CHARACTERIZATION OF A NEWLY DERIVED HUMAN SARCOMA CELL LINE (HT-1080)

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A tumor cell line was derived from the fibrosarcoma of a 35-year-old Caucasian man who died without having received chemotherapy or radiotherapy. The in vitro growth properties and transplantability into antithymocytic sera treated mice were characteristic of these malignant cells. An aberrant karyology with marker chromosomes was present. No virus particles were detected.

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PERMANENT CELL LINES FROM ANIMAL TISSUES were established as early as 1948.[23] Most of these were originated from malignant tissues. Although a number of human cell lines have been established in vitro, very few fibrosarcoma cell lines have been described. As far as we are aware, there are reports of approximately eight cell lines derived from osteogenic sarcomas,[1,4,9,10,13,21] two on rhabdomyosarcoma,[12,26] three on liposarcoma,[1,9,10] and one each on giant cell sarcomas,[1] Kaposi's sarcoma,[8] neurogenic sarcoma,[25] synovioma,[10] and fibrosarcoma.[1] The rest of the reported cell lines are all carcinomas. Only a few of these sarcoma cell lines have been fully studied and characterized. In this paper we describe the properties of a tumor cell line (HT-1080) derived from a fibrosarcoma of a human male.

MATERIALS AND METHODS

Source of Cancer Tissue

A biopsy was obtained in July, 1972, from a fibrosarcoma arising adjacent to the acetabulum of a 35-year-old Caucasian man. The patient died 3 months later without having received chemotherapy or radiotherapy. At death he had clinical evidence of widespread metastatic tumor; an autopsy was not done. The histopathologic diagnosis of the biopsy specimen was poorly differentiated fibrosarcoma.

Tissue Culture Techniques and Nutrient Media

The tumor specimen was trimmed free of connective tissue, washed several times with culture medium, and placed in a wide mouthed sterile vial. The tumor was finely minced with a sharp pair of scissors and suspended in about 10 ml of growth medium. The suspension was allowed to stand for about 1 minute, until the larger pieces of tumor settled at the bottom of the vial. The tumor cell suspension was thinly seeded in 75 cm^2 Falcon flasks and 100 mm dishes, and the excess medium was aspirated. The flasks were left at an angle of 60° for 5–10 minutes before adding 7–8 ml of culture medium. This procedure helped the cells attach to the surface of the flask or dish. Several of the cell suspensions were filtered through a loose mesh gauze to exclude the larger clumps.

Cultures were fed with Eagle's minimum essential medium (EMEM) with Earle's salts, supplemented with 10% heat inactivated fetal bovine serum, 100 units of penicillin, 100 μg/ml streptomycin, and 50 μg/ml gentamicin. The medium was also enriched with 1 ml/100 ml of 100X nonessential amino acids and 1 ml/100 ml of 200 mM glutamine. For subculturing, 0.1% trypsin in calcium- and magnesium-free Hanks' balanced salt solution containing 200μg/ml EDTA (versene) was used throughout. Agar was obtained from

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Difco; a 0.3% solution was used in the nutrient medium for colony formation assay.[16]

The cultures were incubated at 37C in a humidified atmosphere containing 4% CO_2 in air. The cells remained undisturbed for 4 days, after which many colonies of rounded cells appeared all over the culture containers. At this stage of growth the cultures showed very few areas of fibroblastic cells, which were eliminated by two methods: (A) the areas of fibroblastic growth were marked, scraped, and picked by the tips of curved Pasteur pipettes; the flasks or dishes were then washed twice with complete medium to get rid of other dislodged cells which were not aspirated by the pipette; (B) the flasks or dishes were washed twice with 0.1% trypsin, left at room temperature for 1 minute, and then washed again with 0.1% trypsin. As the fibroblastic areas grew in monolayers and most of the tumor cells grew in aggregates or multiple layers, the fibroblasts detached first from the culture containers, and the tumor cells separated after further incubation of the cultures at 37C for 3 to 5 minutes. A combination of these two procedures suppressed the growth of fibroblastic cells. Moreover, the tumor cells grew so rapidly that at the end of the second subculture the cultures consisted almost entirely of tumor cells. Nevertheless, we repeated the "quick trypsinization" and "picking" procedures two or three times to eliminate fibroblastic growth completely.

