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Establishment and Characterization of a Small Round Cell Sarcoma Cell Line, SCCH-196, with t(11;22)(q24;q12)

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A cell line designated SCCH-196 was established from an extraskeletal small round cell sarcoma developed in a 16-year-old Japanese girl. The cells grew as a monolayer, and have been continuously propagated by serial subcultures during the past 26 months. Cells from the primary tumor and those from the SCCH-196 cell line at passage 10 both showed the same karyotype, 51,XX,+8,+20,+21, t(11;22)(q24;q12),+i(1q),+i(1q). Histologically the primary tumor was difficult to classify as either Ewing's sarcoma (ES) or peripheral neuroepithelioma (NE). Neuron-specific enolase-positive cells in the primary tumor and the occurrence in the upper extremity were in favor of NE, while positive reaction of SCCH-196 cells to an ES-specific monoclonal antibody 5C11 suggested a diagnosis of ES. The SCCH-196 cell line may be useful for basic studies on differentiation of neuroectodermal tumors, and for future cloning of still unidentified genes which may be located at the breakpoints of the 11;22 translocation.

Key words: Cell line — t(11;22) translocation — Small round cell sarcoma — Ewing's sarcoma — Neuroepithelioma

Ewing's sarcoma (ES) and peripheral neuroepithelioma (NE) resemble each other histologically although distinction is possible by immunocytochemical staining and electron microscopy.[1] Data are accumulating to indicate that both tumors share many characteristics, i.e. the same chromosomal translocation t(11;22)(q24;q12),[2] similar neurotransmitter biosynthetic enzyme activities,[3] similar reaction patterns to various monoclonal antibodies,[4] etc.

We report a cell line with the 11;22 translocation established from an extraskeletal small round cell sarcoma of an adolescent girl. The primary site of the soft tissue in the upper extremity,[1,5] and neuron-specific enolase (NSE)[6,7] in tumor cells, were in favor of the diagnosis of NE, but immunophenotyping of the established cell line suggested ES. Such ambiguous findings have been reported in a childhood round cell sarcoma,[8] and suggest that both diseases may originate from common neuroectodermal precursor cells.[9]

MATERIALS AND METHODS

The patient A 16-year-old Japanese girl presented with a mass in the left upper arm in September, 1986. Surgery resected a grayish white tumor of 7.7×7.0×3.8 cm in the biceps of the left upper arm and a metastatic lymph node close to the tumor. Hematoxylin-eosin(HE)-stained histologic sections of the tumor revealed diffuse proliferation of small round or oval cells, without any proper structure (Fig. 1). The cells had scanty amphiphilic or basophilic cytoplasm, and a round nucleus with delicate, fine chromatin and prominent nucleoli. Very few neoplastic cells contained periodic acid-Schiff (PAS) staining granules, which were digested with diastase and were considered to contain glycogen. The tumor was tentatively diagnosed as small round cell sarcoma. The patient was treated with combination chemotherapy consisting of cyclophosphamide, doxorubicin, dactinomycin, vincristine, methotrexate and bleomycin, but subsequently developed metastasis to the left 4th rib, left lung, and subcutaneous tissues of the left axillar and mammary regions in June, 1987. The disease progressed to the 8th vertebra and the adjacent portion of the spinal cord, and she died in October, 1987.

Tissue culture Part of the primary tumor was minced finely with scissors in fetal bovine serum (FBS)(GIBCO, Grand Island, NY). Cell suspensions were placed into 80-mm plastic Petri dishes (Miles Lab. Inc., Naperville, IL) containing ES medium (Nissui Pharmaceutical Co., Tokyo) supplemented with 10% FBS, 2 m*M* L-glutamine (GIBCO), kanamycin, and amphotericin B. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. On the 10th day of the primary culture, cells were detached by using 0.25% trypsin-0.01% EDTA, and were transferred into 25-cm^2 flasks (Falcon Plastics Corp., Oxnard, CA). Subcultures were performed every 5–7 days at a 1:8 dilution until passage 10, and then every 7–10 days at a 1:40 dilution thereafter. The cell line thus

