ABSTRACT

A human malignant fibrous histiocytoma cell line was established in vitro. Cells showed a wide variety of morphologies, although the karyotype study showed that the tumor was monoclonal in origin because of the presence of unique marker chromosomes in 100% of the cells examined (50 of 50). Cells were cloned according to their characteristic morphologies and biological behavior in culture. The cloned cells were sparse spindle, packed spindle, epithelioid, and lymphoid. In colonies, sparse spindle cells grew separately from each other without cell to cell contact but produced a cartwheel pattern at confluency. Packed spindle cells grew in a tightly packed fashion and produced a storiform pattern at confluence. Epithelioid cells were spindle shaped as individuals but became epithelioid when in contact with each other and produced many multinucleated giant cells at confluence. Lymphoid cells were spindle shaped as individuals but became spherical at confluence. When tumors were grown in nude mice after transplantation of these cloned cells, the histology was shown to be unrelated to morphology in culture and was epithelioid (histiocytic), as was the original tumor. These results show that (a) a single cell derived from malignant fibrous histiocytoma cells exhibits a wide range of phenotypical expression in vitro, (b) cells have their own morphological and biological characteristics in vitro, which (c) however, are easily influenced by environmental factors and (d) which are unstable and even interchangeable. These characteristics may contribute to the endless variety of cellular forms and growth patterns of malignant fibrous histiocytomas in humans.

INTRODUCTION

MFH is a soft tissue sarcoma arising from mesenchymal cells. Since this tumor arises from fibroblasts, histiocytes, or undifferentiated cells, its histology shows a wide variety of cell morphologies, such as storiform-pleomorphic (fibroblastic or histiocytic cells dominant), myxoid, giant cell inflammatory, and angiomatoid types (1). The precise histogenesis, however, is still unclear.

We established a cell line in vitro from an MFH tumor made up of uniform histiocytic cells and MNGC with prominent vascular proliferation. These cells displayed a wide variety of morphologies in culture, exhibiting such forms as spindle, epithelioid, dendritic, spherical, and MNGC. Cells were cloned according to their morphological characteristics, and biological behavior was investigated in culture and in nude mice.

MATERIALS AND METHODS

Clinical Summary. In September 1986, a 66-year-old female patient came to us exhibiting a large tumor on her left inner thigh. The tumor had been excised 3 months previously but had recurred and was 3 cm in size when presented to us. This recurrent tumor was located in the subcutaneous fatty tissue with sharp demarcation.

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Tissue Culture. Details of the tissue culture methods have been reported previously (2, 3). Briefly, tumor tissue obtained during surgery was minced into 1-mm³ pieces, and a primary culture was initiated in 35-mm plastic dishes (Falcon Plastics, Los Angeles, CA) using Eagle's minimal essential medium (Nissui Seiyaku Co., Ltd., Tokyo, Japan), supplemented with 10% fetal bovine serum (Grand Island Biological Co., Grand Island, NY), penicillin (100 units/ml), and streptomycin (100 μg/ml) (Grand Island Biological Co.) in a humidified incubator with 5% CO₂ in air at 37°C. Tumor cells migrated out from the primary explants on the third day of culture and then propagated continuously in vitro. Subcultures were done every 2-4 weeks using 0.25% trypsin in a 0.5% EDTA solution (Toshiba Kagaku Kogyo Co., Tokyo, Japan).

Morphological Studies. Light microscopic observations of the original tumor were made after staining with HE. For electron microscopic studies, the original tumor was fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and then processed for electron microscopy, as described previously (2, 3). Thin sections were stained with uranyl acetate and with lead citrate (4) and observed in a Hitachi 500 electron microscope at 75 kV. Light microscopic examination of tissue-cultured cells was made with a Nikon phase-contrast microscope. For electron microscopic studies, cells were cultured on thin plastic coverslips (Microbiological Associates, Inc., Bethesda, MD), fixed in situ with 1% glutaraldehyde, and then processed, as described previously (2, 3).

Biological Studies. Population-doubling time was estimated by a daily count of cells up to 14 days after seeding. Plating efficiency was examined after seeding 30-100 cells onto 35-mm plastic dishes. Saturation density was determined by counting cells in the monolayer.

Chromosome Analyses. Chromosome analyses of 50 tissue-cultured cells were done with conventional Giemsa staining and with trypsin-Giemsa banding, according to the method of Seabright (5).

Cell Cloning. Ten to 100 cells/dish were seeded in 35-mm culture dishes. Colonies were dispersed by trypsin treatment and seeded again. This procedure was repeated 4 times. Cells were uniform in shape and biological behavior at the first cloning.

Transfer of Cultured Media to Cloned Cells. Cloned cells were cultured for 2 weeks in media that had been used for culture of other cloned cells, and cell shapes and biological behavior were observed under a light microscope.

Transplantation into Nude Mice. Cloned cells (1-2 x 10⁷) were inoculated s.c. into 20 athymic nude mice (nu/nu, BALB/c strain; Seidokyo, Shizuoka, Japan). Tumors grown in these mice over a 3-month period were examined under light and electron microscopes.