Pathology and Electron Microscopy (EM) Techniques

The primary tumor biopsy and a pellet of the tissue culture cells were processed by standard methods, and sections were stained with hematoxylin and eosin. For electron microscopy, cell pellets were fixed in glutaraldehyde, post-fixed with osmium tetroxide, dehydrated in ethyl alcohol, passed through propylene oxide, and embedded in araldite. Sections were cut on an LKB ultramicrotome, mounted on uncoated copper grids, and stained with uranyl acetate and lead citrate.

Chromosome Techniques

The tissue culture cells at passage nine were exposed to colcemid, trypsinized, and processed according to the air dry method,[22] and stained in Giemsa. Y-chromosome fluorescence was studied after quinacrine mustard dihydrochloride staining.[11,18]

Cloning Efficiency and Doubling Time Assays

The cloning efficiency was determined by two methods: (A) 500 cells/100 mm dish were plated and left undisturbed for 9 days; the cells were stained with May-Grünwald stain and the colonies counted; (B) the cells were diluted to about 10 cells/ml, and 0.1 ml of suspension was seeded in each well of Falcon microtest II plates.

To determine the doubling time, 12 60 mm dishes were seeded with 25,000 cells/dish and at 24, 48, 72, and 96 hours after plating, 1 dish was trypsinized, 1 dish was stained, and all the other dishes were examined microscopically. The number of cells was determined by counting cells from 1 trypsinized dish and 10 randomly selected 1 mm^2 areas in each of the other dishes. At least 1 stained and 4 unstained dishes were counted every day, and an average population doubling time determined by plotting the number of cells counted against the hours.

Iso-enzyme Assay and Other Biochemical Techniques

The glucose-6-phosphate dehydrogenase (G-6-PD) enzyme mobility pattern was determined by Dr. W. D. Peterson, Jr., following his techniques.[20]

Labeling for ^{3}H-uridine uptake and RNA-dependent DNA polymerase assay were performed according to the methods described before.[5,24]

RNA Tumor Virus Infectivity Assay

The susceptibility of HT-1080 cells to feline leukemia virus (FeLV) and RD-114 virus[15] was determined according to the described methods.[14]

Transplantation to Mice

NIH Swiss mice at 10–15 days of age were immunosuppressed with anti-thymocytic serum (ATS)[3] and inoculated with 2.5×10^6 HT-1080 cells subcutaneously.

In vitro passage 13 cells were used for the first litter of ATS mice, and the resultant tumors were subsequently serially transplanted using 10% fresh tumor suspensions.

RESULTS

Histopathology

The primary tumor biopsy (Fig. 1) was very cellular, consisting mostly of elongated tumor

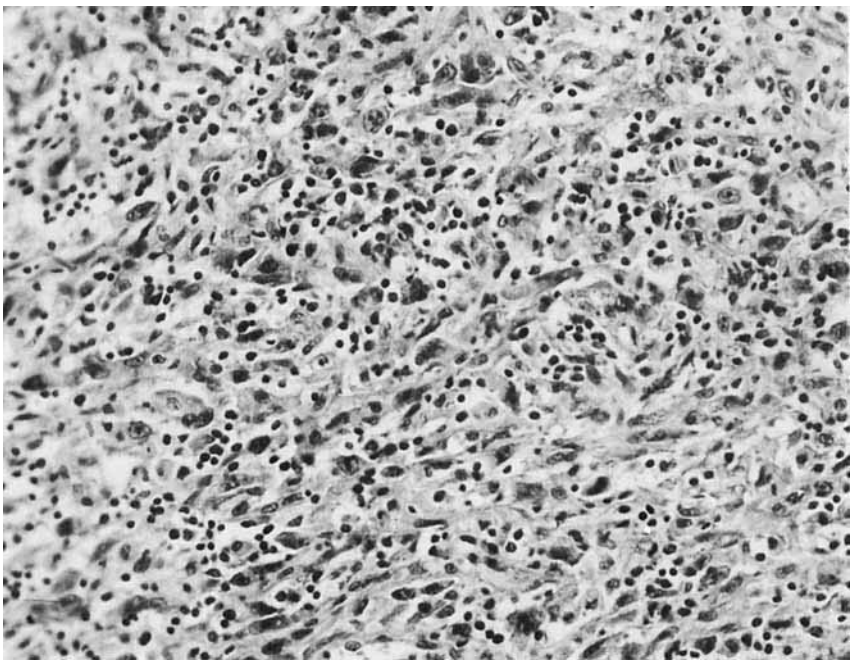

FIG. 1. Primary tumor invading acetabulum. The histopathologic appearance is that of a pleomorphic, anaplastic fibrosarcoma. The tumor is infiltrated with many lymphocytes (H & E, ×250).

cells with little intercellular stroma. It invaded the acetabulum cartilage. Rounded tumor cells were present in lesser numbers. Pleomorphic nuclei, large nucleoli, and frequent mitoses were prominent features. The tumor was infiltrated with many small lymphocytes, but there was no necrosis. The overall appearance was that of a highly malignant fibrosarcoma.

