter M, De JP, Rouleau G, Aurias A, et al (1992) Cloning and characterization of the Ewing′s sarcoma and peripheral neuroepithelioma t(11;22) translocation breakpoints. Genes Chromosom Cancer 5:271–277
40. Zucman J, Delattre O, Desmaze C, Epstein AL, Stenman G, Speleman F, Fletchers CD, Aurias A, Thomas G (1993) EWS and ATF-1 gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. Nat Genet 4:341–345