A New in Vitro Cell Line Established from Human Oat Cell Carcinoma of the Lung

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SUMMARY

A new tissue culture cell line, OAT-1975, has been established from explant cultures of human oat cell carcinoma of the lung, characterized and maintained for more than 1 year. The cells grew in a monolayered sheet with 24 hr of population-doubling time and are capable of clonal growth with about 60% plating efficiency. The cells still retain unique morphological and structural characteristics of oat cell carcinoma and showed "tumor takes" when transplanted into nude mice and conditioned young Syrian Golden hamsters. The chromosome constitution of the cells is hypertriploid with a modal peak at 74. The permanent growth integrity of the present cell line made it possible to utilize it for cell biological studies. Cell inactivation experiments indicated that the cells are sensitive to X-rays and Mitomycin C when compared to the responses of HeLa S3 cells.

INTRODUCTION

Human oat cell-type carcinoma of the lung is an undifferentiated and specific type of tumor in terms of cell and tumor structure (2-4, 12). Because of its high malignancy with rapid and extensive tumor growth, oat cell carcinoma of the lung frequently presents clinical problems from the viewpoints of curability and resectability (14, 26). In spite of various efforts to obtain cultured cell lines from human lung cancer (1, 6-8, 10, 13, 19, 21), the establishment of tissue culture cell lines from human oat cell carcinoma has not been completely successful for the purpose of cell biological studies (13, 19).

In March 1975, tumor cells isolated from a lung of a patient, whose case was diagnosed as oat cell carcinoma with Eaton-Lambert syndrome (myasthenic syndrome), were transferred to tissue culture. After a 5-month latent period, a new permanent cell line was established. Such specific tumor cell lines can be utilized for cell biological studies to provide a basis for human lung cancer therapeutic studies.

The present report deals with the characterization and identification of the cells together with some experimental results on cellular inactivation by X-ray and Mitomycin C.

MATERIALS AND METHODS

Primary Culture. A tumor mass was obtained immediately postmortem from the pulmonary lesion of a 63-year-old male Japanese patient. The patient autopsy showed that the tumor was highly metastatic and had already disseminated to the mediastinum, liver, adrenal gland, and pancreas. Since the tumor cells were only loosely attached to each other in the tissue, neither enzymatic dissociation nor forceful mincing was required for the separation of tumor cells. The tumor was cut into 2 pieces and rubbed softly with the tip of dissecting tools. The spill-out cells with milky fluid were collected and purified by removing large pieces of the tissue and other debris. The cell suspension, free of debris, was collected for appropriate cell density and seeded into 21- or 25-sq cm plastic culture vessels containing Ham's F-10 medium (10) with 10% calf serum, 0.05% penicillin (100 units/ml), and streptomycin (100 µg/ml). The cultures were then incubated in a humidified CO2 chamber, constantly gassed with 5% CO2 in air at 37°. The medium change depended on the condition of the cultures, such as nutritionally starved cells.

Light and Electron Microscopy. Pieces of the tumor and cultured cells were fixed with 10% neutral formalin solution and processed for histological preparations.

Slide preparations of the cultured cells were stained by various methods: Giemsa's and Meyer's hematoxylin-eosin counterstaining for morphology, and argentaffin stains by Fontana-Masson's method and periodic acid-Schiff stains for histochemistry.

Electron microscopy was performed on the pieces of the patient's tumor, and the packed cell pellets were harvested by dispersing monolayered cell sheets with EDTA solution (0.02% disodium EDTA dihydrate in Puck's Saline Solution V). The specimens were fixed in phosphate-buffered glutaraldehyde (3%) for 1 to 2 hr, postfixed with 1% OsO4 for 1 hr, and embedded in epoxy resin. The sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi DS-11 electron microscope. Tumors that developed in the experimental animals as a result of inoculation of the cultured cells were excised and fixed in 10% neutral formalin solution and sectioned for histological studies.

Fluorometry. Autofluorescence of smeared cells was examined under a fluorescent microscope immediately after the fixation of cells in paraformaldehyde moisture for 5 to 6 min. Determination of the total amount of 5-hydroxytryptamine and its derivatives in cultured cells was performed by the direct method of Udenfriend and Weissbach (25). Fluorospectrometry was carried out by using a Hitachi MPF-2A...
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spectrophotofluorometer.

Chromosome Analysis. Mitotic cells were obtained from colcemid-treated cultures (0.1 \(\mu g/ml\) overnight) of the 5th transfer. The cells were treated with 0.75% KCl solution for 15 min and fixed with Carnoy’s solution for about 1 hr. After fixation the cells were spread on a glass slide by the air drying technique (18) and stained with Giemsa’s solution. Karyotype analysis was made by taking photographs of well-spread metaphase plates.

Heterotransplantation. Suspensions of cultured cells were injected into nude mice s.c. or into the cheek pouches of Syrian Golden hamsters. Nude mice were inoculated with \(1 \times 10^6\) cells and carefully kept in a hygienic environment. The young hamsters, weighing about 60 g at the age of 30 days, were irradiated with 500 R 2 to 3 hr before the inoculation of the cell suspension, and treated with 2.5 mg hydrocortisone (24) periodically at 2-day intervals 5 times after the inoculation of cells. About \(2 \times 10^7\) cells were inoculated into the cheek pouches of the conditioned hamsters. The growth of cells was checked every 3 days after inoculation.

