Establishment of a Human Leiomyosarcoma Cell Line

Isamu Ishiwata, Shiro Nozawa, Soichiro Nagai, Soju Kurihara, Atsuo Mikata, and Hideo Okumura

Departments of Obstetrics and Gynecology [I. I., S. N., S. N., S. K.] and Pathology [A. M.], School of Medicine, Keio University, 35, Shinnanomachi, Shinjuku-ku, Tokyo 160, and Department of Virology and Rickettsiology, National Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan (H. O.)

SUMMARY

A cell line designated SKN was established from the human uterine leiomyosarcoma of a 52-year-old female. The cell line has grown well and the serial passages were successively carried out 82 times within 12 months. The monolayer cultured cells revealed anaplastic and pleomorphic features, and they multiplied rapidly without contact inhibition. Electron microscope studies revealed myofibrils but no virus-like particles, while chromosomal studies showed that all cultured cells were hyperploid, the modal number was 112, and the marker chromosome was present. The cells were transplanted into an immune-depressed hamster cheek pouch and produced a histological leiomyosarcoma resembling the original tumor.

INTRODUCTION

The cell lines previously established from human sarcomas include osteogenic sarcoma (2, 3, 7–9, 12), rhabdomyosarcoma (5, 8, 10), liposarcoma (2, 7, 8), giant cell sarcoma (2), neurogenic sarcoma (17), and fibrosarcoma (2, 13). As far as we are aware, however, a human leiomyosarcoma cell line has never been reported.

Among the human neoplastic tumors in the gynecological field, the uterine sarcoma is a very rare lesion and represents 1.1 to 5.5% (12) of all malignant uterine tumors (except for choriocarcinoma). The treatments of the uterine sarcoma are surgical removal, irradiation, or chemotherapy. The best 5-year salvage has been approximately 37.2% (1), but most investigators have reported a very low percentage (4, 16, 19).

Because chemotherapy against soft-tissue sarcomas is generally ineffective, it is necessary to develop new chemotherapeutic agents. The in vitro culture of the uterine sarcoma is a suitable experimental system for the testing of such agents. This paper describes a successful attempt to establish a cell line derived from the uterine leiomyosarcoma and the subsequent detection of myofibrils in the cultured cells. 

MATERIALS AND METHODS

Material. The patient, a 52-year-old female, was admitted to Keio Hospital, Tokyo, on July 2, 1975. She had noted a spotty vaginal bleeding and pain in the left abdomen during the preceding year. A biopsy was obtained from the uterine cervix and placed into culture on July 3, 1975. Histopathological diagnosis of the biopsy specimen indicated leiomyosarcoma (Fig. 1).

Tissue Culture Techniques and Culture Media. The material was rinsed twice with the culture medium and minced with a sharp pair of scissors. The fragments were placed into plastic dishes (3.5 cm in diameter; Falcon Plastics Co., Oxnard, Calif.) and left at an angle of 30° for 15 min before the culture medium was added. Cultures were fed with Ham's F-12 medium (Grand Island Biological Co., Grand Island, N.Y.), 100 units penicillin, 100 μg streptomycin per ml, and 10% inactivated newborn calf serum at a final pH of 7.2 and incubated at 37° in a humidified atmosphere containing 5% CO2 in air. The medium was changed twice a week. After a stationary period of 2 days each specimen developed favorable outgrowth. The cells were dispersed into a single-cell suspension of phosphate-buffered saline (NaCl, 8 g; KCl, 0.2 g; Na2HPO4•2H2O, 0.116 g; KH2PO4, 0.2 g/1000 ml) with 0.1% trypsin (Difco Laboratories, Inc., Detroit, Mich.) and 0.02% EDTA (Sigma Chemical Co., St. Louis, Mo.) in order to transfer them into a monolayer culture and then were replaced in a closed culture system. The cells were transferred at a 1:2 or 1:4 dilution using 0.25% trypsin solution to free the cells.

Growth Curve, Doubling Time, and Plating Efficiency Assays. For studies of the growth curve, about 5 × 104 single-suspension cells per ml were plated onto plastic dishes (3.5 cm in diameter). They were incubated for about 7 days, and the average number of cells was determined every day by counting cells in 3 dishes. The medium was changed every 2 days. The population-doubling time was determined by the growth curve. For the study of plating efficiency, 1 × 102 single-suspension cells were incubated in 5 plastic dishes (6.5 cm in diameter; Falcon Plastics Co., Oxnard, Calif.) for 10 days. Plating efficiency was determined by the ratio of the number of colonies to the total number of inoculated cells.