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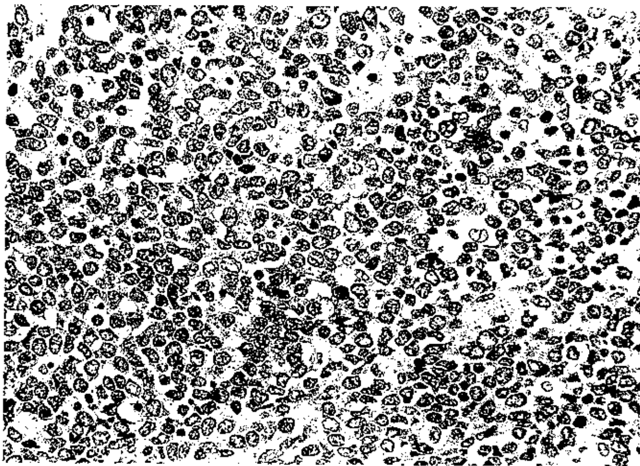

Fig. 1. Histological appearance of the original tumor. Small round tumor cells showed diffuse proliferation. Neither proper structure nor Homer-Wright type rosette formation was noted. HE, ×80.

established was designated SCCH-196. By the time of submission of this paper, the cells have undergone 70 passages in culture.

For the delineation of the growth curve, 2×10^5 viable cells each (at the 61st passage) were inoculated in 2 35-mm plastic Petri dishes (Falcon) with 2 ml of ES medium supplemented with 10% FBS. Cells were harvested after 1 to 9 days of culture and were counted. The average numbers of viable cells per dish assessed by a trypan blue dye exclusion technique were plotted at 24-h intervals.

Transplantation into nude mice Aliquots of 10^8 cells each (at the 50th passage) were transplanted subcutaneously into the backs of three BALB/cA *nu/nu* mice (Nihon Clea, Tokyo). A tumor developed in one of them and reached 20 mm in diameter 108 days after the inoculation, when the tumor-bearing mouse was killed for histological examination of the tumor.

Chromosomal analysis Chromosomes were examined in cells from the primary tumor, and from the SCCH-196 cell line at the 10th passage. After treatment with 1 ng/ml colcemid (GIBCO) for 1 h at 37°C, cells were detached by using 0.25% trypsin-0.01% EDTA, treated with 0.075 *M* KCl for 20 min, and fixed in Carnoy's fixative. Karyotypes were analyzed with trypsin G-banding, and described according to the ISCN (1985).[10]

Histologic, histochemical, and immunocytochemical characterization For immunohistochemical studies of the primary tumor, the peroxidase-antiperoxidase (PAP) method was employed using antisera against NSE, neurofilament (NFP), actin, myosin (Bio Genex, Dublin, CA), myoglobin (Ortho Diagnostics, Raritan, NJ), desmin (Immunon, Detroit, MI), and S-100 (MILAB, Malmö, Sweden). Immunocytochemical characteristics of the SCCH-196 cells (at the 50th passage) were studied with indirect immunoenzyme staining using monoclonal or heteroantiserum antibodies against NFP 68 KD (DAKO, Glastrup, Denmark), NFP 200KD (Labsystem, Helsinki, Finland), vimentin (DAKO), desmin, cytokeratin MAK6 (Triton Bioscience, Almeda, CA), cytokeratin K8.13 (Biomakor, Rehovot, Israel), NSE (DAKO), chromogranin (Incstal, Steelwater, MN), Leu7 (Becton Dickinson, Mountain View, CA) and 5C11. 5C11 is an ES-specific novel monoclonal antibody originated in a mouse sensitized with cells from an ES cell line, RD-ES (obtained from the American Type Culture Collection, Rockville, MD) (S. Hara *et al.*, unpublished results).

Electronmicroscopic study The cells were suspended in PBS followed by centrifugation at 1,000 rpm for 1 h. The cell pellets were fixed with 2.5% glutaraldehyde and 1% OsO_4. The embedded cells were examined by the method described previously.[11]

RESULTS

Histochemical and immunocytochemical findings NSE was positive in the primary tumor cells, but negative in the SCCH-196 cells. The primary tumor cells failed to react with other antibodies examined. The SCCH-196 cells reacted with antibodies Leu7 and 5C11, and that against desmin, but not with those against NFP, vimentin, cytokeratin, and chromogranin.

