View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Kyoto University Research Information Repository
KURENAI 紅
Kyoto University Research Information Repository
KYOTO UNIVERSITY

Title	Establishment and Characterization of A New Human Esophageal Cancer Cell Line (YES-1)
Author(s)	NAKAMURA, MASAYUKI
Citation	日本外科宝函 (1991), 60(1): 3-12
Issue Date	1991-01-01
URL	http://hdl.handle.net/2433/203778
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

Kyoto University

Arch Jpn Chir 60(1), 3～12, Jan., 1991

原　　　著

Establishment and Characterization of A New Human Esophageal Cancer Cell Line (YES-1)

Masayuki Nakamura

Second Department of Surgery, Yamaguchi University School of Medicine
(Director: Prof. Dr. Takashi Suzuki)
Received for Publication, Dec. 13, 1990.

Summary

A new human esophageal cancer cell line (YES-1) was established from a subcutaneous tumor implanted into nude mice, which had been transplanted from a surgical specimen obtained 50-year-old Japanese male patient. This cell line has been maintained for 33 months through 94 passages with stable growth. YES-1 cells are mainly polygonal-to-spindle shaped, have eosinophilic cytoplasm and oval-to-round nuclei with some prominent nucleoli. There are also some cells having clear cytoplasm and round nuclei with prominent nucleoli. The cells proliferate in a pavement-like cell arrangement and show a lack of contact inhibition. The doubling time at the 33rd passage was 35.2 hours.

YES-1 cells produce carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC antigen) and tissue polypeptide antigen (TPA) as tumor markers. Chromosome study have shown that the chromosome number ranges from 47 to 54 with a mode of 51. Tumorigenicity has been identified by the development of tumors after the subcutaneous injection of YES-1 cells into nude mice, which were found to be similar to the original tumor on histological examination. Thus, these findings indicate that the YES-1 cell line is available as a new human esophageal cancer cell line which should be useful for various studies.

Introduction

Establishment of cell lines derived from human esophageal cancer has been reported by many investigators [1, 7, 9, 10, 13]. These cell lines have provided valuable information on various aspect of this tumor, including carcinogenesis, histogenesis, diagnosis and therapy. However esophageal cancer remains one of disease with a poor prognosis, so many more cell lines are need to be obtained in order to study this neoplasm in greater depth.

This paper reports the establishment and characterization of a new human esophageal cancer

Key words: Esophageal cancer, Cell line, Tumor markers, Nude mice, Transplanted tumor.
索引用語：食道癌，細胞株，腫瘍マーカー，ヌードマウス，移植腫瘍．
Present address: Second Department of Surgery, Yamaguchi University School of Medicine, 1144 Kogushi, Ube-shi 755, Japan.

cell line which has been designated as YES-1.

Materials and Methods

Patient A 50-year-old Japanese man was admitted to our hospital because of epigastralgia and retrosternal pain occurring after eating. He was diagnosed as having esophageal cancer on the basis of further investigation. On admission, the serum level of carcinoembryonic antigen (CEA) was elevated to 6.5 ng/ml, while the levels of squamous cell carcinoma antigen (SCC antigen) and tissue polypeptide antigen (TPA) were 2.0 ng/ml and 67 U/l, respectively. At operation, a 4.0×2.3 cm well-defined tumor was found in the middle third of the esophagus. The tumor was ulcerated and the surface was partly necrotic (Fig. 1). After obtaining a specimen from the tumor for heterotransplantation, the remainder of the resected lesion was fixed in the 10% formalin for light microscopic examination.

Transplantation into nude mice A specimen was aseptically obtained from the tumor and was trimmed of necrotic tissue. It was washed three times in Hanks' balanced salt solution (HBSS) (Nissui Seiyaku Co., Japan), and then it was minced with two surgical blades into about 3 mm pieces. These tumor fragments were then transplanted subcutaneously into the backs of 5- to 6-week-old male athymic nude mice (BALB/C, nu/nu, Clea Japan Inc.) using a trocar needle. When the subcutaneous tumor developed to more than 1 cm in diameter, the mouse was sacrificed and the tumor was resected for tissue culture and light microscopic examination.