Morphology of Tissue Culture Cells

Although a large number of giant multinucleated cells was present, the cultures predominantly consisted of one morphological type of rounded cell (Fig. 2). At confluency the cells grew in multiple layers and formed microtumors throughout the culture containers.

Sections of the pelleted tissue culture cells also showed it to consist almost entirely of rounded tumor cells with many mitoses and occasional bizarre giant tumor cells.

By electron microscopy, both rounded and elongated tumor cells were seen. The fine structure of these cells was similar and uncomplicated. The nuclei were generally round or oval with little indentation of the nuclear membrane, and the chromatin was evenly dispersed. One or two prominent nucleoli were present in most of the tumor cells. The cytoplasm tended to show a paucity of organelles, with very little well-formed rough endoplasmic reticulum; it consisted mostly of free ribosomes. Cytoplasmic microfibrils were noted only adjacent to the cell membrane. The cell surface was smooth without microvilli. No virus particles were seen.

Growth Characteristics

This sarcoma cell line exhibited many properties usually associated with continuous cell lines (Table 1). The cells multiplied rapidly with loss of contact inhibition. There was no apparent decrease in the growth rate even when the cells were plated at extremely low densities. The doubling time was 26 hours and the saturation density was $1.5 \times 10^6/cm^2$. More than 15% of the cells grew in agar medium and 3% formed colonies (approximately 0.1–.2 mm in diameter). The cloning efficiency of these cells was 70–75%. The cells have been in culturefor 10 months, through 60 subcultures, and show no decrease in growth rate. No mycoplasma was detected when tested by Dr. L. Hayflick.

Chromosome Studies

Chromosome analysis on the cells by conventional Giemsa staining techniques after nine passages in vitro revealed human chromosomes with a modal number of 46 (Table 2). Pseudodiploidy, however, was common.

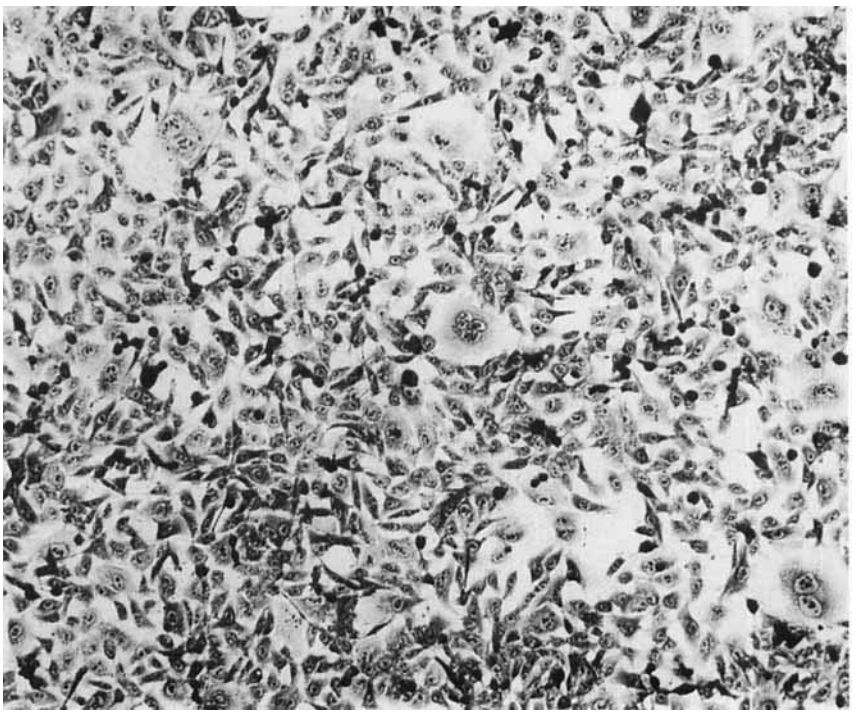

FIG. 2. Tissue cultured tumor consisting of rounded and bizarre cells (×85).