Growth curve and tumorigenicity of SCCH-196 cells The SCCH-196 cells were small, plump, and bipolar at a low cell density in culture (Fig. 2). They grew until they

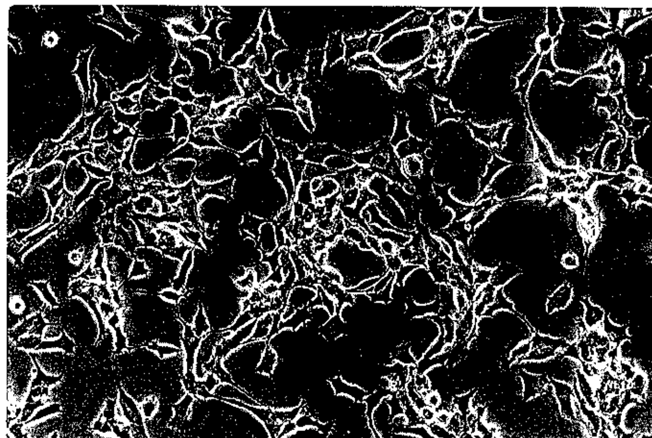

Fig. 2. SCCH-196 cells in culture at a low cell density. The cells were plump and bipolar. ×100.

reached a plateau at 2.3×10^6 cells/dish on day 6 after inoculation of 2×10^5 cells (Fig. 3). Their doubling time was approximately 36 h.

The histology of the tumor developed in the back of a BALB/cA *nu/nu* mouse after inoculation of SCCH-196 cells was similar to that of the original tumor.

Chromosomal findings Of 18 G-banded metaphase cells obtained from the primary tumor, 16 cells had an abnormal karyotype of 51,XX,+8,+20,+21,t(11;22)(q24;q12),+i(1q),+i(1q), and 2 had a normal karyotype of 46,XX. All 20 SCCH-196 cells examined at the 10th passage showed the same abnormal karyotype as the primary tumor (Fig. 4).

Elecronmicroscopic findings The tumor cells had translucent cytoplasm and a round or semiround nucleus. The cytoplasmic organelles were poorly developed; only a few mitochondria or polysomes were seen. No neuritic process or neurosecretory granules were observed (Fig. 5).

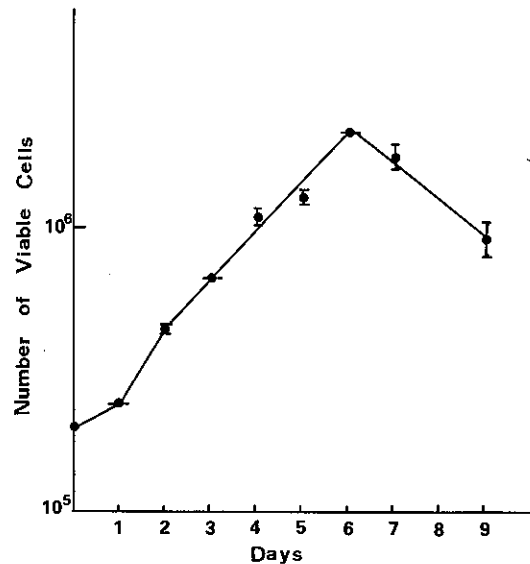

Fig. 3. Growth curve of SCCH-196 cells. When 2×10^5 cells were inoculated with 2 ml of medium per 35-mm dish, they grew at a doubling time of approximately 36 h, reached a plateau at 2.3×10^6 cells per dish on day 6, and regressed thereafter.