RESULTS

Light and Electron Microscopic Studies of the Original Tumor. The tumor was located in the subcutaneous fatty tissue and was solid and well demarcated and lacked a capsule. Tumor cells were atypical and epithelioid in shape and there were no spindle cells. There were many MNGC and slit-like vascular structures (Fig. 1a). As noted by electron microscopy, epithelioid cells contained well-developed rough and smooth endoplasmic reticulum, mitochondria, and lysosomes in the large cytoplasm. Multinucleated cells were also observed (Fig. 1b). Some cells contained many intermediate microfilaments. Cells were tightly attached to each other and often highly inviolated, with occasional high-density areas along cell membranes.

Tissue Culture and Cell Kinetics. Many cells with a wide variety of morphologies migrated out from the primary explants.
Fig. 1. Histological section (a) and an electron micrograph (b) of the original tumor. a, epithelioid cells with a few MNGC and slit-like vascular structures; b, large epithelioid cells and a MNGC with well-developed mitochondria, rough endoplasmic reticula, and lysosomes in a large cytoplasm. a, HE stain, × 250; b, × 2,000.

Fig. 2. A phase-contrasted light micrograph (a) and an electron micrograph (b) of tissue-cultured MFH cells (uncloned). a, variously shaped cells growing; b, MNGC showing well-developed smooth and rough endoplasmic reticula, lysosomes in cytoplasm, and long slender surface microvilli. a, × 25; b, × 4,800.
on the third day of culture and propagated steadily. Subcultures were done every 2 weeks. This cell line (K-MFH-1) had been kept in culture for 4 years after more than 120 subcultures. Plating efficiency was 5–10%. Population-doubling time was approximately 72 h and saturation density was $6 \times 10^4$ cells/cm². Cell viability was >95%.

Morphological Studies of Tissue-cultured Cells. Cells were of various shapes, i.e., thick, thin, long, or short bipolar spindle, and, in addition, dendritic, epithelioid, small spherical, and multinucleated giant cells were also noted (Fig. 2a). As seen under the electron microscope, cells had well-developed smooth and rough endoplasmic reticula, mitochondria, and many electron-dense lysosomes in the large cytoplasm. There were many long slender microvilli on the cell surface. MNGC were also observed (Fig. 2b).

Chromosome Analyses. Fifty metaphase cells were examined with Giemsa and trypsin-Giemsa stains. The chromosome number varied from 39 to 48 with a hypodiploid modal number of 43 (Table 1). Six marker chromosomes were identified by means of trypsin-Giemsa banding (Fig. 3). All of these marker chromosomes were present in 100% of the 50 cells examined.

Cell Cloning. Thirty-five clones were obtained. Among these, 4 clones showed conspicuous morphological and biological features which were characterized as, SS, PS, Ep, and Ly. SS cells were slender and spindle shaped and propagated in colonies without cell-to-cell attachment (Fig. 4a). When cells became confluent, they attached to each other and produced a cartwheel pattern in vitro (Fig. 4b). PS cells were also short, straight, and spindle shaped but propagated with tight cell-to-cell contact (Fig. 5a) with their long axes parallel. When a colony expanded in size, it branched out and created new colonies. Thus, the axes of various colonies crisscrossed. Finally, these cells produced a storiform pattern in culture (Fig. 5b). Ep cells were slender and spindle shaped when there was no cell-to-cell contact but became epithelioid when surrounded by other cells (Fig. 6a). After confluency, many MNGC appeared in this clone (Fig. 6b). Ly cells were slender, straight, and spindle shaped as individuals but when piled up in a colony they became spherical (lymphoid) (Fig. 7). Only Ly cells displayed this tendency to pile up on other cells. Thus, cells at the lowermost layer in the pile were spindle shaped, and cells above the second layer were spherical (lymphoid). Furthermore, these rounded cells showed no tendency to detach from the underlying layer. Cells cloned from this cell line tended to lose their own characteristic morphological and biological behavior after repeated subcultures and, by the 15th subculture, had become a mixture of various cell types just as if they had never been cloned.

Transfer of Cultured Media. Morphological and biological characteristics of each cloned cell type were not altered when cells were cultured in other cloned cell-cultured media.

Tumorigenicity and Cell Morphology in Nude Mice. When <10⁷ cells were injected into 12 mice, no tumor development was observed. When >2 × 10⁷ cloned cells were injected, tumors appeared in 4 mice, i.e., 1 of 2 with SS, 0 of 2 with PS, 1 of 2 with Ep, and 2 of 2 with Ly cell lines. These tumors reached diameters of 1–2 cm within 3 months. All 4 tumors showed very similar histological patterns regardless of the cloned cell type, i.e., there were epithelioid cells with many MNGC and prominent vascular proliferation (Fig. 8) as observed in the original tumor (Fig. 1a). There were also inflammatory cells (mostly lymphoid, as well as a few neutrophils and eosinophils) infiltrated among the tumor cells in these mice (Fig. 8), which had not been observed in the original tumor (Fig. 1a).