Eighty percent of the cells with 46 chromosomes had rearranged karyotypes. All of these cells had an extra C-group-like subtelocentric chromosome, but a missing B-group chromosome (Fig. 3A), and approximately 10% of these cells had, in addition, an extra A-2-like chromosome and were missing a C-group chromosome (Fig. 3B). Although about 50% of the cells examined had more or less than 46 chromosomes, 97% or more metaphases revealed the presence of a Y-chromosome by fluorescence staining. Approximately 7% of the cells exhibited polyploidy.

TABLE 1. Properties of HT-1080 Cell Line

Morphology:	Rounded, polygonal cells with hyperchromatic nuclei
Electron microscopy:	No virus particles observed
Chromosomes:	Aberrant karyology—presence of marker chromosomes
Cell doubling time:	26 hours
Saturation density:	$1.5 \times 10^6/cm^2$
Growth in agar medium:	15% plating and 3% colony formation efficiency
Loss of contact inhibition:	Forms microtumors in vitro
Iso-enzyme pattern:	Type B G-6-PD
RNA-dependent DNA polymerase or reverse transcriptase activity:	Absent
Mycoplasma:	Absent
Susceptibility to RNA tumor virus:	Susceptible to RD-114 and FeLV viruses
Growth in animals:	Transplantable fibrosarcomas in antithymocytic serum (ATS)-treated NIH Swiss mice

Iso-enzyme Pattern and Other Biochemical Studies

Extracts of cells (passage 12) exhibited the Type B band mobility pattern for the enzyme G-6-PD. RNA-dependent DNA polymerase activity was not detected in the culture fluid, and ^{3}H-uridine was not incorporated at 1.16 gm/cm^3 density following the density gradient centrifugation of the culture fluid.

Susceptibility to RNA Tumor Viruses

The HT-1080 cells were susceptible to FeLV and RD-114 viruses as assayed by the induction of the respective complement fixing group specific antigens, and the presence of RNA-

TABLE 2. Chromosome Distribution in 50 Metaphases of HT-1080 Cells

Number of chromosomes/Number of cells				
44	45	46	47	48
1	8	27	11	3

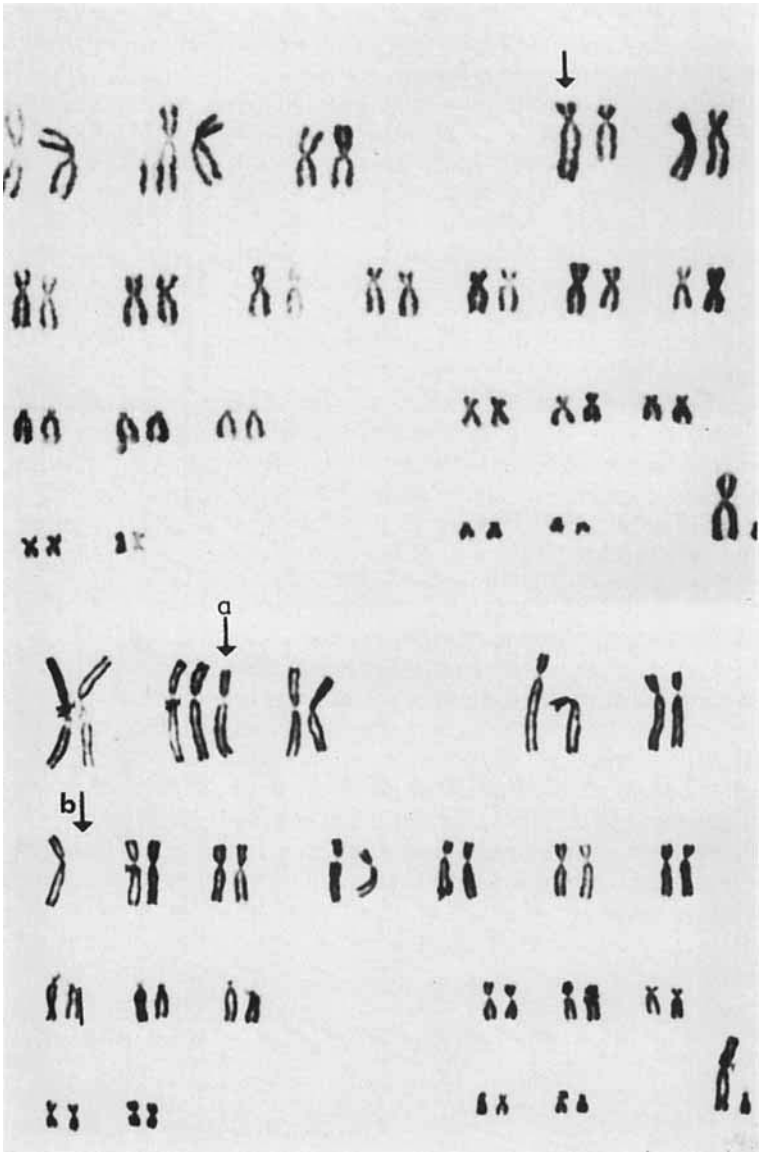

FIG. 3A. Pseudodiploid HT-1080, human male metaphase with unpaired B-group chromosome (arrow) (×1750).