DISCUSSION

We have described here a newly established cell line with a specific 11;22 translocation, derived from a small round cell sarcoma which developed in a biceps brachii

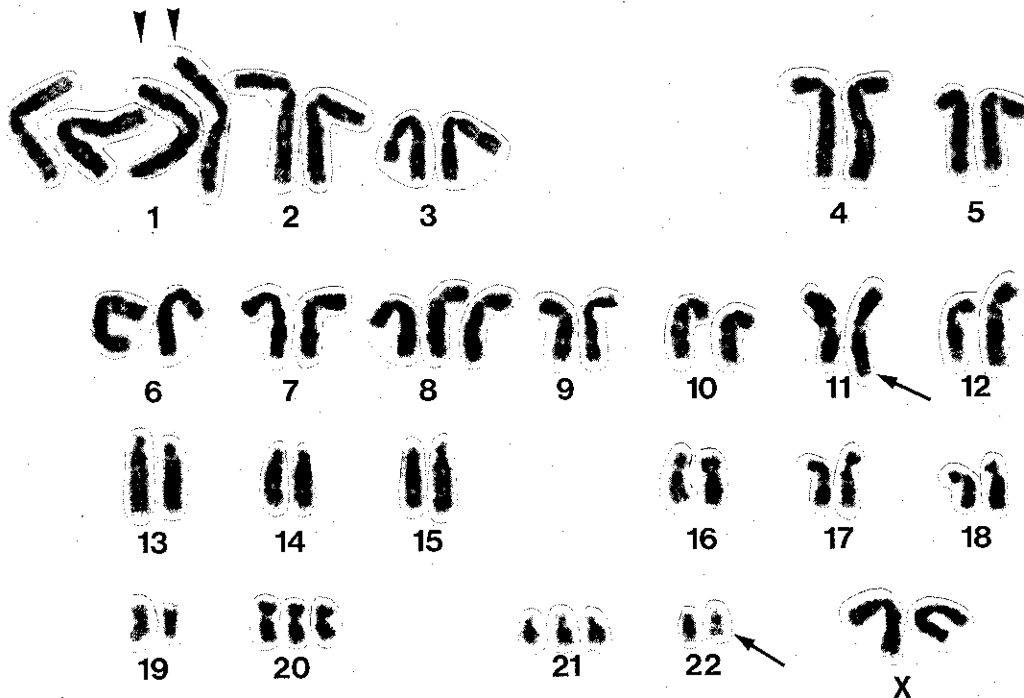

Fig. 4. Karyotype of an SCCH-196 cell at the 10th passage. Arrows indicate the products of the 11;22 translocation. Arrowheads show isochromosomes of 1q. Trisomies of chromosome 8, 20, and 21 are also present. The karyotype was designated 51,XX,+8,+20,+21,t(11;22)(q24;q12),+i(1q),+i(1q).