DISCUSSION

Histopathological analysis of MFH is known to present a striking variety of cells, cellular products, and growth patterns. Generally, MFH is composed of a mixture of fibroblasts, my-
of fibroblasts, and histiocytes associated with a varying number of anaplastic and osteoclast-like giant cells as well as inflammatory cells (1). The exact nature and biological behavior of these cells are still unknown. To further knowledge in this area we undertook the present study.

There have been only a few reports concerning establishment of an MFH cell line and biological studies in culture (6, 7). This is the first study to develop an MFH cell line derived from subcutaneous fatty tissue of the skin. The tumor histology in this study was epithelioid cell dominant, but cells in culture were noted to be spindle cell dominant. Karyotype study disclosed that this tumor was monoclonal in origin. Since cells showed various morphologies and appeared to have different biological behavior in culture, they were cloned according to their particular morphology.

SS cells did not produce a compact colony. This is extremely unusual behavior, because cells almost always grow in contact with one another, e.g., normal fibroblasts, keratinocytes, endothelial cells, melanoma cells, and others. After they reached confluency, these cells exhibited tight cell-to-cell contact and produced a striking cartwheel pattern in vitro. There may be a cell which plays a key role in producing this pattern. A human keratinocytic cell line (8) which differentiated toward an eccrine gland in culture has been reported to produce domes, made up of thousands of cells and containing an empty cavity. Cells with amyl phosphorylase activity are thought to play an important role in the formation of these domes (8). In our MFH cell line, it was unclear what kind of cell played the crucial role in forming the cartwheel pattern. A soluble factor can be ruled out as causative since the transfer of cultured media to other cloned cells did not produce any effect on cell shapes or biological behavior. PS cells exhibited tight contact with each other in colonies and these cells produced a storiform pattern in culture. This storiform pattern is not observed with normal fibroblasts in culture, although the cartwheel pattern does occur. These two unusual growth patterns are characteristic of MFH in vivo. The reason why these cells behaved so differently, although they were both derived initially from a single cell, is unknown at present. Ep cells were spindle shaped but became epithelioid after they became surrounded by other cells. Therefore, these cells were spindle shaped at the periphery but were epithelioid in the interior of the colony. Cell-to-cell contact apparently induced these morphological changes, but again we do not know what kind of information was transferred from cell to cell to induce these changes. Many MNGC appeared in this cell line after confluency, whereas other cloned cells only rarely produced MNGC in vitro. Cells having this biological characteristic could play a role in the formation of MNGC in MFH in vivo.

Ly cells were spindle shaped on the substrate but became rounded when they grew on other cells. The rounding phenomenon and the formation of a cell cluster could suggest that these cells share certain characteristics of hematopoietic (lymphoid) cells since lymphocytes also form such a cluster in culture. However, these cells might be closer to macrophages than to lymphocytes because these clustered spherical cells appeared to have strong adhesiveness. This interpretation may not be in conflict with the mono-histiocytic hypothesis of the origin of MFH. The morphological and biological characteristics of these cloned cells were gradually lost, and cells eventually appeared as if they had never been cloned. This instability appears to be related to the nature of MFH.
BIOLOGICAL BEHAVIOR OF MALIGNANT FIBROUS HistiocYToma

Fig. 6. a, Ep cells in a colony showing spindle-shaped cells at the periphery (left) and epithelioid cells in the interior; b, Ep cells after confluency showing many MNGC. x 25.

Fig. 7. Right upper corner, Ly cells growing in a colony in a pile; left lower corner, spindle shaped cells. Spindle cells are on the substrate. x 25.

Since cartwheel and storiform patterns as well as many MNGC are often observed in MFH in vivo and these appeared to be reproduced with cloned cells in vitro, tumors grown in nude mice were expected to have produced similar histological patterns as cells produced in vitro. The results, however, were disappointing, i.e., tumor histology did not reflect growth patterns in culture and was very similar regardless of the type of clone used. A similar phenomenon was observed in human malignant melanoma (9), i.e., (a) cell morphology in vitro did not correlate with that of in vivo findings and (b) tumor cell morphology in mice was wholly dependent on the original cell morphology of the human tumor used. An interesting observation regarding tumor histology in the nude mice was the appearance of inflammatory cells among tumor cells, which was not observed in the original tumor. MFH cells may have released chemotactic factors for these inflammatory cells because human trichilemmoma cells have been reported to release neutrophil or eosinophil chemotactic factors (10). This capacity may have been inherent in the original tumor and became apparent in the nude mice.

We could not elucidate the precise histogenesis of MFH in this study. However, by using viable cells in vivo and in vitro, we have found that (a) a single cell derived from MFH cells showed a wide variety of phenotypical expression, (b) cells had their own morphological and biological characteristics in vitro, which, (c) however, were easily influenced by environmental factors, and which (d) were unstable and even interchangeable. These findings may explain the endless variety of cellular forms and growth patterns of MFH in humans.

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Biological Behavior of Cloned Cells of Human Malignant Fibrous Histiocytoma  *in Vivo* and *in Vitro*

Tamotsu Kanzaki, Shinji Kitajima and Kaoru Suzumori


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