Some Properties of a Newly Established Human Cell Line Derived from a Oral Squamous Carcinoma

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SUMMARY

A human squamous carcinoma cell line was established from a metastatic oral squamous carcinoma. The cell line, designated as OSC-20, grew in cobblestone pattern demonstrating their epithelial origin. Electron microscopy revealed the presence of tonofilaments and desmosomes. OSC-20 cells were unable to form colonies in an anchorage-independent semisolid support system of soft agar. Immunofluorescence study indicated that the cells contained cytokeratins and vimentin. When transplanted into athymic nude mice, OSC-20 cells produced tumors which on histopathological examination were shown to be moderately differentiated squamous carcinomas. OSC-20 cells secreted GST-π into the culture medium. This cell line may provide a useful model for the study of human oral cancer.

Key words : Squamous cell carcinoma, Cell line, GST-π

INTRODUCTION

Recently, better results of treatment for patients with oral squamous cell carcinoma (SCC) have been gradually obtained, reflecting a few significant advances in treatment modalities, especially in chemotherapy and immunotherapy. Although a drastic clinical trial was attempted, basic research to investigate the biology of this type of tumor has been limited. In part, this has been due to the relative lack of well characterized oral SCC cell lines (1, 2, 9, 11, 14, 15, 17, 18). The establishment of human tumor cell lines in culture plays an impor-

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Abbreviations :
 SCC, squamous cell carcinoma ; GST, glutathione S-transferase

tant role in the investigation of the cellular biology.

The glutathione-S-transferases (GSTs) are a family of enzymes which are involved in the drug biotransformation and the metabolism of xenobiotics(8). The human anionic GST, GST-π has been recently found to serve as a new tumor marker of many malignant diseases, especially head and neck tumors(12). In this report, we describe the establishment and characterization of a new human oral SCC cell line, OSC-20, and GST-π production of these cells in vitro.

MATERIALS AND METHODS

Clinical course

A 58-year old woman was admitted to the hospital with a complaint of a mass with ulcer on the left lateral margin of the tongue. Excisional biopsy was done and the histopathological examination revealed a well differentiated SCC. Ten months after the operation, a tumor in the left oro-pharyngeal region was detected and total resection of the tumor was carried out. The pathologic study showed a moderately differentiated SCC. Five months after, she was readmitted because of a metastasis from the tongue or the oro-pharyngeal tumor to a sub-mandibular lymph node in the left side. After chemotherapy, a left radical neck dissection was performed. A portion of the metastatic tumor was used for culture. The patient shows no evidence of recurrence 2 years and 7 months after the surgery.

Establishment of a cell line

Primary culture was performed by the method as described previously(23). After 7 days of primery culture, the cells outgrowing from a tissue fragment formed a cell sheet with a diameter of 1 cm. The cell sheet was subcultured using 0.01% trypsin (2914 units/mg ; GIBCO) and 0.01% EDTA in Ca^{++}, Mg^{++} -free Hanks' balanced salt solution. The mesenchymal cells were removed with a rubber policeman as throughly as possible. At the 5 to 7 passages, the cells were cloned using a dilution plating technique. A single-cell suspension was diluted and dispensed into wells of 96-well tissue culture plates (Falcon), yielding on the average 1.5 cells/well. The wells containing only one cell as ascertained by microscopic observation were marked. The cells from a selected well were subcultured. Thus, one clonal cell line was established and designated as OSC-20.

Growth characteristics

To determine the population doubling time, the cells were plated at a density of 1×10^5 cells/60-mm dish. At intervals, the viable cells were counted in tripli-

cate. The plating efficiency on the plastic surface was determined from the plating of 1×10^2 cells/60-mm dish. After a 2 week cultivation, the cells were stained with Giemsa solution and the colonies formed were counted macroscopically. Anchorage independent growth was assayed by plating the cells in 0.3% agar on 35-mm dishes containing a 0.5% agar base. The cells were cultured for 30 days and colonies were counted under an inverted microscope.