Tissue culture medium 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 Falls, OH), 12 mM sodium bicarbonate and 20% fetal calf serum (FCS) (M. A. Bioproducts, Walkersville, MD) was used as primary culture medium (PCM). DMEM+5%FCS was used for the maintenance culture medium.

Tissue culture A specimen was aseptically obtained from tumor transplanted into the nude mice and was washed and minced into about 1 mm pieces in the same manner. The small tissue fragments were washed in Ca- and Mg-free phosphate buffered saline (PBS) (Nissui) and were centrifuged at 800 rpm for 5 min at 4°C. The pellet was resuspended in 5 ml of PCM containing

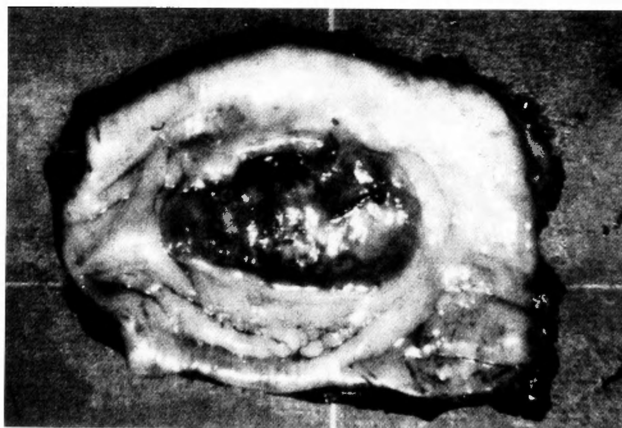

Fig. 1 The macroscopic appearance of the resected tumor showing ulceration.

dispase (1000 U/ml, Godo Syuei Co. Ltd, Japan) and incubated in a 37°C water bath for 30 min. After removing the undigested tissue with a 100-μm-pore mesh, the cell suspension was centrifuged at 800 rpm for 5 min at 4°C. It was then resuspended in PCM, and placed in T-25 flasks (Falcon; Becton Dickinson Labware, Oxnard, CA) at 37°C in an atmosphere of 5% CO_2 in air. The medium was changed three times a week. For subculture, the cells were detached by treatment with trypsin-EDTA (GIBCO). During the early passages, cells were frozen and stored in liquid nitrogen in a medium composed of 10% dimethyl sulfoxide in the maintenance culture medium.

Observations YES-1 cells were observed daily using a phase-contrast microscope (Nikon). For light microscopic observation, cells grown on Lab-Tek chamber slides (Miles Laboratories, Naperville, IL) were washed with PBS three times, fixed in absolute methanol for 20 min, and then stained with hematoxylin-eosin. For electron microscopic observation, confluent monolayer cells grown on Lab-Tek chamber slides were washed with PBS three times, and then fixed in Karnovsky's fixing fluid for 1 hour at room temperature. After rinsing three times with 0.1 M sodium cacodylate buffer, the samples were postfixed in 2% osmium tetraoxide with Millonig's buffer (1 : 1), dehydrated in a graded series of ethanols and embedded in Epon 812. Cell monolayers were then separated from the slides, sectioned on an ultramicrotome, stained with uranyl acetate and examined under a Hitachi H-500 electron microscope.

Growth curve Growth curve was obtained by seeding the YES-1 cells at the 33rd passage at 1×10^5 cells/60 mm culture dish in 5 ml of DMEM+5%FCS 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 counting cells that excluded trypan blue in a Bürker-Türk hemocytometer. The doubling time of the cell population was estimated in the logarithmic growth phase.

Tumor markers The secretion of tumor markers was assessed using YES-1 cells grown for 48 hours in 5 ml of DMEM+5%FCS, when growth curve was obtained. Supernatants were obtained by centrifugation (3000 rpm 10 min at 4°C) of spent medium, and were assayed for CEA, SCC antigen and TPA using radioimmunoassasy kits (CEA, SCC antigen: Dainabot, Japan; TPA: Daiichi isotope Lab, Japan).

