A New Cell Line (TE-3) Derived from Human Squamous Cell Carcinoma of the Esophagus

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KURIYA, Y., KITAMURA, M., AKAISHI, T., HIRAYAMA, K., SEKINE, Y., NISHIHIRA, T. and KASAI, M. A New Cell Line (TE-3) Derived from Human Squamous Cell Carcinoma of the Esophagus. Tohoku J. exp. Med., 1983, 139 (4), 377-387 — A new epithelial cell line (TE-3) has been established from a metastatic lesion at the right chest wall which was originated from a well differentiated human squamous cell carcinoma of the esophagus. TE-3 has been subcultured more than 150 times for 3 years and 1 month. Cultured cells have grown as isolated colonies of epithelial cells. The average doubling time of the TE-3 cell line was 48 hr and the plating efficiency was 10 to 40% in MEM supplemented with 8% fetal calf serum. Distinctive marker chromosomes and a male karyotype were present in TE-3. Electron microscopic examination of the TE-3 cells disclosed the presence of desmosomes and microvilli in connection of the cells which were rich in cell organelles. In early passages of culture, the cytoplasm of cells was slightly positive with PAS stain but negative with Sudan III or mucicarmin stain. Heterotransplantation of the culture cells to BALB/c nude mice produced tumors, the histological appearance of which was similar to that of the original one. The carcinoembryonic antigen level of the medium in the confluent culture of TE-3 was 2 ng/10^4 cells. — esophagus; squamous cell cancer; cell line

Although a number of human cell lines have been established in vitro, very few epithelial cell lines derived from human esophageal cancer have been described. In 1976, Bey et al. reported a cell line derived from human esophageal cancer, and Nishihira et al. (1977, 1978, 1979) reported more detailed properties of two newly established cell lines (TE-1, TE-2). In this paper, we also describe some characteristics of another newly established cell line (TE-3) derived from human esophageal cancer.

MATERIALS AND METHODS

Source of tumor

A 48 years old Japanese male was admitted to Tohoku University Hospital with a chief complaint of difficulty in swallowing. Barium swallow study showed a filling defect of the esophageal wall between the upper and the middle third of the esophagus. Metastatic lesions were palpable in the left supraclavicular region and at the right

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anterior chest wall. A biopsy specimen from esophageal tumor was histologically diagnosed as squamous cell carcinoma.

The patient underwent surgical excision of the tumor at the right chest wall on March 30, 1979. Half of the tumor specimen was subjected to cell culture and the other half was fixed in formalin for histological examination. On histological examination, the metastatic tumor tissue was well differentiated squamous cell carcinoma. Necrotic part was scanty. Cancer pearls with parakeratosis or cell rich nests were seen throughout the tissue. Most of tumor cells were pleomorphic with strong atypism. Intercellular bridges were not evident. Cancer cell nests were surrounded by dense and fibrous stroma (Fig. 1).

Fig. 1. Sections of metastatic carcinoma of the esophagus at the right chest wall showing the histology of well-differentiated squamous cell carcinoma and a typical cancer pearl. Hematoxylin-eosin stain. $\times$ 110 (a), $\times$ 420 (b).
Primary culture and establishment of the TE-3 cells

The viable tissue was minced with scissors into 1 mm³ cubes in a small volume of RPMI-1640 supplemented with 10% fetal calf serum with streptomycin sulfate (100 µg/ml). After centrifugation of the tissue suspension at 800 rpm for 5 min, the tissue pellet was resuspended in culture medium and adjusted to about 5 tumor pieces per ml. Then 5 ml of tissue suspension was seeded to TD-40 glass culture bottles (13 x 4.5 x 2 cm, Ikemoto Co., Japan). A part of the medium was replaced by fresh medium 2 or 3 times a week. Tissue pieces were discarded 3 weeks after explanation. The bottles were then examined microscopically to determine attachment and growth of the tumor cells. Fibroblasts were removed carefully by scraping with Pasteur capillary pipets (Cat. no. 70958 Corning Glass Works, USA). This procedure was repeated every few days until 70% of the bottom was occupied with the tumor cells. On the 45th day after initiating primary culture, the first passage was performed. The cells were trypsinized into cell suspension, pelleted, and replanted into new bottles with small amount of culture medium.