Morphological observations

Ultrastructural examination and immunofluorescence were performed by the previously described method(23). The antibodies were purchased from commercial sources as follows: mouse monoclonal antibody to cytokeratins-PKK1 from Labsystems Oy (Helsinki, Finland); mouse monoclonal antibody to vimentin from DAKOPATTS a/s (Glostrup, Denmark); biotinylated anti-mouse IgG and FITC-conjugated avidin from Vector Labs. (Burlingame, USA).

Tumorigenicity

The cells were subcutaneously transplanted into the back of five athymic nude mice (CLEA Japan Co., Shizuoka, Japan) at 1×10^7 cells/mouse.

Chromosome analysis

Chromosome analysis was performed on culture at exponential growth phase. The cells arrested at metaphase with Colcemid (10^{-7} M for 2 hr) were harvested by trypsinization and treated with 0.075 M KCl for 20 min at 37℃. Following hypotonic treatment, the cells were fixed with Carnoy's fixative and Giemsa staining was done with the use of both conventional and G-banding(19) methods. We analyzed 20 well spread metaphases.

Assay for GST-π

GST-π level in culture media was assayed by a sandwich enzymeimmunoassay method of Takahashi *et al.*(21). Conditioned medium obtained by incubating ·cultures of OSC-20 for 24 hr was assayed for GST-π and the cells were counted simultaneously.

RESULTS

Morphology

The appearance of OSC-20 cells was shown in Fig. 1. They were epithelial in shape and grew in cobblestone patterns. Electronmicroscopy indicated that these cells possessed mitochondria, rough endoplasmic reticulum, a large number of free ribosomes, scattered tonofilaments, and desmosomes in the intercellular
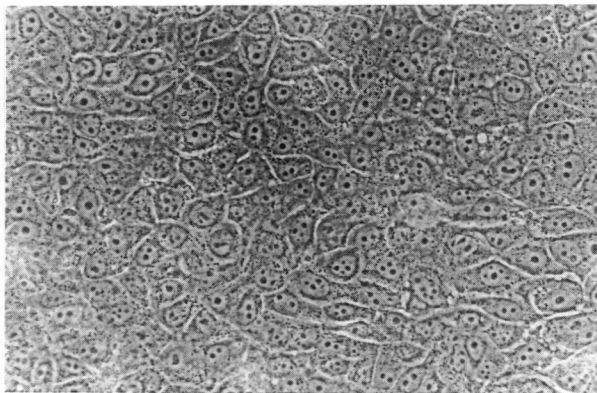
Fig. 1 A phase contrast microphotograph of OSC-20 cells. ×200.

connection (Fig. 2A and 2B). Indirect immunofluorescence revealed that OSC-20 cells were positive for cytokeratins and vimentin (Fig. 3A and 3B).

Growth characteristics

The population doubling time at the 35 th passage was estimated as 27.4 hr. The plating efficiency on the plastic surface was 4.7% at the 30 th passage, and the colony forming efficiency in semisolid agar was less than 0.001% at the 25 th passage.

Tumorigenicity

OSC-20 cells formed tumors in 4 of 5 nude mice inoculated. The tumors grew to approximately 10 mm in diameter in 2 months after transplantation. They remained encapsulated and did not invade neighboring tissues. Histologically, these tumors showed moderately differentiated SCCs (Fig. 4B). On the other hand, histologic examination of the original metastatic tumor removed from the patient demonstrated a poorly differentiated SCC (Fig. 4A).

Chromosome analysis

Chromosomes varied within a range from 59 to 76 with a modal number of 72 (Fig. 5). Giemsa-banded karyotype of OSC-20 was shown in Fig. 6. Distinct chromosome 3 was not found and many unidentified marker chromosomes (M1-M10) were observed.