Chromosome study YES-1 cells at the 7th passage were used for the chromosome study. Three days after seeding, the cells into a T-25 flask, colcemid was added to the flask (0.04 μg/ml) and incubation was performed at the 37°C for 2 to 4 hours. Cells were detached with 0.25% trypsin and treated with hypotonic 0.075 M KCl solution for 10 min. They were then centrifuged and fixed with a mixture of methanol and acetic acid (3 : 1) twice. Cells suspended in fixative were placed onto slides, flame dried and stained with Giemsa. G-banding was carried out using the method of Seabright [19], by staining the cells with Giemsa after 5 min of 0.0125% trypsin treatment.

Heterologous transplantation Five- to six- week-old male athymic nude mice were used to examine the tumorigenicity of YES-1 cells. 3×10^6 cells at the 21st passage were suspended in 200 μl of PBS and were inoculated subcutaneously into the backs of two mice. The mice were killed 4 months after inoculation and the subcutaneous tumors were routinely processed for light microscopy.

Mycoplasma detection After YES-1 cells were passed twice through the 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, California).

Results

Morphology of the original tumor The resected tumor was composed of spindle-shaped cells having eosinophilic cytoplasm and round-to-oval nuclei with some prominent nucleoli. These cells were arranged in nests and showed focal keratinization (Fig. 2).

Morphology of the tumor transplanted into nude mice The transplanted tumor consisted of two types of cells. Most of the cells had eosinophilic, spindle-shaped cytoplasm and round-to-oval nuclei with some prominent nucleoli. The other cells had clear and polygonal cytoplasm and round nuclei with some prominent nucleoli. Thin bundles of collagen fibers were present among the tumor cell nests (Fig. 3).

Establishment and morphology of the cell line A few days after primary culture, small colonies of epithelial-like cells were observed in several flasks. These colonies increased in size and piling up of the cells was observed in the center of the colonies. Growth of fibroblast-like cells, which were easi-

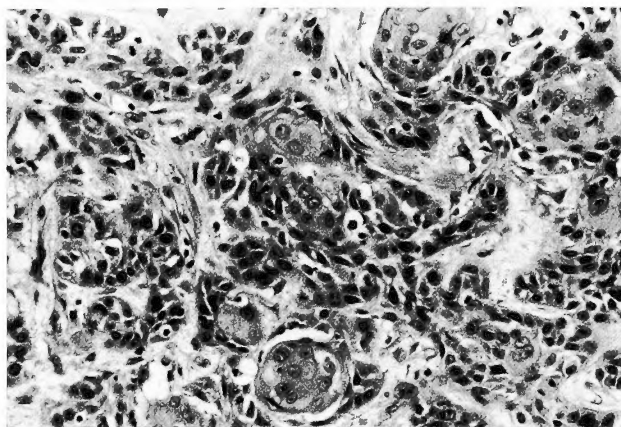

Fig. 2 Histological appearance of the original tumor showing nest formation with focal keratinization (H&E stain, ×100).

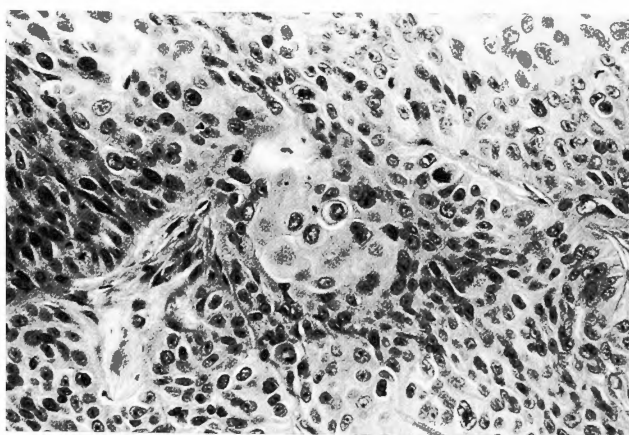

Fig. 3 Histological appearance of the transplanted tumor showing two types of cells (H&E stain, ×100).