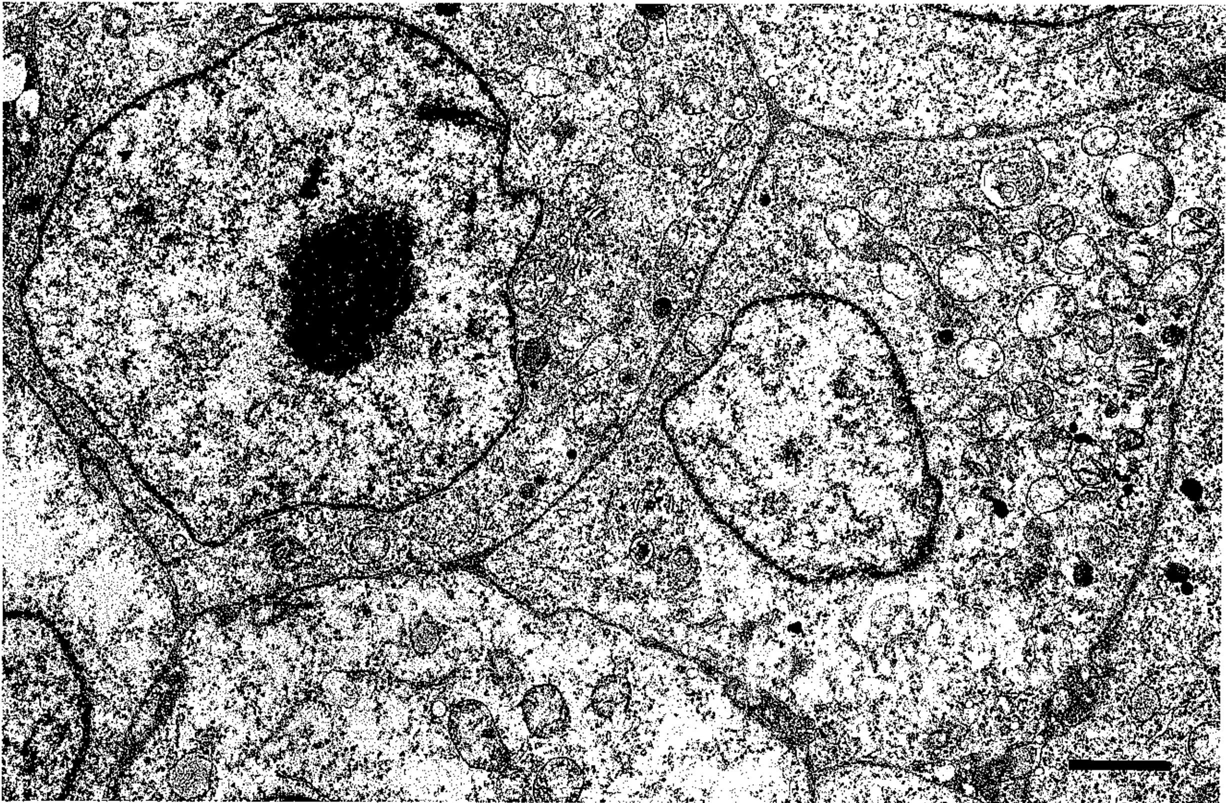

Fig. 5. Ultrastructure of tumor cells. The cells had translucent cytoplasm, containing poorly developed organelles, and a round nucleus with prominent nucleoli. The horizontal bar at the bottom right indicates 2 μm. ×6000.

muscle of an adolescent girl. Several cell lines with the 11;22 translocation have been reported[2, 12]; all of these originated from ES or NE developed in American or European patients. The SCCH-196 is the first cell line with this translocation established in Japan.

It was difficult to determine the origin of this tumor from the histological and ultrastructural findings. NSE-positivity was in favor of NE, while monoclonal antibody 5C11 suggested ES.

Recently, a poorly differentiated NSE-positive round cell tumor was reported with 11;22 translocation.[8] Although the tumor originated from a bone and the cells had glycogen-containing granules, the absence of vimentin and presence of NSE prevented a definite diagnosis of either ES or NE. In addition, *in vitro* induction of immunocytochemical neural differentiation of ES cells by cyclic AMP has also been reported.[13] Thus, the difficulty in more defined diagnosis of t(11;22)-associated small round cell sarcoma of childhood, even with the use of immunocytochemical techniques, may be explained by the hypothesis that the 11;22 translocation occurs in a primitive, pluripotential cell which can differentiate into both mesenchymal and neuroepithelial cells,[9] and that some tumors with the translocation could show intermediate or aberrant differentiation.

Both the primary tumor and the cell line had the same karyotype, i.e. 51,XX,+8,+20,+21,t(11;22)(q24;q12),+i(1q),+i(1q). Trisomy 8 in addition to the t(11;22) is the most frequent numerical change in ES[14] and is also frequently reported in NE.[2] An isochromosome of the long arm of chromosome 1 is a common abnormality observed in various pediatric solid tumors.[15] Since the 11;22 translocation has been consistently observed in ES and NE, oncogenes and/or genes related to differentiation of progenitor cells common to either of these tumors may be present in the breakpoints of the chromosomes forming the translocation. Oncogenes c-*ets*-1 and c-*sis* are located in 11q23 and 22q11, the bands including or adjacent to the breakpoints of the t(11;22), respectively.[16] However, the involvement of these genes is unlikely in the tumorigenesis.[17, 18]

The SCCH-196 cell line may be useful for basic studies on the differentiation capability of sarcomatous small round cells in childhood, and for future cloning of still

unidentified genes located at the breakpoints of the 11;22 translocation.

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