Further subculture was repeated when the growth of the cells continued. About 4 months later, the cells adapted to tissue culture conditions were passaged at greater dilutions. The culture was considered to be fully adapted to tissue culture after 7 passages. It was performed on the 160th day after the initiation of the primary culture.

Cytological studies

For microscopic examination, cultures of tumor cells in Lab-Tek chamber (Cat. no. 4808, Lab-Tek Products, Naperville, Ill., USA) were prepared by trypsinizing the cells in the confluent condition. The cultures were incubated 2 days at 38°C in the presence of 5% CO₂ in air. Lab-Tek chamber cultures were then dipped in 2% formalin for 5 min at room temperature. The cells on the bottom of chambers were then stained with hematoxylin and eosin, May-Grunwald-Giemsa, and PAS.

For examination by scanning electron microscopy, the cells on cover slips were fixed in 1.6% glutaraldehyde and 2% osmium tetroxide, and were observed under a Hitachi, S-450 scanning electron microscope. For examination by transmission electron microscopy, monolayer cells on the surface of Falcon plastic dish were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1% cacodylate buffer, and postfixed in 2% osmium tetroxide at room temperature. The fixed cells were dehydrated with graded ethanol series (50-100%) at room temperature, and the cells were embedded in Epon 812. Ultrathin sections were made with an LKB ultramicrotome and double stained with uranyl acetate and lead acetate.

Chromosome analysis

The cultured cells were treated with colchicine at a final concentration of 2 µg/ml for 4 hr. Then, the cells were dispersed by trypsinization, washed with PBS, and resuspended in a hypotonic solution containing 0.1 g/liter of KCl, 2.0 g/liter of NaCl, 1.0 g/liter of D-glucose, and 5.0 g/liter of sodium citrate dihydrate, for 15 min. Fixation was made 3 times in Carnoy’s fixative, and the cells were dropped on a chilled wet slide glass (50% ethanol) and stained with Giemsa solution. Fifty metaphase plates were analysed.

Heterotransplantation

The cell suspension containing 6 x 10⁶ cells in 0.5 ml of PBS was transplanted into the subcutis of BALB/c nude mice. When the tumor reached the size of 10 mm³, tumors were excised and examined histologically.

Detection of carcinoembryonic antigen

The CEA level in the used media was measured by radioimmunoassay (Special Reference Laboratory Co., Tokyo).
Fig. 2. Hematoxylin-eosin staining of TE-3 cells at the 120th passage. ×85 (a), ×360 (b), ×900 (c).
Detection of Epstein-Barr virus associated antigen (EBNA)

EBNA in TE-3 cells was examined by the method of Reedman and Klein (1973).

Detection of mycoplasma

Detection of mycoplasma in cultures was made by Hoechst Stain Kit (Cat. No. 30-100-00).

RESULTS

Primary culture and establishment of TE-3 cell line

Epithelial cells had started to migrate out of tissue cubes on the third day of culture in all of the original 6 bottles. Fibroblasts originating from residual connective tissue grew rapidly at this stage and the epithelial cell colonies were surrounded by fibroblast sheets after 2 weeks. The fibroblast sheet was eliminated with Pasteur capillary pipets under a phase-contrast microscope. This process was repeated once a week until the first subculture.

Fig. 3. A scanning electron micrograph of TE-3 cells.
Fig. 4. Transmission electron micrographs of TE-3 cells. $\times 4,250$ (a), $\times 8,500$ (b), $17,000$ (c).
The first successful subculture was done on the 45th day by trypsinization. During this first subculture, fibroblasts were markedly diminished. The second subculture was made on the 85th day after cultivation. Thereafter, the cells were successively transferred at a splitting ratio of 1:2 every 7–10 days. After 7 months in culture the cells could be stored frozen and later recovered. This cultured cell

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Fig. 5. Distribution of chromosome numbers of TE-3 cells.