ly distinguishable from the epithelial-like cells under phase contrast microscopy, was also observed around the colonies. After removing the fibroblast-like cells from a T-25 flask with pipettes, the epithelial-like colonies were subcultured to two T-25 flasks after treatment with trypsin-EDTA at 53 days. This cell line grows continuously and has been passed for 94 generations over 33 months period, it has been designated the YES-1 cell line.

YES-1 cells were found to be proliferate in a pavement-like arrangement. After reaching con-

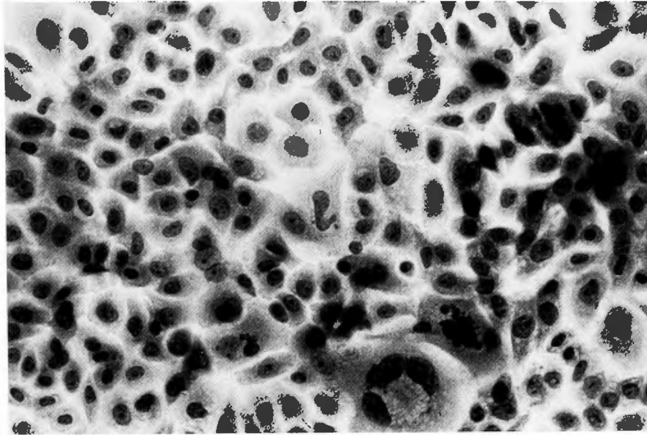

Fig. 4 Light microscopic view of YES-1 cells showing their pavement-like arrangement (H&E stain, ×200).

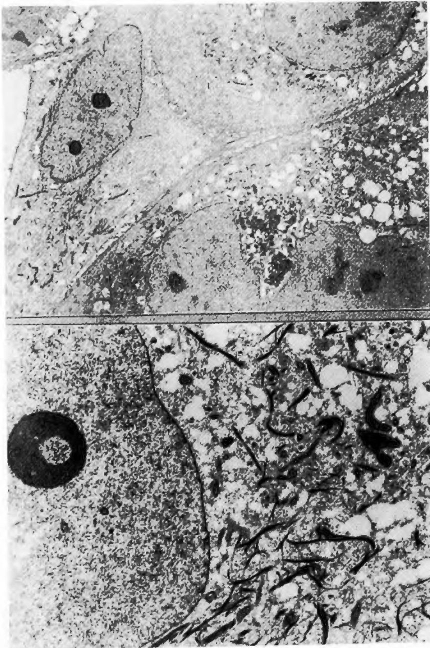

Fig. 5 Electron microscopic view of YES-1 cells showing poorly developed desmosomes and abundant tonofilaments (Upper ×1500, lower ×4000).

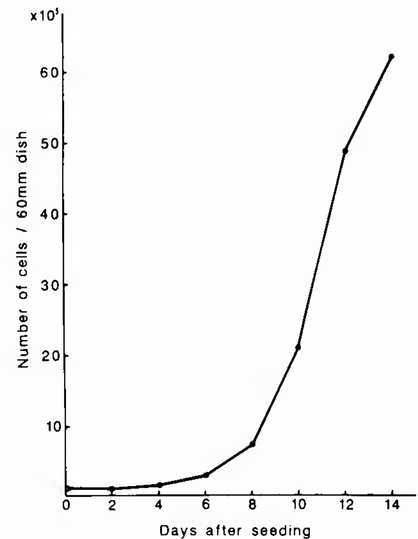

Fig. 6 Growth curve of YES-1 cells in DMEM +5%FCS at the 33rd passage.

fluence, the cells had a tendency to pile up and showed lack of contact inhibition. These cells were shown to have pleomorphic features, being mainly polygonal-to-spindle shaped, with eosinophilic cytoplasm and oval-to-round nuclei with some prominent nucleoli. There were also cells having clear cytoplasm and round nuclei with some prominent nucleoli. Occasionally, multinucleated giant cells were observed (Fig. 4). These cells resembled the original tumor cells morphologically. Electron microscopy demonstrated abundant tonofilaments, but few organelles in the cytoplasm. Desmosomes between adjacent cells were poorly developed (Fig. 5).