Cell Growth and Survival. Inocula of \(2 \times 10^8\) cells were grown on the surface of 21-sq cm plastic dishes in a 5% CO\(_2\) atmosphere. The population-doubling time was determined by counting the cells in a series of duplicated dishes at daily intervals.

Inactivation of the cultured cells by X-irradiation and 1-hr pulse treatment with Mitomycin C was investigated by assaying the colony-forming ability of surviving cells. A series of experimental cultures of singly dispersed cells was prepared and incubated for 2 to 3 hr in a CO\(_2\) chamber for cell attachment. Then, a group of cultures was irradiated with various doses of X-rays delivered under 200 kVp and 20 ma conditions at a dose rate of 85 R/min. After the X-irradiation the cultures were kept in a CO\(_2\) chamber for the formation of surviving cell colonies. Survival of cells was determined by counting the number of colonies containing more than 50 healthy cells after 2 weeks of posttreatment incubation.

Mitomycin C was added to the cultures in graded concentrations. The drug-treated cultures were washed twice with drug-free medium and replenished with fresh growth medium. The results obtained in both X-ray and drug treatment experiments were compared with those obtained with HeLa S3 cells, which were treated in the same way in our laboratory.

RESULTS

Morphology and Other Characteristics of Cultured Tumor Cells. The cells in the initial phases of the primary culture were not attached to the surface of the culture vessels and showed no significant growth during the 1st 4 months. After the latent period, 1 of 6 bottles of the primary cultures showed a strong acidification of the culture medium. The culture was found to contain a significantly increased number of cells and was then subcultured. The subsequent cultures gave a dominant growth of epithelial-like cells replacing the nonattached cells during the next 1-month period. Sequential passages have been successful for over 40 transfer generations until May 1976. Microscopic observation of cultures during that period showed no sign of contaminated growth with other kinds of cells like fibroblasts.

Morphologically, cells are characterized by an epithelial-like shape with prominent nuclei and rather scanty cytoplasm (Figs. 1 to 3). Nuclei, generally found localized at the end of the cytoplasm and pleomorphic in shape, usually contained 2 or 3 prominent nucleoli (Figs. 1 and 2). The presence of numerous mitochondria and Golgi complexes was commonly observed. However, the presence of neurosecretory granules that are frequently observed in such tumor cells was not clearly identified by electron microscopy (Figs. 2 and 3).

Chromosome analysis of cells was performed by observing 50 metaphase plates. Chromosome numbers in each plate showed a wide variation from 70 to 77, and the mode
was found at 74 (24% frequency). Most of the cells were in the hypotriploid range in chromosome number but contained no useful marker chromosome as evidence that cells were of male origin except for the Y-chromosome (Figs. 4 and 5). Some 4th-generation cultures were found to retain aberrant chromosomes. Most of these were identified as chromosome fragments without a centromeric region or chromosomal aberrations. This suggests that an alteration of chromosomes occurred during the course of the establishment of the present cell line.

The histochemical observations were carried out to elucidate unique cell properties. The cells were found to be positively argyrophilin, being stained positively in 2 hr to Fontana-Masson’s stain, whereas HeLa S3 cells used as a negative staining control did not show any positive reaction. The reaction to periodic acid-Schiff staining was not found positive in either OAT-1975 cells or HeLa S3 cells. Autofluorescent reaction of cells under paraformaldehyde fixation was positive only for OAT-1975 cells but not for HeLa S3 cells. However, typical signs of the presence of serotonin granules were not detected. Spectrofluorometry of cellular extracts by the method of Udenfriend and Weissbach (25) demonstrated the presence of 5-hydroxytryptamine derivatives in the cultured cells. The presence of a substance related to 5-hydroxytryptamine was suggested by fluorometry. A fluorometric peak was demonstrated at 340 nm. The position of the peak was located at the same position for serotonin-containing control samples treated in the same way. From the height of the peak for the cellular samples, the amount of the serotonin-like substance contained in the cultured oat cell carcinoma cells was tentatively estimated as 0.0132 μg/10⁶ cells. In HeLa S3 cells, however, there was no response at the 340-nm region when the cells were treated in the same way. A kinetic study on the in vitro growth of the cultured oat cell carcinoma cells is shown in Chart 1. The cells used for these experiments were 35th-transfer generation cells, completely adapted to the in vitro conditions and growing vigorously. The experimental cultures were initiated with an inoculum of 2 × 10⁶ cells and incubated in a CO₂ chamber at 37°. The experimental cultures were initiated with an inoculum of 2 × 10⁵ cells and incubated in a CO₂ chamber at 37°. The dispersed cells adapted to the in vitro conditions and growing vigorously.