Light and Electron Microscopic Observations. Sections of the biopsy specimens were stained with hematoxylineosin, Mallory, Van Gieson, PTAH,2 periodic acid-Schiff, and silver impregnation. The monolayer-cultured cells were stained with Giemsa, Papanicolaou, Mallory, PTAH, and silver impregnation. For transmission electron microscopy, the cultured cells were fixed in 2.5% glutaraldehyde buffered at pH 7.4 with 0.05 M phosphate for 30 min, washed in phosphate buffer, postfixed with 1% osmium buffered at pH 7.4, dehydrated in a graded series of ethanol, and prepared for electron microscopy.

Received July 28, 1976; accepted November 16, 1976.

1 This work was supported by a Grant for Cancer Research from the Japanese Ministry of Education.
tetroxide for 1 hr, and dehydrated step by step with ethanol. The capsules were then placed directly on the cultured cells before polymerization of the Epon 812 for 2 days at 60°C. Subsequently, they were peeled off the glass at 90°C. Sections were stained with uranyl acetate and lead citrate and studied in Japan Electron Optical Laboratory 100B (Tokyo, Japan).

**Chromosomal Analysis.** Cells were treated with Colcemid (1 × 10^{-7} M) Ciba Ltd., Basle, Switzerland) for 4 to 6 hr at 37°C, placed in a hypotonic solution (0.2% potassium chloride) for 15 min, and then fixed in alcohol:acetic acid (3:1). After being air-dried the cells were stained with a 1:10 dilution of Giemsa stock solution. More than 100 metaphase plates were counted for the study of distribution of chromosomal number. Their karyotypes were analyzed strictly in accordance with the Paris Conference recommendations.

**Heterotransplantation.** Four Syrian golden hamsters (Nippon Bio-Supplies Center, Tokyo, Japan) weighing about 50 g were inoculated with 2 × 10^6 cells into the submucosa of their cheek pouches. The hamsters were given hydrocortisone acetate (2.5 mg/50 g body weight) 2 times every week after transplantation (11,14).

## RESULTS

**Histopathology of Biopsy Specimens.** The tumor was composed of elongated spindle-like cells with large irregular hyperchromatic nuclei. Cytoplasm was stained red by Mallory, yellow by Van Gieson, and brown by silver impregnation and contained fibrillar apparatus that was stained deep blue by PTAH, but without cross-striation. The filaments measured 100 Å in diameter by electron microscopy. Periodic acid-Schiff stain was negative. Pleomorphic multinucleated giant cells and mitosis were also seen. There was some hemorrhage within the tumor. The histopathological appearance was interpreted as leiomyosarcoma (Fig. 1).

**Morphology of Cultured Cells.** The monolayer cultured cells were spindle-shaped or fibroblast-like but were anaplastic and pleomorphic. The cytoplasm contained fibrillar apparatus stained deep blue by PTAH. Multinucleated giant cells were noted. The nuclei revealed neoplastic features such as a bizarre aggregation of chromatin granules, an irregular thickening membrane, and multiple large nucleoli (Fig. 2). At confluency, the cells became multilayered. Electron microscopy revealed most individual cells to be characterized by highly indented nuclei with multiple large nucleoli. The cytoplasm showed a paucity of organelles except for the rough endoplasmic reticulum. Often the cells contained myofibrils running along the longitudinal axis. The cell surface was smooth, and desmosomal junction was not seen at the adjacent membrane (Fig. 3). These features suggested that the cells were mesenchymal in origin. No virus-like particles were observed.

**Growth Characteristics.** Growth curve was examined at passages 8 and 29. Eighteen hr after inoculation these cells grew logarithmically, and after 5 days they reached a plateau or stationary phase. The doubling time of passage 8 was 60 hr and that of passage 29 was 36 hr (Chart 1). The growth rates were then accelerated with subcultivation. The plating efficiency of passage 29 cells was 21.3%. The SKN cells grew well without interruption for over 12 months, and 82 serial passages were successively carried out. They continue to be stable in growth.

**Chromosomal Analysis.** Chromosomal analysis was done at passages 6, 19, and 29; the distribution of chromosomal number is described in Chart 2. About 95% of the cells showed a hyperploidy of more than 100. A majority of
the cells were found in the range from 105 to 125. An exact count of this modal range was done and showed the modal number to be 112 (Chart 2). Karyotype analysis was done at passages 6, 29, and 71. Constitutions of every group indicated abnormality in all passages. The marker, large subtelocentric and minute, chromosomes were present in all passages (Figs. 4 and 5).