Secretion of GST-π

As shown in Fig. 7, GST-π level in the culture media was elevated as the number of cells increased. The concentration of GST-π at exponential growth
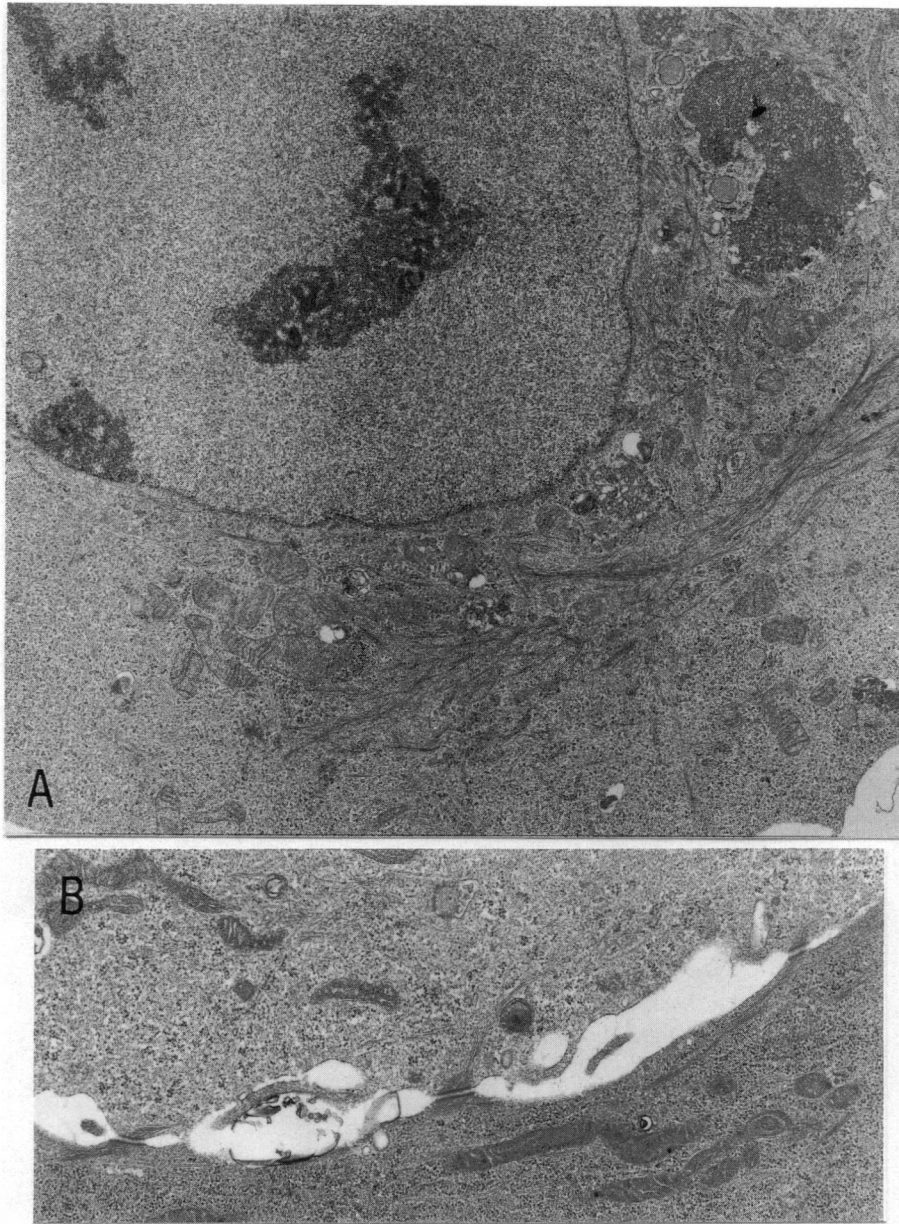
Fig. 2 Electron microphotographs of OSC-20.
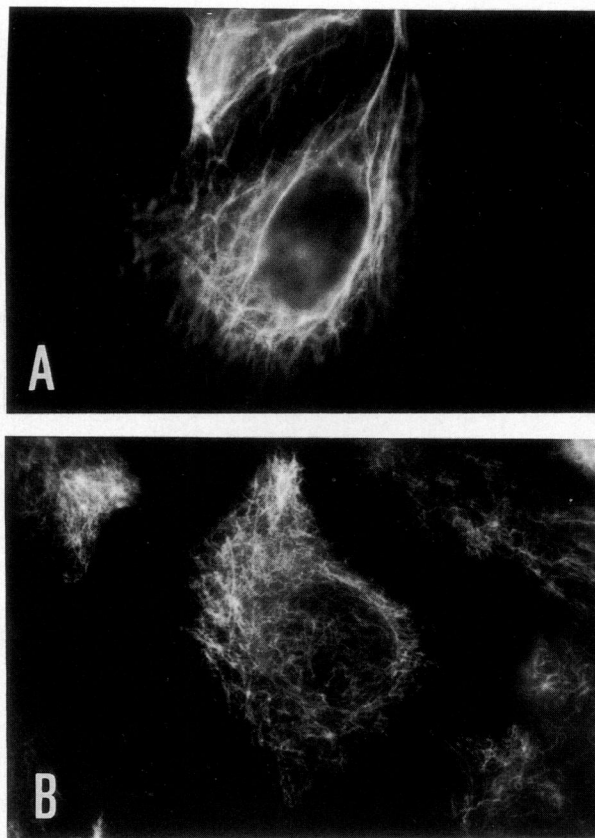   A. ×6,400.
         B. Desmosomes, ×15,000.