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terms of the colony-forming ability of the cultured cells. Charts 2 and 3 show the results of the experiments on the survival of OAT-1975 cells irradiated with graded doses of X-rays or treated with the medium containing various concentrations of Mitomycin C for 1 hr. Each chart contains the results obtained with HeLa S3 cells treated with both of the agents in the same way. The dose-effect curves showed that the inactivation of cells was of a threshold type with these 2 kinds of agents. OAT-1975 cells were found to be much more sensitive to both X-ray and Mitomycin C treatment than HeLa S3 cells. The response of HeLa S3 cells to Mitomycin C treatment was consistent with the results obtained in a previous work by Ohara and Terasima (20). The parameters of the X-ray survival curve for the oat cell carcinoma cells are 85 R for mean lethal dose (Do), 229 R quasi-threshold dose (Dq), and 10.5 for extrapolation number (n), whereas those for HeLa S3 cells are 130 R, 125 R, and 2.5. The curves of these 2 kinds of cells are significantly different.

The response of the oat cell carcinoma cells to Mitomycin C treatment was also found to be more sensitive than that of HeLa S3 cells (Chart 3). The Do for the present lung cancer cells was 0.11 µg/ml, whereas that of HeLa S3 cells was about 0.25 µg/ml. The survival of the oat cell carcinoma cells decreased in an exponential manner to levels as low as 0.2%. Mitomycin C was found to inactivate the reproductive integrity of the oat cell carcinoma cells effectively in a dose-response manner.

DISCUSSION

So far as we know, 2 human oat cell carcinoma cell lines have been established by Japanese investigators (13, 19). These 2 lines are different from the present line in morphology and other characteristics. Briefly, one line, derived from a metastatic lymph node tumor, is in suspension form with minor changes in chromosome constitution and some unique immune properties (19), and the other line, derived from a pulmonary tumor, grows loosely attached to the surface of culture vessels and is characterized by its slow growth and extraordinarily multiplied chromosomes over a hexaploid range (13). Both of these lines are not useful for cell kinetic studies because of their inefficient colony-forming ability.

The character of a cell line established from malignant tumor tissue should be identified with respect to cellular structure, morphology, chromosome constitution, tumor growth, and other specific properties. The characteristics we have observed in the present cell line may constitute the histologically unique structures of oat cell carcinoma. The close similarity of cell and histological structures will give credence to the view that the present cell line did indeed originate from the tumor of the patient concerned, although a clear-cut demonstration of the presence of neurosecretory granules was not obtained in the tissue-cultured cells. This may be due to the vigorous integrity of cellular growth in vitro, since cells in an actively growing phase usually are much less functional in terms of secretion or production of particular substance.

The production of ectopic hormones by malignant cells in many bronchial tumors and carcinoids has been known for many years (5, 9, 15, 17). The origin of oat cell carcinoma and its relation to bronchial carcinoids have been studied in terms of ultrastructural similarities (2–4). Ultrastructural observations of the present cells suggest that the cultured cells maintained close similarities to oat cell bronchial tumor cells except for the lack of neurosecretory granules in the cellular cytoplasm.

The neurosecretory granules frequently observed usually consist of serotonin granules (5-hydroxytryptamine), but the presence of serotonin granules is not universal in all of the cases of oat cell carcinoma, although its frequency is very high (2, 12). Argentaffin cell properties may also suggest a possibility that the cultured cells are or were a kind of secretory cell. Instability of such functional properties in the...
cultured cells growing in vitro is quite possibly a result of the vigorous growth of the cells. In general, establishment of a cultured cell line for the purpose of cell biological studies requires stable cell growth. In this sense, most of the established lines from a variety of normal and malignant tissues retain unlimited growth integrity, and the cells are not fully functional. The demonstration of the fluorescent peak as well as positive argentaffin property of the OAT-1975 cells probably supports the view that the secretory function, retained by the original tumor cells, may have been ruined during the course of cellular adaptation to an in vitro environment. The establishment of suitable conditions for the functional culture of the present cell line has not yet been successful in either in vitro or in vivo situations.

Chromosome analysis showed that the cells had a hypertriploid chromosomal constitution. Such abnormal chromosomal constitution may be admitted to be a feature of cancer cells, but nothing definite can be said concerning the original tumor of this line. According to studies on chromosomal abnormality in human malignant tumors in solid form (16), it has been shown that 61% of the tumors contain a hyperdiploid number of chromosomes varying between 47 and 57, whereas only 14% are in the hypertriploid range. Other studies (18, 22) have indicated that alteration can occur almost as soon as in vitro conditions exist. Therefore, the hypertriploid constitution may account for clues to the establishment of the present cell line.

Finally, the experimental results on cellular inactivation by X-rays and Mitomycin C indicated that the cells were highly sensitive to both agents, although the cells were rather insensitive to lower doses. Cell inactivation with increased dosage was seen to be remarkable for the present lung cancer cell line. The finding that undifferentiated-type, small-cell human lung cancer is much more susceptible to X-irradiation than differentiated tumors such as adenocarcinoma (23) is interesting in the light of the present experimental results. Rapid and vigorous growth of tumor cells in such undifferentiated small-cell tumors may be a reason for the remarkable response of the tumor to X-irradiation and its rapid recovery by surviving cells. Similar kinds of cell inactivation experiments with other chemotherapeutic agents are now under way.

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