Fig. 6. Karyotype of TE-3 cells.
Morphology

Light and electron micrographs of cultured cells are shown in Figs. 2, 3 and 4. The cells were epithelial in shape. The cytoplasm of TE-3 was weakly positive for PAS in early stages of culture but negative in late stages and negative for Alcian blue, and mucicarmin stain. The scanning electron micrographic feature of TE-3 cells was flat and polygonal. Transmission electron micrographs of TE-3 showed abundant mitochondria but few tonofilaments in the cytoplasm. Desmosomes were noted between adjacent cells and intranuclear chromatin was distributed uniformly. No virus-like particles or other inclusion bodies were seen in the cytoplasm or intercellular space.

The doubling time of the cells was approximately 48 hr as calculated from the growth curve of the cells which have been maintained for 2 years and 6 months after the initiation of the culture. Chromosomes were hypotetraploid with the modal number 72 (Fig. 5). Distinctive marker chromosomes and male karyotype were present (Fig. 6).

Tumors, 10 mm in diameter, were produced 9 days after inoculation of TE-3 into the back of nude mice. Histological picture of the tumors closely resembled that of the original one (Fig. 7). The transplanted tumor tissue revealed marked keratinization and the central portion of tumor tissue was occupied by keratin. The shape of tumor cell was pleomorphic or spindle. The CEA level in the medium of 7 day old confluent culture was 2 ng/10^4 cells. EBNA was not detected in TE-3. Detection of mycoplasma in the cultures was negative.

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Starting dates of culture</th>
<th>Origin of culture specimen</th>
<th>Morphology</th>
<th>Histochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE-1</td>
<td>58/M Jan. 27 1976</td>
<td>Primary tumor</td>
<td>Well differentiated</td>
<td>Sudan III stain positive bodies in the cytoplasm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rich in cell organelles</td>
<td></td>
</tr>
<tr>
<td>TE-2</td>
<td>57/M Dec. 1 1976</td>
<td>Primary tumor</td>
<td>Poorly differentiated</td>
<td>PAS and Alcian blue stain positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Scanty cell organelles</td>
<td>Mucicarmin stain negative</td>
</tr>
<tr>
<td>TE-3</td>
<td>48/M Mar. 30 1979</td>
<td>Metastatic tumor</td>
<td>Well differentiated</td>
<td>PAS stain slightly positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rich in cell organelles</td>
<td>Sudan III and mucicarmin stain negative</td>
</tr>
</tbody>
</table>

The newly established cell line was designated TE-3.
There are very few accounts in the literature of the establishment of a new cell line from a human esophageal cancer (Bey et al. 1976; Nishihira et al. 1977, 1978, 1979). In 1977, 1978 and 1979, Nishihira and coworkers reported two newly established cell lines (TE-1, TE-2) from human esophageal cancer. A cell line TE-3, described here, is the third cell line established in our laboratory from human esophageal cancer.

Some morphological and biological characteristics of TE series are summarized in Table 1. The period needed for the first subculture was 45 days in TE-3, which was longer than in TE-1 and -2. Fibroblast sheets were diminished by scraping with Pasteur pipets. By this method, the fibroblast sheets were torn off like a sheet of paper, and after that, the tumor colonies grew up moderately and new fibroblasts enclosed the colonies. Fortunately, the fibroblasts were diminished after the first subculture. With scrupulous care against contamination, this scraping method is useful for eliminating fibroblasts.

PAS stain of TE-3 in the early stage revealed weakly positive, but negative in the recent examination. Transmission electron micrographs of TE-3 showed abundant cell organelles, such as mitochondria, free and membrane bound ribosomes, like TE-1, while Golgi apparatus and microtubules were not prominent in TE-3.

Heterotransplantation to BALB/c nude mice produced tumors without pretreatment, and the doubling time of the transplanted tumor was about 14 days. The time for tumors to reach 10 mm in diameter is about 9 days so that transplantability of TE-3 to nude mice is greater than that of TE-1 and TE-2. Karyotype of TE-3 is hypotetraploid similar to TE-1 and TE-2.

Further studies on other properties of these cells are being carried out.
Acknowledgment

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Fig. 7. Histological picture of the tumor of TE-3 cells grown on the back of a nude mouse. Hematoxylin-eosin stain. ×80 (a), ×420 (b).
References


