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Establishment and Characterization of a New Human Esophageal Cancer Cell Line (YES-2)

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Abstract A new human esophageal cancer cell line (YES-2) was established from a surgical specimen obtained from an 81-year-old male patient. This cell line has been maintained for 68 months through 196 passages. YES-2 cells have clear polygonal shaped cytoplasm, round nuclei and prominent nucleoli. The cells proliferate in a pavement-like cell arrangement and show a lack of contact inhibition. The doubling time at the 102nd passage was 23.7 hours.

YES-2 cells produce squamous cell carcinoma antigen (SCC antigen), tissue polypeptide antigen (TPA) and carcinoantigen 125 (CA 125) as tumor markers. Chromosome analysis has shown that the chromosome number ranges from 53 to 62 with a mode of 60. Tumorigenicity has been demonstrated by development of tumor after the subcutaneous injection of YES-2 cells into nude mice. These findings indicate that the YES-2 cell line is a new human esophageal cancer cell line which may be useful for various studies.

Key words:Esophageal cancer. Cell line.

Introduction

Establishment of cell lines from esophageal cancer has been reported by many investigators[1-6]. A previous paper from our laboratory reported the successful establishment of a cell line, YES-1[7], derived from a human esophageal cancer. These cell lines have provided information of human esophageal cancer, however, more cell lines are needed to study this neoplasm in greater detail since esophageal cancer remains a disease with poor prognosis.

This report describes the establishment and characterization of a new human esophageal cancer cell line, YES-2.

Material anmd methods

Patient An 81-year-old man was admitted to our hospital for dysphagia and was subsequently diagnosed as having esophageal cancer. On admission, the serum level of squamous cell carcinoma antigen (SCC antigen) was 12 ng/ml, while the levels of tissue polypeptide antigen (TPA) and carcinoantigen 125 (CA125) were not tested. At surgery, he was diagnosed to have a A_3N_4 (+) M_0Pl_0 stage IV lesion, according to general rules for the description of esophageal cancer[8].

Transplantation into nude mice A specimen of the primary tumor was aseptically obtained and trimmed of necrotic tissue. It was washed three times in Dulbecco's

modified Eagle's medium (DMEM) (Nissui Seiyaku Co., Japan) supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin (GIBCO, Chagrin Walkersville, MD), 12 mM sodium bicarbonate and 1% heat-inactivated (56°C, 30min) fetal bovine serum (FBS) (M. A. Bioproducts, Walkersville, MD), and minced with two surgical blades into 3 mm pieces. These tumor fragments were then transplanted into the back of 5 to 6 week old male athymic nude mice (BALB/C, nu/nu, Clea Japan Inc.) using a trocar needle. When subcutaneous tumor developed, the mouse was sacrificed and the tumor was resected for tissue culture.

Tissue culture A sample of the tumor was washed and minced into 1 mm pieces. The small tissue fragments were washed in Ca- and Mg-free phosphate buffered saline (PBS) (Nissui) and centrifuged at 1500 rpm for 5 min at 4°C. The pellet was resuspended in 20 ml of PBS containing collagenase type IV (236.5U/ml, Sigma Chemical Co., St. Louis, MO) and incubated at 37°C for 120 min. The fragments were then filtered through a 90 μm-pore-mesh and the undigested tissue fragments were placed in 35 mm culture dish (Falcon; Becton Dickinson Labware, Oxnard, CA) and cultured with DMEM+20%FBS at 37°C in a humidified atmosphere of 5% CO_2 in the air. The medium was changed three times a week. For subculture, the cells were detached by treatment with trypsin-EDTA (GIBCO).