Growth curve Cells grew rapidly after a lag phase of 48 hours and entered a logarithmic growth phase (Fig. 6). The doubling time of the cell population in the logarithmic phase was 35.2 hours.

Tumor markers The presence of CEA, SCC antigen and TPA was detected in the spent media. The relationship between cell growth and the secretion of tumor markers was as follows: pro-

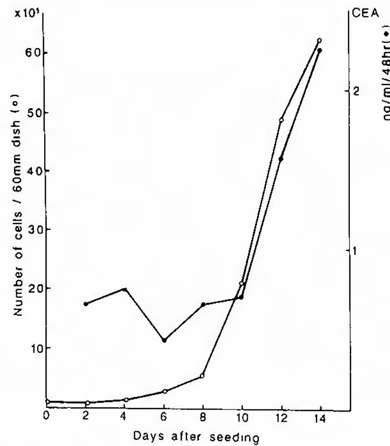

Fig. 7 Correlation between CEA production and the growth curve.

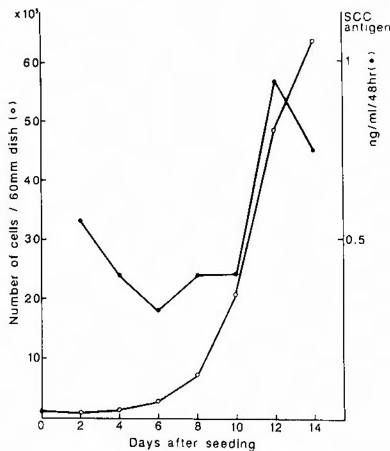

Fig. 8 Correlation between SCC antigen production and the growth curve.

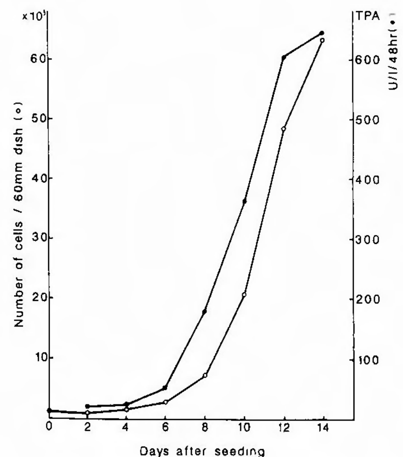

Fig. 9 Correlation between TPA production and the growth curve.

duction of CEA levels arose at the lag phase transiently, and paralleled the growth curve except for the early logarithmic phase (Fig. 7), SCC antigen levels did not parallel the growth curve (Fig. 8), and TPA levels paralleled the growth curve in all phases (Fig. 9). CEA, SCC antigen and TPA were not detected in the fetal calf serum.

Chromosome study The number of chromosomes was ranged from 47 to 54, with a mode of 51 (Fig. 10). Analysis of cells containing 51 chromosomes by the G-banding method revealed 14 marker chromosomes and common structural abnormalities (1q−, 6q−, 7p+, 10p+, 13p+,

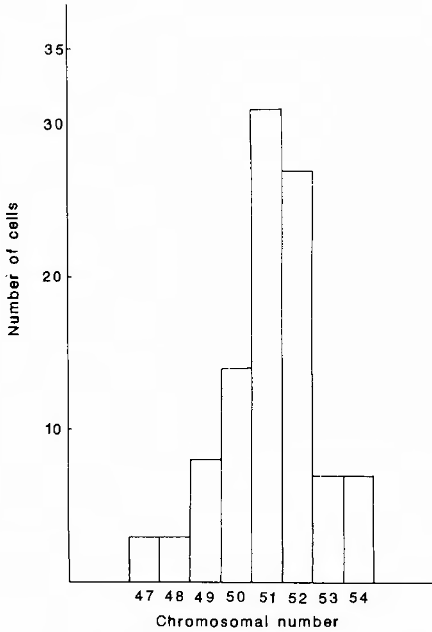

Fig. 10 Distribution of chromosomal numbers at the 7th passage of YES-1 cells.