Fig. 3 Immunofluorescent microphotographs of OSC-20 cells.
　　　A. Cytokeratins.　×790.
　　　B. Vimentin.　×790.

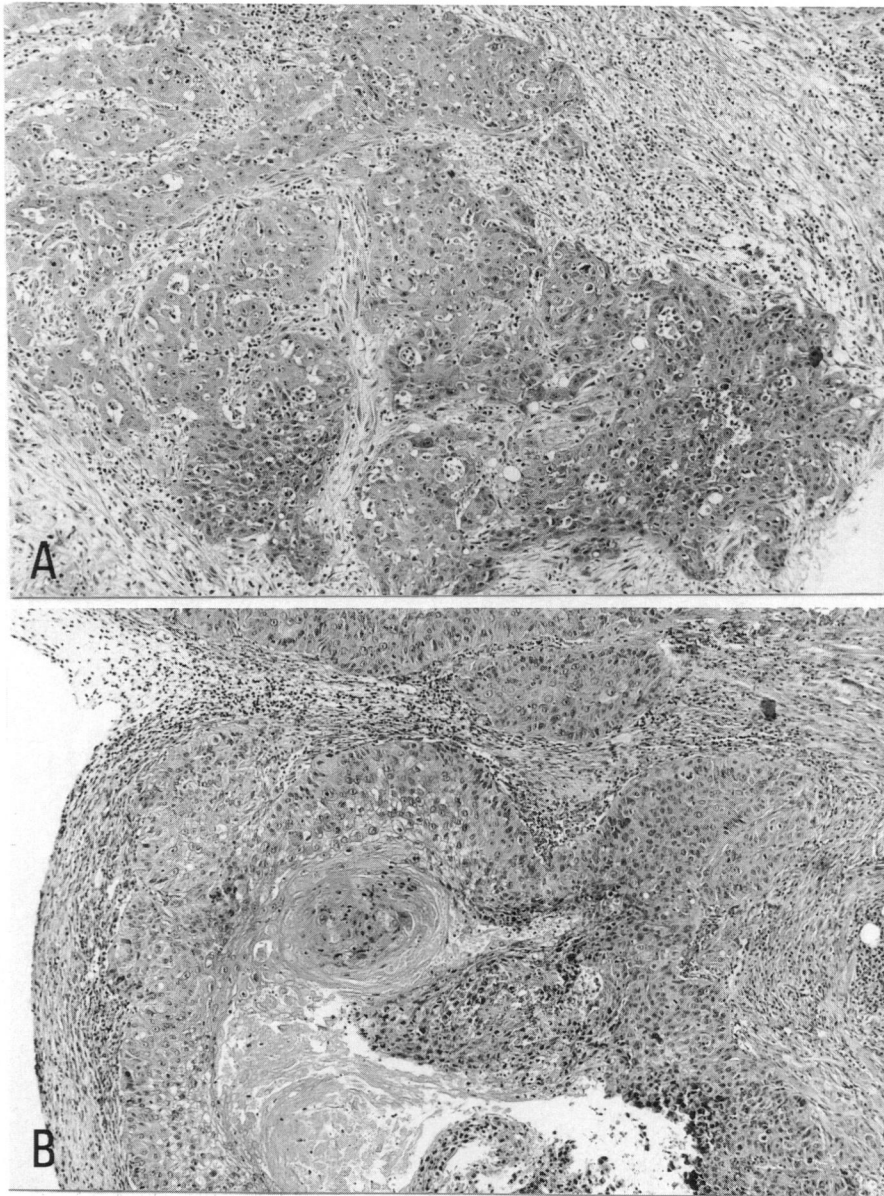**Fig. 4** Histological microphotographs of the tumors, H.-E. staining.