Morphological studies For light microscopic observation, cells grown on Lab-Tek Chamber Slide (Miles Laboratories, Naperville, IL) were washed with PBS three times, fixed in absolute methanol for 20 minutes, stained with hematoxylin and eosin. For electron microscopic observation, cells grown on 60 mm culture dish (Falcon) were washed with PBS three times, and then fixed in 2.5% glutaraldehyde for 2 hours at 4°C. After rinsing seven times with 0.1 M cacodylate buffer, the samples were postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanols and embedded in Epon 812. Cells were then separated from the slides, cut with an ultramicrotome, stained with uranyl acetate and lead citrate and examined under JEM-1200EX electron microscope (Nihon densi).

Growth curve A growth curve was obtained by seeding the YES-2 cells at the 102nd passage at 1×10^5 cells / 60 mm culture dish in 5 ml of DMEM+10%FBS in duplicate. The medium was changed every 2 days after seeding. Cells were detached with trypsin-EDTA and the average number of viable cells from two dishes was determined by trypan blue exclusion test in a Bürker-Türk hemocytometer.

Tumor markers Secretion of tumor markers by YES-2 cells was assessed at the 102nd passage in duplicate. Cells were grown near to confulency for 48 hours in 5 ml of DMEM+10%FBS. The media from these cells were centrifuged (3000 rpm 10 min at 4°C) and the supernatant was assayed for the presence of SCC antigen, TPA and CA125.

Chromosome study YES-2 cells at the 30 th passage were used for the chromosome study. G-banding was carried out using the method of Seabright[9].

Heterologous trasplantation Five to six week old male athymic nude mice were used to examine the tumorigenicity of YES-2 cells. 1×10^6 cells at the 46th passage were suspended in 200 μl of PBS and inoculated subcutaneously into the back of nude mice.

Mycoplasma detection After YES-2 cells at the 35th passage were passed twice through antibiotic free medium, the medium to be tested was exposed to the cells for 3 days. Mycoplasma contamination was examined by Gen-Probe Mycoplasma T. C. Rapid Detection Kit (Gen-Probe Co., Inc, CA).

Results

Original tumor Histologic study of the resected tumor revealed a moderately differentiated squamous cell carcinoma (Fig. 1).

Tumor transplanted into nude mice Four months later, subcutaneous tumor greater than 2 cm in diameter developed (Fig. 2).

Establishment of cell line A few days after initiation of the primary culture, small colonies of epithelial like cells were observed arround undigested tissue fragments. These colonies increased in size and pilling up of cells was observed in the center of colonies. Growth of fibroblast like cells, which were

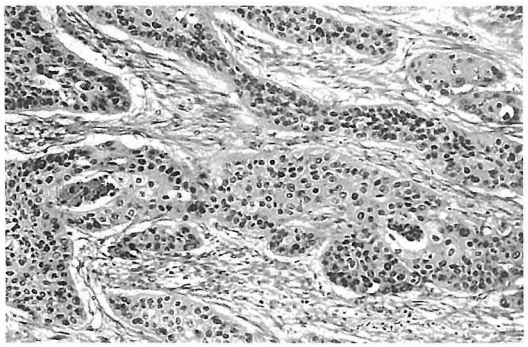

Fig. 1 Histological appearance of the original tumor (H & E, original magnification × 100).

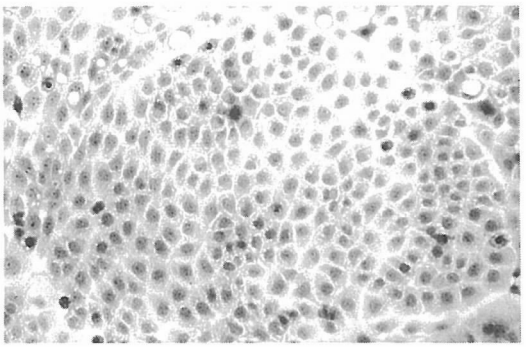

Fig. 3 Light microscopic view of YES-2 cells (H & E, original magnification ×100).