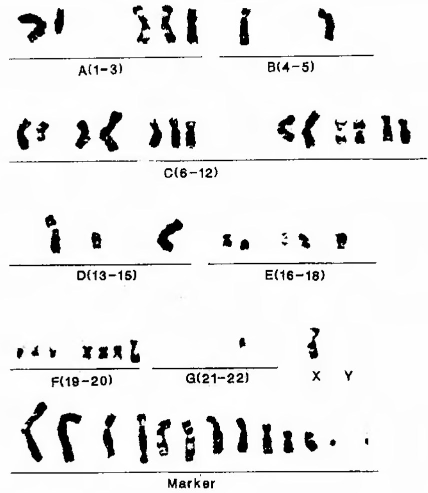

Fig. 11 A G-banded karyotype obtained at the 7th passage from YES-1 cells with 51 chromosomes.

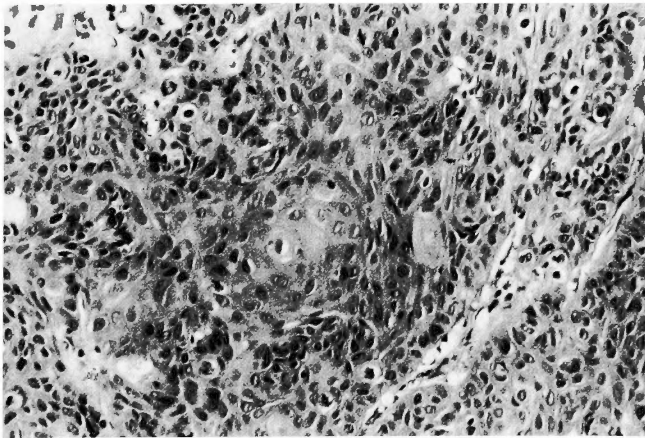

Fig. 12 Histological appearance of a subcutaneous tumor showing spindle-shaped cells with focal keratinization.

15p+, 20q+, norisomy of 2, 9, 10, 13, 15, 21, monosomy of 6, 7, 14, 18, trisomy of 8, 20, and deletion of Y) (Fig. 11).

Heterologous transplantation Visible subcutaneous tumors were detected in nude mice about 2 months after inoculation with YES-1 cells, and gradually increased in size. These tumors were composed spindle-shaped cells having eosinophilic cytoplasm and round-to-oval nuclei with some prominent nucleoli. These cells grew in nests with focal keratinization, which were separated by thin fibrous stroma (Fig. 12).

Mycoplasma detection Mycoplasma contamination was not present.

Discussion

Since the first report of the transplantation of a human colon cancer into nude mice by *Rygaard* and *Povlsen* [16] in 1969, transplantation of human malignant tumors into nude mice has been reported by many authors [5, 14–18]. Since the transplanted tumors retain the morphological and functional characteristics of the original tumor, transplantation into nude mice is recognized as one of the useful in vivo tumor experimental models. We subcutaneously transplanted the human esophageal cancer cells that we obtained into nude mice, because transplantation is a simple procedure, and if the transplanted tumors develop, the chances of obtaining fresh cancer cells are increased. The overall success rate for the transplantation of esophageal cancer is 83% at our laboratory (unpublished data).