A. The original tumor from which OSC-20 was derived, ×85.
B. The tumor developed in nude mouse, ×85.
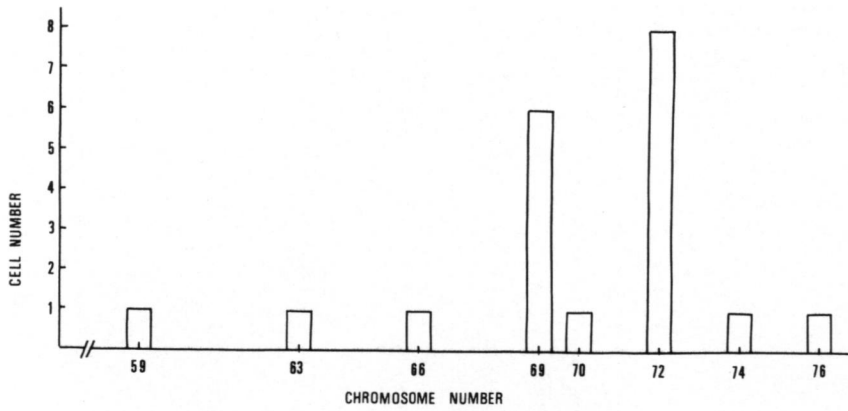
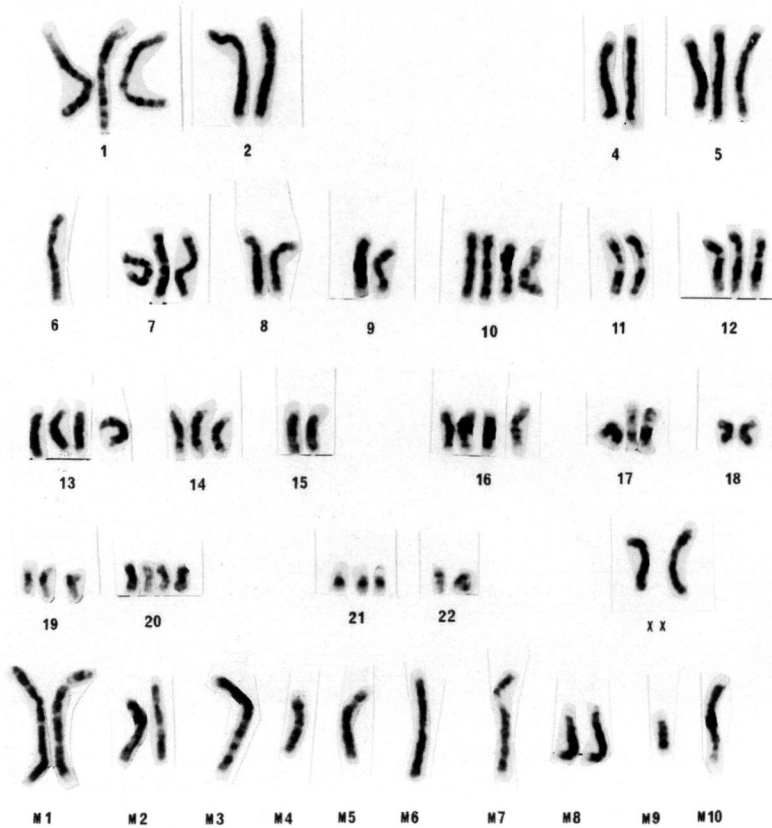**Fig. 5** Chromosome distribution in OSC-20 cells.