**Heterotransplantation.** Ten days after inoculation these cells produced a tumor in all cases (4/4), measuring 0.4 to 0.6 cm in diameter. The histology of the tumors indicated leiomyosarcoma and the tumors closely resembled the original (Fig. 6). Necrosis was seen in the central area.

**DISCUSSION**

Sarcomas arise from muscle, connective tissue, vascular elements, endometrial stroma, and other areas of mesodermal origin. The majority of uterine sarcomas are leiomyosarcomas, originating as a malignant alteration in a previously benign myoma. Leiomyosarcomas may sometimes be mistaken for fibrosarcomas, neurogenic sarcomas, rhabdomyosarcomas, or other sarcomas. However, there should be little difficulty in diagnosing leiomyosarcoma with the use of specific stains such as PTAH. SKN cells contained myofibrils in cytoplasm detected by electron microscopy and PTAH stain.

This paper has described a successful attempt to establish a cell line derived from the human uterine leiomyosarcoma. The SKN line will be suitable experimental material for the testing of chemotherapeutic agents and the analysis of myosin in smooth muscle tumor.

Some cell lines have been established by the colony isolation technique. In the primary culture they contained normal cells such as fibroblasts. The normal cells grew rapidly, and thus malignant cells became detached from the surface of the culture bottle. The SKN line, however, consisted of pure leiomyosarcoma cells without the development of normal cells in the primary culture. As evidence, all cells revealed anaplastic, pleomorphic features, and the chromosomal numbers were of hyperploid range with no diploid cells such as fibroblasts. The normal cells grew rapidly, and thus malignant cells became detached from the surface of the culture bottle. The SKN line, however, consisted of pure leiomyosarcoma cells without the development of normal cells in the primary culture. As evidence, all cells revealed anaplastic, pleomorphic features, and the chromosomal numbers were of hyperploid range with no diploid-range cells.

That the SKN line was established from human leiomyosarcoma is suggested by the following facts: (a) the material was human leiomyosarcoma; (b) the cells had a malignant morphology and grew multilayered without contact inhibition; (c) the cells were transplantable and produced the tumor that was histologically leiomyosarcoma; (d) as revealed by electron microscopy, the cells contained myofibrils in cytoplasm, and desmosomal junction was not seen; (e) the chromosomes of the cells showed a hyperploidy of more than 100, and the marker chromosomes were present; (f) the cells grew without interruption for over 12 months; and (g) contamination by other previously established human sarcoma never occurred because there were no other human sarcoma cells in the laboratory where SKN was isolated and grown.

**ACKNOWLEDGMENTS**

We are grateful to N. Komatsu of the Department of Pathology, Tokai University for his skillful technique of electron microscopy and to K. Yamada of the Department of Virology and Rickettsiology, National Institute of Health, Japan, for his generous advice on this report.

**REFERENCES**

Fig. 1. The original tumor was composed of spindle-like elongated cells with large irregular hyperchromatic nuclei. Pleomorphic giant cells and mitosis were also seen. The histopathological appearance was leiomyosarcoma. H & E, × 400.

Fig. 2. The monolayer-cultured cells of passage 6 were spindle or fibroblast-like but were anaplastic and pleomorphic. Multinucleated giant cells were noted. Papanicolaou, × 400.
Fig. 3. The microstructural findings of passage 8 cells were characterized by highly indented nuclei with multiple large nucleoli, cytoplasmic myofibrils with focal densities, a smooth surface, and no desmosomal cell contact. Electron microscopy. × 5,000. Inset A, the area enclosed by the rectangle on the low-magnification micrograph, shows filaments measuring 100 Å in diameter. Electron microscopy. × 30,000.
Fig. 4. Karyotype and metaphase chromosomes of passage 71. Note the marker, large subtelocentric (M, or large arrow) and minute (m, or small arrow), chromosomes.
Fig. 5. Metaphase chromosomes of passage 29. Note the marker, large subtelocentric (large arrow) and minute (small arrow), chromosomes.

Fig. 6. The histology of the tumor produced by heterotransplantation of passage 26 cells into hamster cheek pouches revealed leiomyosarcoma and resembled the original tumor. H&E, x 400.
Establishment of a Human Leiomyosarcoma Cell Line

Isamu Ishiwata, Shiro Nozawa, Soichiro Nagal, et al.