Morphologically, YES-1 cells exhibit an epithelial cell growth pattern with a pavement-like arrangement and show a lack of contact inhibition. Light and electron microscopy showed these cells to have the feature of squamous cells and to resemble the original tumor cells. YES-1 cells have shown stable growth for more than 33 months. through 94 passages, and tumorigenicity was shown by the development of tumors after subcutaneous injection of the cells into nude mice. It was confirmed that these cells were of human origin by the chromosome studies, so we concluded that the cell line we established was derived from a human esophageal cancer.

Recently, many tumor markers have been used for diagnosis and management of malignant tumors. In particular, serial determinations of tumor markers have been widely accepted as a promising method for monitoring disease progression and for the detection of recurrence. CEA, SCC antigen and TPA have been used as tumor markers after being identified by *Gold* and *Freedman* [6], *Kato* and *Torigoe* [8], *Björklund, B.* and *Björklund, V.* [2], respectively. High serum levels of these tumor makers are sometimes found in patients with esophageal cancer. In our patient, the serum level of CEA was elevated but levels of SCC antigen and TPA were not so high, although the YES-1 cells were shown to produce CEA, SCC antigen and TPA. Though the reason for this discrepancy is uncertain, the following hypotheses can be suggested. First, during culture TPA producting subclones may have been mainly selected and/or better adapted to the tissue culture medium. YES-1 cells show pleomorphic features, so they may have been subcloned further during the serial passages. Changes of tumor marker production in the course of passage have been reported by *Yano* et al [21]. Accordingly, it is necessary for us to examine this factor further in cells from passages. Second, as in the lag phase the production of CEA was noted while TPA secretion was absent, most of the original esophageal cancer cells might have corresponded to this phase of the YES-1 cells. A correlation between production of tumor markers and the cell cycle has been reported by *Davis* et al [4], but its mechanism is not yet clearly understood. Third, some substance(s) which induce YES-1

cells to produce TPA may be contained in fetal calf serum. Changes of tumor marker production with different tissue culture media have been reported by *Yano* et al [20. 21]. To test this hypothesis, we plan to culture YES-1 cells in serum-free medium with various hormones and/or growth factors, following methods previously reported [3. 11. 12. 19]. If materials which enhance the expression of tumor markers without assisting the development of tumors can be purified, they will be useful for detecting malignant disease at a very early stage. Furthermore if materials which inhibit tumor cell growth are discovered, they might be therapeutically valuable.

In conclusion, the YES-1 cell line retains the morphological and functional characteristics of the original human esophageal cancer. This cell line should therefore be a useful experimental model not only for investing esophageal cancer but also for studying the production of tumor markers.

Acknowledgement

The author acknowledges Prof. T. Suzuki of Yamaguchi University for helpful comments, Dr. T. Murakami of Yamaguchi University for helpful discussions. This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References