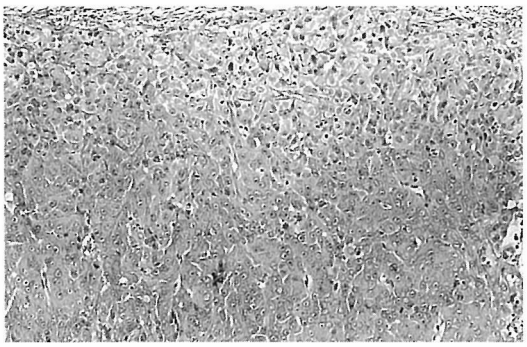

Fig. 2 Histological appearance of the transplanted tumor (H & E, original magnification ×100).

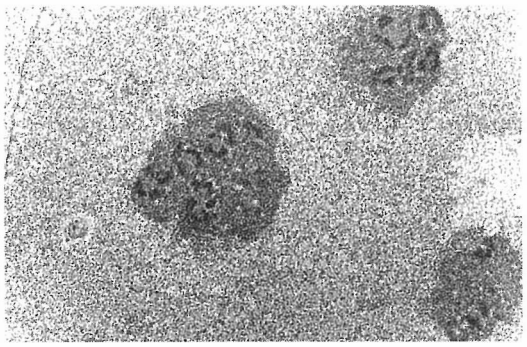

Fig. 4 Electron-microscopic view of YES-2 cells, Bar, 1 μm. (original magnification × 9000).

easily distinguishable from the epithelial like cells under phase contrast microscopy, was also observed around the colonies. At 36 days cells were treated with trypsin-EDTA and subcultured in T-25 flasks (Falcon). Fibroblast like cells were removed by differential trypsinization and serial passages. After obtaining a stable cell growth the cells were cultured and maintained in DMEM+5% FBS in T-25 flasks. This cell line grew continuously and was passaged for 196 generations over 68 months. It has been designated the YES-2 cell line.

YES-2 cells proliferate in a pavement-like arrangement. After reaching confluence, the cells had tendency to pile up and showed a lack of contact inhibition. These cells had clear polygonal shaped cytoplasm, and round nuclei (Fig. 3) and then electron-microscopy demonstrated prominent nucleoli with high density (Fig. 4).

Growth curve The doubling time of cell population in the logarithmic phase was 23.7 hours (Fig. 5).

Tumor markers The presence of SCC antigen, TPA and CA125 were detected. These antigens were not detected in DMEM+10%FBS without cells.

Chromosome study Chromosomal analysis at the 30th passage revealed that the number of chromosomes ranged from 53 to 62, with a mode of 60 (Fig. 6). Analysis of cells

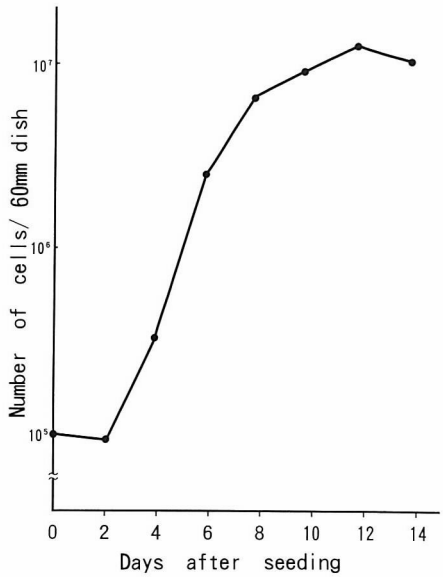

Fig. 5 Growth curve of YES-2 cells in the DMEM+10%FBS at the 102nd passage.