Fig. 6 Karyotype of OSC-20 cells at the 30th passage analyzed by G-banding.
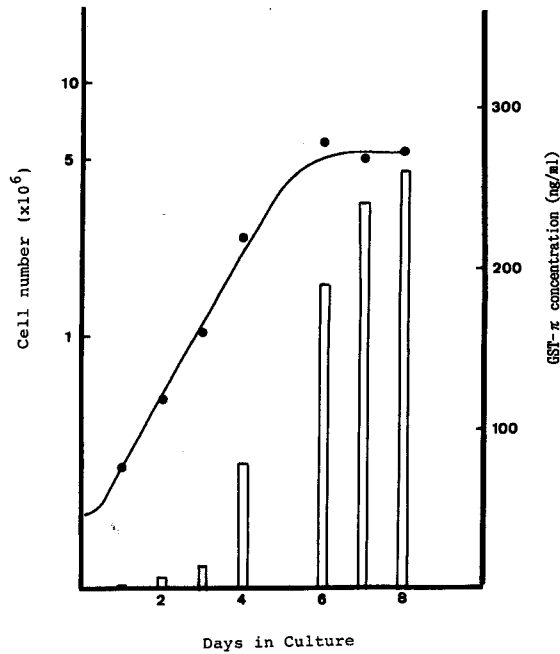
Fig. 7 Growth curve and GST-π levels in culture media. Black circle and open bar represent the mean value of duplicate dishes.
　● : Cell number.
　☐ : GST-π concentration.

phase (day 3) and at confluent phase (day 7) were 1.75 and 4.33 ng per 10^5 cells for 24 hr, respectively.

DISCUSSION

Human squamous cell carcinomas have been generally difficult to establish in culture as cell lines. However, trials to establish SCC cell lines have recently become more successful because of the development of cell culture technique(3, 9, 15). We have established a SCC cell line designated as OSC-19 which derived from a metastatic oral SCC(23). This line was considered to have little differentiated ability, because they had few tonofilaments and produced poorly differentiated SCCs in nude mice. OSC-20, a newly established cell line, had number of tonofilaments in their cytoplasm and produced tumors with cancer pearl like structures. Therefore, OSC-20 was thought to have more differentiating ability than OSC-19. These characteristics of this line appeared to be in contrast to the histology of the original tumor. However, there were also no apparent correlation between the histology of nude mice tumors and the

original tumor specimens in other SCC cell lines(15, 16).

Interestingly, immunofluorescence analysis revealed that OSC-20 contained both cytokeratins and vimentin. Although cytokeratins are typical of various epithelial cells and vimentin is routinely detected in mesenchymal cells, the co-expression of cytokeratins and vimentin is commonly seen in cultured cells, including human fetal keratinocyte(6) and rat hepatocyte(4). OSC-20 cells were polygonal in shape and grew in cobblestone pattern. Ultrastructurely, they had desmosomes and tonofilaments. These characteristics indicated strongly their epithelial origin despite of the vimentin expression.

Although it is well known that there is a correlation between tumorigenicity and growth in soft agar(5, 20), the correlation is not always found in cases of epithelial cell lines(10). OSC-20 cells were also unable to form colonies in soft agar despite of their tumorigenicity in nude mice. Therefor, OSC-20 cells are thought to be highly anchorage dependent.

Recently, GST-π has been considered to be a useful marker for patients with various types of cancers(13, 22). Hirata *et al.*(7) reported that elevated levels of GST-π were found in 24 of 32 patients with primary oral SCCs. We examined whether our established cell line, OSC-20 also produced GST-π in vitro or not, and found that OSC-20 cells produced GST-π into culture media. GST-π levels appeared to correlate with cell number. This may indicate that plasma GST-π levels in patients also correlate with the tumor size.

In conclusion, OSC-20 cell line appears useful for biological studies and GST-π production.

ACKNOWLEDGEMENTS

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