1) Bey E, Alexander J, Whitcutt JM, et al: Carcinoma of the oesophagus in Africans: establishment of a continously growing cell line from tumor specimen. In vitro. **12**: 107, 1976.
2) Björklund B, Björklund V: Antigenicity of pooled human malignant and normal tissue by cytoimmunological technique; Presence of an insoluble, heat-labile tumor antigen. Int. Arch. Allergy and Appl. Immunol. **10**: 153, 1957.
3) Bottenstein J, Hayashi I, Hutchings S, et al: The growth of cells in serum-free hormone-supplemented media. Methods Enzynol. **58**: 94, 1979.
4) Davis KM, Zurawski VR, Bast RC Jr, et al: Characterization of the CA 125 antigen associated with human epithelial ovarian carcinomas. Cancer Res. **46**: 6143, 1986.
5) Giovanella BC, Yim SO, Stehlin JS, et al: Development of invasive tumors in the nude mouse after injection of cultured human melanoma cells. JNCI. **48**: 1531, 1972.
6) Gold P, Freedman SP: Demonstration of tumor specific antigen in human colonic carcinoma by immunological tolerance and absorption technique. J. Exp. Med. **121**: 439, 1965.
7) Hu CP, Hsieh HG, Chien KY, et al: Biologic properties of three newly established human esophageal carcinoma cell lines. JNCI. **72**: 577, 1984.
8) Kato H, Torigoe T: Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. Cancer. **41**: 1621, 1977.
9) Laboratory Section, Department of Surgery, People's Hospital, Peking Medical College: Establishment of a cell line from human esophageal carcinoma. Chinese Med. J. **2**: 357, 1976.
10) Matsuoka H, Sugimachi K, Ueo H, et al: Sex hormone response of a newly established squamous cell line derived from clinical esophageal carcinoma. Cancer Res. **47**: 4134, 1987.
11) Murakami H, Masui M: Hormonal control of human colon carcinoma cell growth in serum-free medium. Proc. Natl. Acad. Sci. USA. **77**: 3464, 1980.
12) Nakabayashi H, Taketa K, Miyano K, et al: Growth of human hepatoma cell lines with differentiated functions in chemically defined medium. Cancer Res. **42**: 3858, 1982.
13) Nishihira T, Kasai M, Mori S, et al: Charactereristics of two cell lines (TE-1 and TE-2) derived from human squamous cell carcinoma of the esophagus. Gann. **70**: 575, 1979.
14) Povlsen CO, Rygaard J: Heterotransplantation of human adenocarcinomas of the colon and rectum to the mouse mutant nude. A study of nine consecutive transplantation. Acta Path. Microbiol. Scand. **79**: 159, 1971.
15) Robinson KM, Maistry L: Tumorigenicity and other properties of cells from ten continuous human esophageal carcinoma cell lines in nude mice. JNCI. **70**: 89, 1983.
16) Rygaard J, Povlsen OC: Heterotransplantation of a human malignant tumor to nude mice. Acta Path. Microbiol. Scand. **77**: 758, 1969.

17) Seabright M: A rapid banding technique for human chromosomes. Lancet. 2: 971, 1971.
18) Sharkey FE, Fogh J: Considerations in the use of nude mice for cancer research. Cancer Metastasis Rev. 3: 341, 1984.
19) Shimosato Y, Kameya T, Nagai K, et al: Transplantation of human tumors in nude mice. JNCI. 56: 1251, 1976.
20) Simms E, Gazdar AF, Adrams PG, et al: Growth of human small cell (oat cell) carcinoma of the lung in serum-free growth factor-supplemented medium. Caner Res. 40: 4356, 1980.
21) Yano H, Kohjiro M, Nakashima T: A new human hepatocellular carcinoma cell line (KYN-1) with transformation to adenocarcinoma. In vitro. 22: 637, 1986.
22) Yano H, Maruiwa M, Murakami T, et al: A new human pleomorphic hepatocellular carcinoma cell line, KYN-2. Acta Pathol. Jpn. 38: 953, 1988.

和文抄録

ヒト食道癌細胞株 (YES-1) の樹立と性状

山口大学医学部外科学第2講座（指導：鈴木　敞教授）

中村真之

50歳日本人男性食道癌症例より採取した腫瘍細胞をヌードマウス背部皮下に移植し，生着した腫瘍組織よりヒト食道癌細胞株 (YES-1) を樹立した．YES-1 細胞は培養開始後33ヶ月が経過し，その間94回の継代に成功しており安定した増殖性を示している．

YES-1 細胞は，主に，明瞭な核小体をもった卵型から類円型の核とエオジン好性の多角型から紡錘型の胞体を有する細胞よりなり，明瞭な核小体をもった類円型の核と明調な多角型の胞体を有する細胞も存在しており，それらは敷石状に増生している．33代めにおける倍加時間は35.2時間であった．

YES-1 細胞は carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC antigen), tissue polypeptide antigen (TPA) を産生する．染色体分析で染色体数は47から54に分布しており，モードは51であった．異種移植能は YES-1 細胞をヌードマウス背部皮下に移植することによって確認された．また，移植腫瘍の組織像は食道癌原発巣の組織像と類似していた．

このような性格を有する YES-1 細胞は，食道癌研究のさまざまな分野において有用であると思われる．