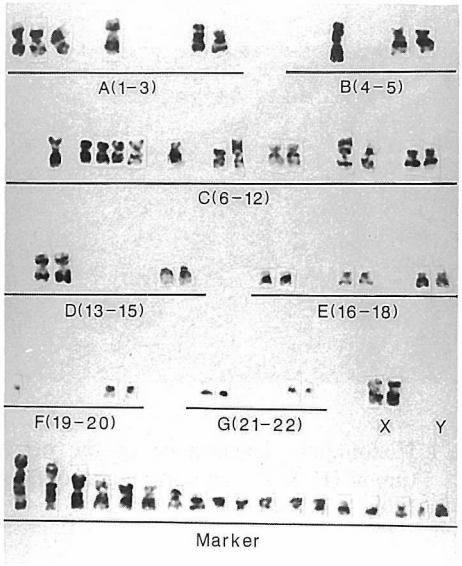

Fig. 7 A G-banding karyotype obtained at the 30 th passage from YES-2 cells with 60 chromosomes.

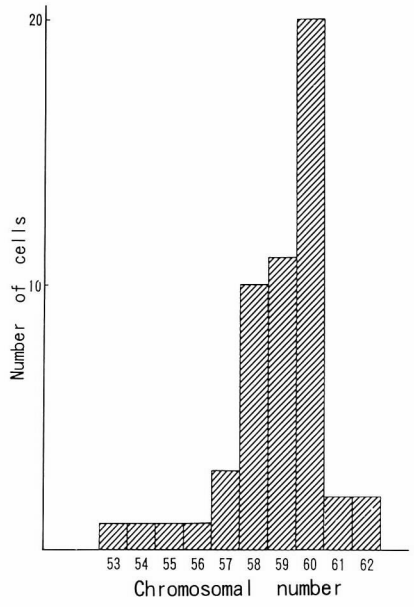

Fig. 6 Distribution of chromosomal number at the 30th passage.

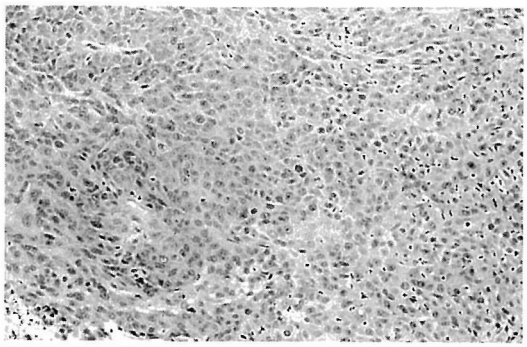

Fig. 8 Histological appearance of the subcutaneous tumor (H & E, original magnification ×100).

containing 60 chromosomes revealed a human karyotype with common structural abnormalities ($3p^-$, i4q, $6p^+$, $7q^-$, $11p^+$, $11p^-$, $13p^+$, norisomy of 4, 6, 11, 13, 14, monosomy of 2, 3, 8, 9, 19 trisomy of 1, and deletion of Y) with 18 marker chromosomes (Fig. 7).

Heterologous trasplantation Visible subcutaneous tumors were detected about 2 weeks after inoculation and gradually increased in size (Fig. 8).

Mycoplasma detection Mycoplasma con-

tamination was not present.

Discussion

Morphologically, YES-2 cells exhibited an epithelial cell growth pattern with a pavement-like arrangement and show a lack of contact inhibition. Light and electronmicroscopy showed these cells to have the feature of squamous cells. YES-2 cells have shown stable growth for more than 68 months through 196 passages, and tumorigenicity was shown by the development of tumor after subcutaneous injection of the cells into nude mice. It was confirmed that these cells were human origin by the chromosome studies, so we concluded that the cell line we established was derived from a human esophageal cancer.

As to the secretion of tumor markers by YES-2 cells, SCC antigen, TPA and CA125 were produced. These tumor markers have been used for diagnosis and management of malignant tumor. However, the secretion mechanism still remains unclear. It may be shed by further investigation by using YES-2 cells. By contrast, we successfully established a human esophageal cancer cell line (YES-1). As to morphology, growth curve and chromosomal study, there are some characteristic differences between YES-1 and YES-2. In general, clinical courses of esophageal cancer are various. This means more cell lines which have different character are necessary.

In conclusion, YES-2 cell line is a new human esophageal cancer cell line which should be a useful experimental model for human esophageal cancer.

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