FIG. 3B. Pseudodiploid HT-1080, human male metaphase with extra A-2 chromosome (arrow a) and missing C-group chromosome (arrow b) (×1750).

dependent DNA polymerase activity in the culture fluids 14–21 days after infection.

Transplantation to Mice

Tumors appeared in all subcutaneously inoculated ATS-treated mice by day 7 and grew rapidly thereafter as long as the ATS treatment was maintained. No tumors could be detected in similarly inoculated mice not treated with ATS.

Tumors were harvested on the 15–25th day after transplantation from each of four consecutive transplant passages, and were histologically poorly differentiated fibrosarcomas in each case. Cells propagated in tissue culture from the sarcoma pools of the mice in all transplant passages were karyotypically human and histologically indistinguishable from the purely in vitro grown cultures of HT-1080.

DISCUSSION

Our results strongly suggest that this cell line is composed of malignant cells. The cells grow to high saturation density in vitro, show lack of contact inhibition, have a high plating efficiency, and form colonies in agar medium. Cytologically they appear to be a uniform population of undifferentiated tumor cells with an abundance of normal and abnormal

mitoses. We do not have karyotype information on close relatives of the donor, nor on any of his own non-tumor tissues, and cannot therefore rule out chromosomal peculiarities which may have been unrelated to neoplasia. However, the aberrant karyotypes of cells at an early passage in vitro, as well as the presence in different cells of identical marker chromosomes, are characteristics consistent with a tumor origin of this cell line. Interestingly, the chromosome alterations (loss or gain of chromosomes) did not involve Y-chromosomes as described by Peterson.[19]

It is not likely that HT-1080 cells are "contaminants"[7] of other previously established human tumor cell lines such as HeLa, from a Negro woman,[2] or RD, from a Caucasian girl.[12] There are no HeLa cells in the laboratory where line HT-1080 was isolated and grown. Line HT-1080 cells exhibit a Y-chromosome, while both HeLa[18] and RD[12] do not. HeLa cells display Type A mobility pattern for G-6-PD,[20] and while RD[12] and HT-1080 are both Type B, their respective karyotypes are as different as their morphology. An osteogenic sarcoma line from a 15-year-old male described by Ishihara et al.[10] not only differs from HT-1080 in its histology and pathology but is also heteroploid and does not contain any chromosome markers. The fibrosarcoma line reported by Aaronson et al.[1] is from a female and has not been reported to be tumorigenic upon transplantation.

The HT-1080 cells grow into large tumors within a few days after transplantation into ATS-treated NIH Swiss mice. These transplant tumors can be serially propagated in ATS-treated mice. The transplant tumors show invasion of the surrounding tissues and progressive growth. "Normal" or non-tumor cell cultures have never produced tumors in this system.[3] Although the HT-1080 cells are now in their 60th subculture passage, their population doublings have already exceeded 200. This line should therefore have the potential to grow indefinitely in vitro.[6] The cell line is available upon request from the Naval Biological Laboratory in Oakland, California, or the American Type Culture Collection, Rockville, Maryland.

This particular tumor cell line may prove useful in searching for hypothetical endogenous RNA tumor virus gene expressions. Our studies thus far have failed to show any evidence of Type C virus particle production from these tumor cells. Further attempts to activate the production of Type C virus particles by chemical mutagens or by interspecies genetic complementation are underway.

ADDENDUM

Using a modification of the trypsin-Giemsa banding technique for chromosome identification,[23a] Dr. William Benedict, of Childrens Hospital of Los Angeles, has found that the chromosome originally referred to as "unpaired B-chromosome" was, in fact, a C-11 chromosome with a translocation on its long arm. Four chromosomes in the B-group were also found in all metaphases analyzed with a mode of 46 chromosomes. One B-5 chromosome had an additional dark band on the short arm. No other abnormalities were observed.

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